MICROBIOLOGY RESEARCH ADVANCES

MICROBIAL COMMUNICATION AND MICROBIOTA-HOST INTERACTIVITY

NEUROPHYSIOLOGICAL, BIOTECHNOLOGICAL, AND BIOPOLITICAL IMPLICATIONS

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MICROBIAL COMMUNICATION AND MICROBIOTA-HOST INTERACTIVITY

NEUROPHYSIOLOGICAL, BIOTECHNOLOGICAL, AND BIOPOLITICAL IMPLICATIONS

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A fascinating and contemporary book about networks and how microorganisms communicate and interact. The book has a plethora of interesting and though provoking facts. Although it contains many technical words and biological jargon the text is still understandable and keeps the reader captivated. The analogy between the microorganism and humans is allegoric. It is slightly apprehensive to read that we are at the mercy of our microbial guests. The understanding of how bacterial communicate could lead to new treatments and chemicals to fight pathogens, and help our symbiotic microorganisms. This highly informative book should be made compulsory reading for anyone interested in microbiology, gastroenterology, bacteriology, endocrinology and neurology. It could be used as a supplementary text in teaching biology, medicine, and even ethology.

Dr. Ronald Herman
Humanities, Science and English Teacher, DIS

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INTRODUCTION

Starting from the early 1980's, much attention has been given by the global microbiological community to cell-cell interaction and signal exchange in the microbial world as well as to the structure and functioning of microbial colonies and biofilms. This area of research is referred to herein as the *population organization and communication-centered paradigm (POCCP)* in microbiology.

Historians of science know that, before a new paradigm takes shape in a field of science, several decades are spent on disseminating new ideas that challenge pre-existing views. This trend was also characteristic of the development of POCCP. The main proponents of this novel paradigm include James Shapiro, Martin Dworkin, Eshel Ben-Jacob, Ian Sutherland, and other prominent researchers. However, their indisputably important contributions to the paradigm were antedated by the work of a whole school in Russian microbiology including Nikolai Yerusalimsky, Nikolai Krasil'nikov, Stanislav Smirnov, Galina El'-Registan, Vitaly Duda, Robert Pshenichny, Arsen Kapreliants, and others. Their studies were conducted in the 1950s-1980s; a recently defended dissertation on the history of microbiology describes their contributions (Kirovskaya, 2005).

These studies were foreshadowed by still earlier research that addressed the organization of life on the population and suprapopulation (ecosystem) levels as well as biological communication mechanisms in more general

terms, with respect to a wide spectrum of forms of life. As early as at the turn of the 20th century, Vladimir Vernadsky considered the whole biosphere as one coherent system. Leontii Ramensky emphasized the continuity of the plant formations that cover the whole planet. Biological evolution was envisioned as a result of the formation of symbiotic systems by diverse biological species (Andey Famintsyn and Boris Kozo-Polyansky). The similarities between human society and the biosocial systems formed by various life forms were highlighted in the works by Peter Kropotkin as well as the representatives of the Russian "phytosociology" school of thought. Or more direct relevance to microbiology were the ideas put forward by Vasily Kedrovsky in 1910 who emphasized the similarity beween the structure of a microbial colony and that of a multicellular organism. These ideas foreshadowed Nikolai Yerusalimsky's views set forth in his dissertation (1952) as well as Shapiro's relatively recent hypothesis envisaging "bacteria as multicellular organisms" (1988).

A number of scientists around the globe were interested in collective microbial behaviors and microbial communication at the beginning of the 20th century. William Penfold (1914) revealed that the culture liquid at the initial growth stage (the lag phase) of a bacterial culture contained substances promoting the culture's transition to the next stage (the exponential phase). Otto Rahn (1906) investigated the substances that were produced by microbial populations and accelerated or decelerated their development. Drawing on these data, microbiologists were conducting research on the development of cultures of prokaryotic and eukaryotic microorganisms for almost a century.

In the 1930s, Rahn investigated the phenomenon called "mitogenetic radiation". For several decades, this area of research was in the focus of attention of Alexander Gurwitch (1944) who presented his data on radiation that was produced by living cells and induced the division of other cells. Subsequently, these data provided the foundations for studies conducted in the 1990s by Yuri Nikolaev with the bacteria *Vibrio costicola, Pseudomonas fluorescens,* and others.

In the late 20th century and at the turn of the 21st century, extensive studies were carried out on the processes of communication, cooperation,

and regulation in microbial populations and associations, including colonies, biofilms, flocs, etc. It was revealed that advanced social organization is characteristic of a large number of microorganisms, and their biosocial systems are, in important ways, similar to eukaryotic multicellular organisms (Shapiro, 1988; Vysotsky et al., 1991; Gray, 1997; Losick & Kaiser, 1997; Shapiro, Dworkin, 1997; Shenderov, 1998, 2008, 2013,2016; Shenderov et al., 2016, 2017; Oleskin, 1994, 2001, 2009, 2014; Oleskin et al., 2000, 2016, 2017a, b; Greenberg, 2003; Waters & Bassler, 2005; Nikolaev & Plakunov, 2007; Oleskin & Shenderov, 2013, 2016, 2019). Currently, the idea that a bacterial culture is a homogeneous "soup" in which solitary cells independently develop is being replaced by a new concept that focuses on coherent associations of communicating cells which are differentiated in functional terms within the whole supracellular "organism" composed of many microbial cells (Voloshin & Kapreliants, 2004). To re-emphasize, these data and concepts evoke the idea that bacteria actually are multicellular organisms (Shapiro, 1988; Shapiro, Trubatch, 1991; Shapiro, Dworkin, 1997).

Recently, microbiologists have been paying increasing attention to the diversity of microorganisms at the interspecies and also at the intraspecies level: the microbial population is envisioned as a system based on the *unity in diversity* principle. Emphasis is placed on the presence of several different types of microbial cells inside many populations; this enables some degree of functional differentiation and specialization among them. Over 50 years ago, this issue was in the focus of attention of Nikolai Yerusalimsky and other researchers of the same historical period. This enabled them, as early as in the 1950s, to set forth the main principles of the population organization and communication-centered paradigm (POCCP) in microbiology that is still valid and includes the following main points:

- *Phenotypic heterogeneity of the cells of a microbial population (culture)* exemplified by the formation of spores and other dormant forms as well as filterable cells and other L forms.
- *Integrity of a microbial population as a coherent system.* As pointed out by Yerusalimsky (1952), "under appropriate cultivation

conditions, bacterial cells develop at different rates but in the same direction. Therefore, the totality of these cells, i.e., the bacterial culture as a whole, undergoes certain developmental changes referred to as the culture's *ontogeny*<emphasis added, *O.A. & S.B* >… A manifestation of the ontogeny process is a gradual increase in the number of mature cells in the bacterial culture".

- *Microcolonial lifestyle of most microorganisms in nature*. Microorganisms form compact cell aggregates (microcolonies) that are separated by void areas. In multispecies microbial associations, the microcolonies of different species are spatially segregated, and there often are "no man's land" zones between them. Microcolonies also form within microbial biofilms.
- *Release of chemical factors that are produced by the cells of a microbial population, influence its development (enable the population's autoregulation), and, in many cases, enable the population to estimate its own density*; this phenomenon came to be known as "quorum sensing" long after Yerusalimsky's seminal work.
- *Cyclic pattern of microbial population development under natural conditions.*
- *Constant interaction between a microbial population and environmental factors.* "In order to understand the driving forces of a microbial culture's ontogeny, account should be taken of the fact that it is a coherent system including both microbial cells and environmental factors that undergoes the development process" (Yerusalimsky, 1952)

Indesputably, Yerusalimsky's ideas were ahead of time; after a relatively short lag, they were confirmed in studies that were conducted by a large number of research teams around the globe.

ABBREVIATIONS

3-OHHL	N-(3-oxohexanoyl)-L-homoserine lactone
5-HIAA	5-hydroxyindoleacetic acid
ACTH	adrenocorticotropic hormone
ADHD	attention deficit hyperactivity disorder
AI	autoinducer (in a QS system)
AR	adrenoreceptor
BA	biogenic amine
BAS	biologically active substance
BBB	blood-brain barrier
BDNF	brain-derived neurotrophic factor
c-di-GMP	cyclic diguanylphosphate
CFU	colony-forming unit
CNS	central nervous system
CRF	corticotropin-releasingfactor
DCreg	regulatory dendritic cell
DHPA	dihydroxyphenylacetic acid
DOPA	3,4-dihydroxyphenylalanine
DSF	diffusible signal factor used by QS systems
EAE	experimental autoimmune encephalomyelitis
EEC	enteroendocrine cell
ENS	enteric nervous system

FA	ferulic acid
FMT	fecal microbiota transplantation
FOXO	Forkhead box (a gene locus)
GABA	γ-aminobutyric acid
GALT	gut-associated lymphatic tissue
GBL	γ-butyrolactone, a QS signal
GDNF	glial cell line-derived neurotropic factor
GF	germ-free (animal)
GHBA	γ-hydroxybutyric acid
GI tract	gastro-intestinal tract
GLP	glucagon-like peptide
GPCR	G-protein coupled receptor
HE	hepatic encephalopathy
HO	heme oxygenase
HPA	hypothalamus-pituitary-adrenal (axis)
IBD	inflammatory bowel disease
IBS	irritated bowel syndrome
IBS-D	irritated bowel syndrome with diarrhea
IF	interferon
Ig	immunoglobulin
IL	interleukin
LCFA	(mostly unsaturated) long chain fatty acid
LPS	(microbial) lipopolysaccharide
MAMP	microbe-associated molecular pattern
MAO	monoamine oxidase
MDSC	myeloid derived suppressor cell
MS	multiple sclerosis
α-MSH	α-melanocyte-stimulating hormone
N-AHL	N-acylhomoserine lactoneNCD noncommunicable disease
NLR	nucleotide oligomerization domain-like receptor
NO	nitric oxide
NOS	NO synthase
PAMP	pathogen-associated molecular pattern
PMS	premenstrual syndrome

POCCP	population organization and communication-centered paradigm in microbiology
PYY	peptide YY
QS	quorum sensing
SAD	seasonal affective disorder
SCFA	short-chain fatty acid
SERT	serotonin transporter
TGF	tumor growth factor
TLR	Toll-like receptor
TNF	tumor necrosis factor
Treg	regulatory T lymphocyte
VEGF	vascular endothelial growth factor

Chapter 1

THE SOCIAL BEHAVIOR, COMMUNICATION, AND SUPRACELLULAR STRUCTRES OF MICROORGANISMS

> "…We must be prepared to learn some day, from the students of microscopical pond-life, facts of unconscious mutual support, even from the life of micro-organisms."
>
> Peter Kropotkin (1902)

This Chapter is focused on basic concepts dealing with microbial *social behavior*. In Chapter two, these concepts will be applied to the complex gamut of interactions between the host organism and its microbiota. Needless to say, microorganisms are very distant in evolutionary terms from higher animals with which classical studies on social behavior were conducted. However, recent data demonstrate the great complexity of the social organization of a large number of prokaryotic and eukaryotic microorganisms. Many bacteria form supracellular structures, such as compact colonies on the surface of the medium or in its bulk, biofilms, and local cell aggregates in a liquid medium, including microcolonies, flocs, and larger formations that are exemplified by millimeter granules formed by methanogenic microbial associations. Flocs in the form of long bundles

develop in the culture of *Zoogloae ramigera* in a liquid medium (Pavlova et al., 2007). Recently, much attention has been given to microbial biofilms that can be envisaged as advanced biosocial systems (see 1.3.9 below).

Microbial supracellular structures are comparable to multicellular organisms in terms of their complexity and cell differentiation (Shapiro, 1988), although this comparison has been recently called into question (see below, 1.3). Microscopic fungi form complex collectives striving to retain their identity in spite of other organisms' attempts to merge with them or assume control over them (Rayner, 1988).

The term "sociomicrobiology" has recently been coined to denote the subfield of microbiology that is concerned with communication and collective behavior in microorganisms (Sekowska et al., 2009). Importantly, collective behavior is characteristic not only of free-living organisms such as bacteria, fungi, algae, and protozoans, but also of the cells of some of the tissues of multicellular plants and animals.

It should be emphasized that microorganisms possess important advantages as research subjects for studies on social behavior: (i) such studies are relatively economical in terms of material resources, time, and effort; (ii) microorganisms provide opportunities for molecular and genetic studies; (iii) new forms of behavior evolve within a short period of time; and (iv) many complex behaviors in animals have their relatively simple analogs in microorganisms. For instance, the complex behavior form referred to as *affiliation* (see below) in animals is analogous to microbial cell aggregation that proceeds under the influence of chemical stimuli.

1.1. Collective Activities (Social Behavior) in Microorganisms; The Ethological Approach

1.1.1. Ethology

Ethology according to its classical meaning is construed as the field of science that deals with animal behavior. It includes social ethology (research

on interaction among individuals and groups in animal communities), comparative ethology (comparative research on the behavior of different biological species), ethological endocrinology (studies on the interaction between behavior and the secretion of hormones and other intraorganism regulatory factors), and neuroethology (neurophysiological underpinnings of behavior; see Dewsbury, 1978; Gorokhovskaya, 2001; Deryagina & Butovskaya, 2004). Ethology interacts and competes with behavioral ecology that considers behavior as a factor contributing to the viability and adaptedness of living organisms as they deal with environmental factors (Gorokhovskaya, 2001).

Are ethological concepts applicable to microbial systems? Many microbiologists tend to answer this question in the affirmative. They take into consideration "the ethology of protozoans" or even "the ethology of bacteria" (Smirnov, 2004) that focuses on behavioral responses at both the individual and the collective level as exemplified by bacterial cell aggregation in response to a signal (pheromone). Cooperation among bacteria in a colony enables them to move in formation over the surface of a substrate: this was demonstrated in a study with bacilli over 50 years ago (Sherstobaev, 1961). Importantly, "intercellular chemical signals in bacteria correspond to pheromones in social insects and animals. Cooperative feeding in myxobacteria has been compared with pack hunting in wolves and lions" (Velicer, 2003, p.330). Coordinated cell movement detected in myxobacteria (Alberts et al., 1983) points to a similarity between bacterial colonies and multicellular organisms (Shapiro, 1988).

Stanislav Smirnov defined the term *bacterial ethology* as "the developing concept that sums up the behavioral responses of prokaryotes at the cellular and the population level". He emphasized the "goal-directedness" of bacterial social behavior. The "goal-directedness" was based on "a complex hierarchical structure of behavioral responses aimed at attaining the genetically programmed goal of securing species survival under diverse environmental conditions by accumulating a maximum biomass amount" (Smirnov, 2004, p.9). Importantly, social behavior is to be defined, in light of classical sociobiological works, as a behavior that affects another cell‟s evolutionary fitness (Hamilton 1964; Wilson 1975; Ulvestad, 2009).

"Social behavior does not only happen in higher organisms. In the microbial community, single-celled microbes have developed the capacity to work together for the common good through sophisticated cell-to-cell communication" (Zhao et al., 2017, p.516). Much evidence has been presented that "microbes indulge in a variety of social behaviors involving complex systems of cooperation, communication, and synchronization" (West et al., 2007). Typical examples of microbial social behavior include collective hunting by *Myxococcus* spp., aggregation and subsequent programmed cell death as a stage of the development of the stalk in the fruiting body of *Dyctiostelium discoideum*, and biofilm formation, e.g., in *Pseudomonas fluorescens* and *Bacillus subtilis* (Tarnita, 2017).

In ethology (and in the social sciences including social psychology), social behavior is often classified into the following two types (each type is subdivided into a number of behavior forms):

(a) *Agonistic behavior*. This type of behavior is associated with conflict among living organisms (Dewsbury, 1978). Importantly, "like any society… microbes face conflict" (Foster, 2010);
(b) *Loyal* behavior including the totality of friendly interactions among living beings that consolidate their groups, families, colonies, or other *biosocial systems*. The characteristic forms of both agonistic and loyal behavior are schematically represented in Figure 1.

It should be noted that other microbiologically relevant classifications of social behavior are suggested in the literature. Depending on the positive or negative outcome for both the initiator and the target partner in social interaction, social behavior breaks down into *exploitation* (the initiator gains an advantage, and the target partner sustains costs), *altruism* (the initiator provides benefits for the target partner at some cost for itself), *win-win behavior*, or *mutualism* (both partners stand to gain), and *spite*, or *double lesion* (both partners are disadvantaged, see: Foster, 2010; Zhou and Cai, 2018). This classification will be taken into consideration further in this section.

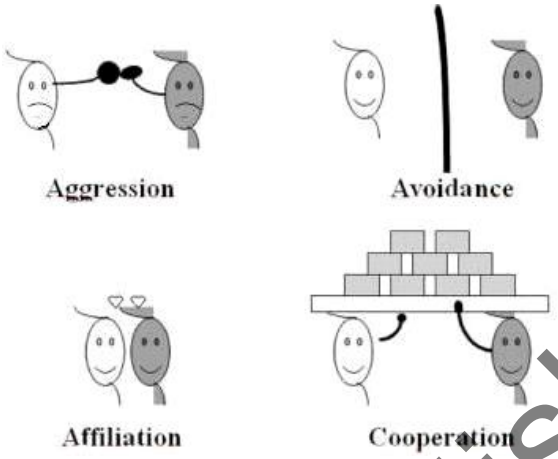

Figure 1. Main forms of social behavior (according to: Oleskin, 2012).

1.1.2. Aggression

The classical definition given by Niko Tinbergen (1968) with regard to animals is "approaching an opponent and inflicting damage on him or at least generating stimuli that cause him to submit." Analogous behavior in the microbial realm includes, e.g., the production of antibiotics (including bacteriocins), toxins, or surfactants for destroying or inhibiting competitors. The cyanobacteria of the genus *Anacystis* suppress the growth of the green algae *Scenedesmus*, *Chlamydomonas*, *and Haematococcus* (Ostroumov, 1986). Undoubtedly, many antibiotics are not only "chemical weapons" because they also function as important developmental regulators in the antibiotic producer culture.Their involvement in microbial aggressive behavior, nonetheless, is consistent with the data that antibiotics are actually released in response to the presence of a competitor. Of special interest in this context is the fact that the fungus *Trichotecium roseus* produces 1.7 times more trichotecin (an anitibiotic) if its culture is supplemented with that of a competitor (*Penicillium chrysogenum*, Egorov & Landau, 1982).

Regulatory substances produced by many microorganisms can be involved in agonistic interaction with the populations of other microbial strains or species. For instance, autostimulator APK-1, a complex of low

molecular weight metabolites contained in the culture liquid of *E. coli* M-17, strengthens the liquid's inhibitory effect on other bacteria, e.g., *Staphylococcus enteritidis*. The same substance also has other effects. On a nutrient-poor medium, APK-1 increases the viability of an *Escherichia coli* culture and decreases that of *Staph. enteritidis* (Vakhitov, 2019).

These findings have direct relevance to the human gastro-intestinal (GI) tract that contains useful (symbiotic) and potentially pathogenic (opportunistic) microorganisms.[1] Timur Vakhitov (2019, p.195) emphasizes that agonistic interaction between several bacterial species results in the competing species producing additional amounts of growth stimulators. A useful (probiotic) *E. coli* strain more efficiently develops in the presence of a pathogenic bacterium (*Staph. enteritidis*) than without it. If *E. coli* grows together with other symbiotic microorganisms such as lactobacilli (see 1.1.5), the same growth stimulators positively influence both symbiotic partners.

There at least 4 different strains of the potential pathogen *Staphylococcus aureus.* Each strain produces a cyclic peptide. The peptide functions as a signal in the culture of the producer strain (as a quorum sensing autoinducer), but it also disrupts similar quorum sensing systems in all other strains (see 1.2.3 below).

"The toxin-antitoxin systems carried by many bacteria are probably the closest thing that microbes have to aggression... In their simplest form, these systems comprise two neighboring genes: one encoding a toxin (bacteriocin) that kills other strains <or slows down their growth – O. A.>, and the other an antitoxin, or immunity protein, that protects the toxin-producing strain. The cystic fibrosis pathogen *Pseudomonas aeruginosa* carries multiple toxin secretion systems, which evolve rapidly, and may again be indicative of arms races" (Foster, 2010). For instance, the VI secretion system (T6SS) of *Ps. aeruginosa* can transfer a toxic protein to the adjacent T6SS-lacking cells and suppress their growth (reviewed, Zhou & Cai, 2018).

Such aggressive behavior often results in destroying "outsiders", in an analogy to similar behavior in other forms of life including, e.g., social

[1] Notably, the boundary between these two types of microrganisms is somewhat arbitrary; "friends" can become "enemies" if the host organism is weakened by stress.

insects. However, a competitor can be inactivated in a more "subtle" way. Some bacilli produce antibiotics that convert the cells of competing bacterial species into dormant spores (Bushell, 1989). As a result, the bacilli monopolize all available nutrient substrates.

Aggressive behavior in the microbial world does not only take the form of exchanging destructive/incapacitating chemical agents A series of micrographs in the work by Väth (1992) demonstrated the dynamics of a "battle" between an amoeba, the predator, and an infusorian, the prey. The fighting continued for 20 minutes and resulted in the death of both opponents.

All forms of aggression are considered costly and risky behaviors by animal ethologists, and evolution promotes the formation of aggression-mitigating mechanisms. Microorganisms do not lie on their back like wolves, exposing their vulnerable body parts to the aggressor as an appeasement signal. There are, however, microbial analogs of the aggression-preventing strategy based on niche separation, e.g., in the human GI tract where different representatives of the microbiota tend to inhabit different parts of the gut.

Generally, competition tends to select for individuals (cells) that utilize different resources than their competitors (Foster, 2010). If competition is mitigated, this may promote *cooperation* among former competitors. For instance, the product synthesized by one of the strains/species is utilized as a substrate by another strain/species (or by the host organism).

A large number of behavioral phenomena in the microbial world are based on analogs of aggressive interactions: microorganisms recognize potential "enemies" ("outsiders" as contrasted with "insiders"), use camouflage to prevent an immunocyte attack while struggling with the host organism, and resort to "weaponry" such as antibiotics and other toxic chemicals during conflicts with their competitors (Ulvestad, 2009); however, microorganisms also engage in loyal behaviors (see below).

1.1.3. Isolation (Avoidance)

In the animal kingdom (and in human society), avoidance behavior often manifests itself in marking the boundaries of one's own territory. Isolation in the microbial world is based on strain- or clone-specific interaction among microbial cells. As an analog of behavior aimed at avoiding outgroup individuals in various animal species (including humans), isolation promotes the spatial structuring and segregation of microbial biosocial systems.

Avoidance behavior is displayed by various microbial species, including *Proteus mirabilis, E. coli, Vibrio alginolyticus, Bacillus subtilis, Pseudomonas putida, Rhodospirillum rubrum*, and *Rhodobacter sphaeroides*; it manifests itself, for instance, in the "colony separation" phenomenon: microbial colonies that share one petri plate typically do not merge even if they grow towards one another (Dienes, 1946; Shapiro, 1985; Budrene, 1985; Novikova, 1989; Oleskin, unpublished data). Moreover, the expansion of a single microbial colony on the agar surface may result in the formation of protrusions that separate from the original colony and never merge with it.

The wood-destroying fungus *Stereum hirsutum* forms spatially isolated mycelia that do not merge. Local aging proceeds in hyphae that grow towards a neighboring mycelium, and such hyphae contain pigments that are characterstic of an aging mycelium. Aging prevents the hyphae of the two mycelia from coming into contact (Rayner, 1988). Analogous local aging occurs during the tissue repulsion process in animals and the hypersensitive response of the plant immune system.

1.1.4. Affiliation

Affiliation is defined as a form of social *behavior* involving an individual *animal's* tending to approach and remain near conspecifics (*Dewsbury*, 1978), particularly those belonging to the same family or social group. Animals engage in greeting, play, and grooming behaviors. The cohesion of

the cells of one clone and of one tissue in a muticellular organism is an obvious analog of animal affiliation. If cultivated kidney and liver cells are mixed experimentally, they tend to form separate aggregates, "like attracts like".

The colonies of many bacterial species contain cell aggregates (microcolonies). Interestingly, the addition of the brain neurochemicals dopamine, norepinephrine, serotonin, and histamine to an *E. coli* culture results in changing the ratio between solitary and aggregated cells (Anuchin et al., 2008; see Chapter three for details).

The ameboid vegetative cells of the slime mold *Dictyostelium discoideum* feed on bacteria. After all available bacteria have been consumed, the starving cells aggregate to form the motile pseudoplasmodium ("the slug") and, subsequently, the fruiting body with spores. Cell aggregation depends on chemical regulators such as cyclic adenosine monophosphate (cAMP) and chlorinated hexaphenones termed differentiation-inducing factors (DIF-1, DIF-2, and DIF-3). About 20% of the cells undergo programmed cell death (apoptosis), and the dead cells constitute the stalk of the mushroom-like fruiting body; this is considered an example of altruistic behavior in microrganisms (Ben-Jacob et al., 2016).

Another microbial analog of affiliation is to be found in myxobacteria, the prokaryotes that strikingly resemble eukaryotic myxomycetes in terms of social behaviors. When depleted of nutrients, the cells of *Myxobacterium xanthus* release factor A (a mixture of hydrophobic amino acids and short peptides) that induces cells to form compact groups. Subsequently, contact cell-cell communication comes into play. It involves non-diffusible factor C (attached to the cell producing it) that initiates fruiting body formation. Up to 90% of the cells involved undergo programmed cell death during this process (Ulvestad, 2009).

1.1.5. Cooperation

In ethological terms, this kind of loyal behavior implies *interaction between two or more individuals or groups for the purpose of solving a*

problem or carrying out a task. An alternative, although in principle similar, approach to defining cooperation involves considering it from the viewpoint of a whole group (community). In these terms, *cooperators* are contrasted with *cheaters (free riders*, see below, 1.1.7*)*: cooperators contribute to the collective good within a distinct group at an individual cost, and cheaters exploit it (Hochberg et al., 2008, modified).

There are analogous phenomena at the cellular level (Crespi, 2001). Of relevance is the behavior of immune cells (macrophages and lymphocytes) inside an animal organism in response to a foreign invader. Macrophages bind the agent that has penetrated into the organism and present it to T lymphocytes. The activated T lymphocytes interact with B lymphocytes that produce antibodies neutralizing the agent.

Cooperation is widely spread among free-living prokaryotic and eukaryotic organisms. Like multicellular organisms, microorganisms cooperate to build a shelter, to forage, to reproduce, and to spread in the available area (Crespi, 2001; Ulvestad, 2009). Cooperation is characteristic of myxobacteria that coordinately move over the surface of the nutrient medium and pursue their prey, i.e., other bacteria. Filamentous cyanobacteria form associations and display sophisticated behaviors aimed at securing the survival and integrity of the whole association. If a cyanobacterial biofilm is damaged (ruptured), it tends to regenerate: filaments actively move towards the gap and close it (Sumina, 2006).

Cooperation often implies some degree of functional differentiation and specialization of the individuals (microbial cells) involved. "Nitrogen-fixing cells of *Rhizobium* and cyanobacteria filaments are specialized food providers analogous to the foraging classes of social insects" (Velicer, 2003, p. 330).

The prominent scholar and social activist Peter Kropotkin (1972 [1902, 1st edition]) dreamed of establishing a new social and political system that would be based on voluntary cooperation; he corroborated his ideas with numerous examples of "unconscious mutual aid" characteristic of living nature. Kropotkin believed that microorganisms also engage in cooperation.

Nikolai Yerusalimsky (1952) called into question the hypothesis that all interactions among microbial cells should be considered in terms of

competition and "the survival of the fittest". In contrast to this hypothesis (denoted as 'the population theory" by him), Yerusalimsky emphasized that microbial cells often interact for the benefit of the whole system (the "microbial culture"). In line with these views, it was established that cooperation among the cells of a colony of bacilli enables the colony to coherently move on the substrate surface (Sherstobaev, 1961). Similar data were subsequently obtained with myxobacteria (Alberts et al., 1983). According to James Shapiro (1988), such facts signify that bacterial colonies bear considerable similarity to multicellular organisms.

To reiterate, cooperation results in accumulating *public goods*, i.e., "costly resources produced by the cooperator and freely available to others" (Tarnita, 2017). "Cooperation is conceived positively as any behavior that is costly to the individual but that helps to generate a public good that otherwise would not exist or would be inaccessible" (Velicer, 2003, p.330). This is exemplified by the release of substances that help other cells and the whole population fulfil their functions. Such substances are exemplified by bacterial cells-produced antimutagens that decrease the gene mutation rate and also reactivate other bacterial cells under stress (Vorobyeva et al., 1993; 1995a, b). In line with these data, endogenous stress protectors cooperatively produced by all bacterial cells in a population, have been revealed in a large number of bacterial species (Rowbury & Goodson, 2001; Nikolaev et al., 2006).

Cooperative behaviors may be based on *altruism* that was defined in 1.1.1 as providing benefits for a partner at some cost for the provider. There is a sufficiently large body of evidence for the existence of altruistic phenomena in the microbial realm as exemplified by stalk-cell death in *Dictyostelium* fruiting bodies, cell autolysis during the development of *Myxococcus*, self-destructive toxin production by bacteria, and *E. coli* phage exclusion involving the programmed death of the phage-infected bacterial cells that prevents the spread of the phage in the bacterial population (Velicer, 2003).

Interspecies cooperation takes place whenever a substance produced by a microbial species is utilized as a substrate by other species. In the termite

gut, protozoans break down cellulose, and their products are metabolized by spirochetes and other bacteria (Foster, 2010).

Apart from influencing producer cells, regulatory substances released by microorganisms may exert an effect on other microbial species. *E. coli* M-17-produced growth stimulators (the aforementioned APK preparation) also promote the growth of *Bifidobacterium bifidum* in mixed culture. That is why the combined probiotic preparation Bificol that contains both species develops very efficiently. Likewise, APK *E. coli* M-17 stimulates the growth of the probiotics *Lactobacillus acidophilus* and *Lact. delbrueckii* subsp. *bulgaricus* (Vakhitov, 2019). The latter species does not form a part of the normal gastro-intestinal microbiota but is, nevertheless, considered a valuable probiotic. This bacterium was advertized in the form of an aging-decelerating preparation by Ilya Mechnikov over one century ago. In line with this, the data obtained by the authors of the present work demonstrate that *L. delbrueckii* subsp. *bulgaricus* produces sufficiently large (micromolar) amounts of γ-aminobutyric acid (GABA), an essential neurochemical that exerts protective and antioxidant effects (Oleskin et al., 2014a, b).

In light of these data on the beneficial effects of probiotic bacteria on the host organism, cooperation between the host and its microbial consortium seems to be of paramount importance. For instance, microorganisms provide the host organism with otherwise unavailable resources, including biologically active substances (see Chapter two for details).

Apart from the human intestinal microbiota, demonstrative examples of cooperation in the microbial world include (a) the production of antifungal substances by bacteria that protect the fungus gardens of ants from extraneous fungi; (b) nitrogen fixation by root nodules and free-living bacteria that supply plants with nitrogen; and (c) mycorhiza (growth of fungal hyphae around or inside plant roots).

Cooperation may result in *symbiogenesis*, i.e., the formation of a new coherent system that may be more evolutionarily advanced than its components per se (Ulvestad, 2009). Serial (repetitive) symbiosis, in all likelihood, played an important role in biological evolution. While

eukaryotic cells arose as a result of cooperation among free-living bacteria (Ulvestad, 2009), further symbiogenesis stages gave rise to colonial and, subsequently, multicellular organisms (see 1.4 below).

1.1.6. Swarming

Cooperation among microbial cells underlies a collective behavioral process known as cell *swarming,* i.e., group migration away from high-density areas (Foster, 2010) that involves highly motile swarmer cells. "Microorganisms use collective migration to cross barriers and reach new habitats, and the ability to form motile swarms offers a competitive advantage" (Ben-Jacob et al., 2016, p.257).

One of the reasons why bacterial cells convert into swarmers is that this helps the colony spread on the solid medium. Swarmers migrate to vacant medium areas where they revert to vegetative cell that occupy the new niches. The pathogenic representatives of the genus *Proteus* (e.g., *P. mirabilis*) that cause urinary tract and kidney infection in humans, produce swarmers that spread in the human organism and invade the epithelial cells of the urinary tract (reviewed, Oleskin, 2001). Many different swarmer types and swarming microbial species have been detected. All swarming phenomena have a number of common features (Ben-Jacob et al., 2016):

(i) There is a triggering factor, e.g., nutrient depletion, that starts the swarming process;
(ii) Swarming involves collective behaviors that are coordinated within the framework of a colony or a biofilm;
(iii) Behavior coordination does not depend on any central leader (pacemaker) because it is based on a network-type mechanism (see below, section 2.3).

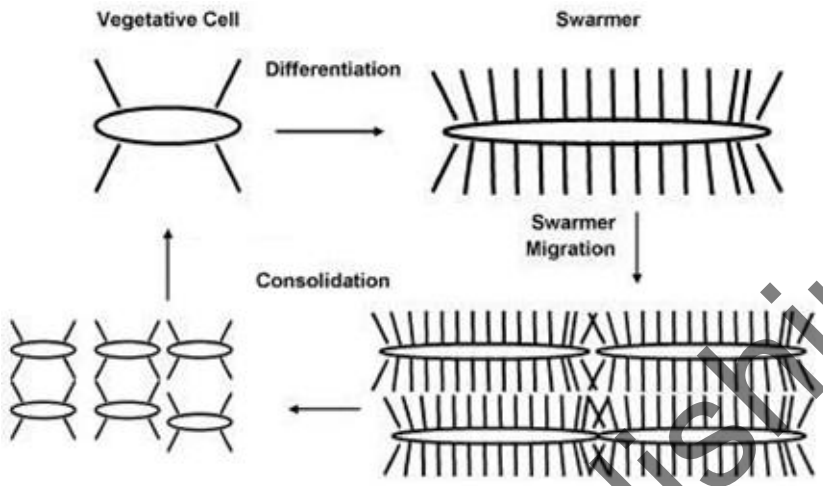

Figure 2. Stages of swarming behavior (according to: Oleskin, 2001).

The swarmer cells of *Proteus* sp. form a colony (Figure 2) with concentric rings. This is due to the alternation of the following processes: (a) the growth and division of vegetative cells (this is the lag phase that precedes swarmer formation); (b) the production of a large number of centrifugally moving elongated swarmer cells; (c) the conversion of swarmers into vegetative cells that form a new ring (the consolidation stage).

Swarming sensu stricto is based on cell migration by flagellar movement, but swarmer cells can also be pulled by pili and pushed by the slime that is released by them (Foster, 2010). Cells cooperate in secreting enzymes and surface tension-reducing substances (surfactants).

Swarming can be considered an example of a more general phenomenon, *pattern formation* by groups of cooperating cells. Of note is the formation of macroscopic waves (rippling) in the myxobacterium *Myxococcus xanthus*. Such rippling takes place if *M. xanthus* cells directly contact prey cells (Stevens et al., 2012). Cell groups can form more elaborate collective structures that function as organs of whole colonies, biofilms, or other biosocial systems in the microbial world (see below, section 1.3).

1.1.7. Cheaters (Free Riders)

This issue concerns both human society and all kinds of biological systems that include individuals benefiting from the goods produced by cooperators without cooperating. Cooperation is a costly kind of behavior, and this provides an incentive for defecting (free riding) that implies the "absence of cooperative behavior coupled with exploitation of its benefits" (Tarnita, 2017). The issue faced by microbial cells and other kinds of living organisms is "Why would an individual give up part of its own fitness to confer fitness benefits on other individuals?" (ibid.) In fact, noncooperators do not spend their resources and possess competitive advantages over "honest cooperators" (Özkaya et al., 2017). "Cheaters are individuals that reap the benefits of cooperation without contributing or by contributing less" (Stevens et al., 2012). They "disrupt cooperative systems by unfairly procuring an excessive share of group-generated resources while making disproportionately small contributions" (Velicer, 2003, p.330). Cheating is possible in the biosocial systems of all kinds of living organisms. "Cheating even occurs in eusocial insect colonies <e.g., ant and bee societies – O.A.>, which are often viewed as archetypes of social cooperation, but in fact are susceptible to many forms of social conflict" (Velicer, 2003, p.331).

Therefore, it is not surprising that "microbial communities are susceptible to the public goods dilemma, whereby individuals can gain an advantage within a group by untilizing but not sharing the cost of producing public goods" (Zhao et al., 2019).

A demonstrative example in the microbial realm is provided by siderophore synthesis. Siderophores enable *Pseudomonas aeruginosa* cells to take up vitally important iron ions. Some wild-type cells convert into mutants that do not release siderophores into the medium but take up iron by means of siderophores produced by other cells. In the long term, this decreases the metabolic efficiency of the whole cell population and, therefore, attenuates the virulence of *Ps. aeruginosa* (Foster, 2010). In a similar fashion, defectors exploit public goods produced by cooperators in *Myxococcus xanthus* (Stevens et al., 2012).

Apart from free-living cells, cheating behavior can be displayed by cells inside a multicellular organism, the most notorious example probably being cancerous cells. They "ignore the developmental program and reproduce uncontrollably to the detriment of the group" of cells to which they belong and of the whole organism (Tarnita, 2017).

Behavior researchers pay much attention to factors that prevent the spreading of cheaters in populations which are otherwise composed of "honest" cooperators working for the benefit of the whole biosocial system. For instance, free riders will not be selected for if their cheating endangers the survival of all group members, including the free riders themselves (Corning, 1983, 2003). As mentioned above (in the example of siderpohores), the spread of noncooperative free-riders that "freely" consume public goods (enzymes, nutrients, protective mechanisms, etc.) results in depleting these resources, so that both the cheaters and the cooperators run out of them (Özkaya et al., 2017).

Overall, if systems that predominantly consist of cooperators and prevent cheaters from spreading have a longer life-span than those allowing for cheater proliferation, living nature should gradually develop mechanisms that eliminate free riders. In microorganisms, such mechanisms include:

- Decrease in mutation frequency that depends on natural antimutagens in some bacterial populations; this slows down the accumulation of mutant cells that lack cooperation-promoting genes;
- DNA redundancy: traits that are important for cooperation (e.g., the production of relevant enzymes) can be encoded in several DNA loci, and the operation of at least one of them is sufficient for the manifestation of these traits;
- Pleiotropic gene effects: a mutation that prevents cooperation also produces effects that inhibit the growth of cheater cells.

A starving *E. coli* culture assumes the state of dormancy. However, mutants of the GASP (growth at stationary phase) type continue actively growing even at low nutrient concentrations. The same mutation enhances

the cells' sensitivity to medium acidification. Therefore, defectors (cheaters) cannot compete with wild-type cooperators under acidic conditions, and the normal transition to the stationary phase takes place in this situation (Foster, 2010). Even without medium acidification, it is obvious that defectors can survive as long as their percentage in the population is sufficiently low. "In pure cultures, the GASP strategy results in higher death rates (upon resource depletion) than" in "wild-type cells" (Velicer, 2003, p.334).

In *Ps. aeruginosa,* a mutation results in the formation of cheater cells that do not synthesize costly proteases and rely on proten-degrading wild-type cooperators. The same mutation blocks the synthesis of nucleoside hydroxylase by cheater cells. Therefore, they cannot grow and displace wild-type cells on a medium with adenosine (reviewed, Zhao et al., 2017). Some defectors (with disrupted *lasI-lasR* quorum-sensing systems, see 1.2.3 below) also lack wild-type cyanide detoxification systems and, therefore, can be "socially punished" by wild-type cooperators that release cyanide into the medium (Zhao et al., 2019).

Cooperation is favored over cheating if there is "some form of assortment that makes cooperators more likely to interact with other cooperators" (Tarnita, 2017). Possible mechanisms of such assortment include (i) a spatial structure that limits the dispersal of microbial cells (population viscosity), (ii) selective adhesion of cells with a cooperative phenotype to a substrate or to one another (cohesion) and (iii) other forms of selective interaction with those sharing the same phenotype, while avoiding cells with a different phenotype (Stevens et al., 2012; Tarnita, 2017).

Recently, it has been suggested that cooperators and cheaters can coexist for a long time in a population, and this behavioral heterogeneity can actually stabilize social cooperation. A widespread mechanism involved in communication is based on quorum-sensing (QS) systems, which will be discussed in detail below (1.2.3). Cooperation or cheating behavior may be displayed depending on whether such systems are on or off; conditional turning them on and off by inducing changes in relevant genes can account for the coexistence of cooperators and cheaters in a population (Zhao et al., 2019); in other words, individual cells can switch between cooperative and

cheating behaviors. "Signal-blind" mutants do not respond to social signals and play the role of cheaters but they may become cooperators if the signals change and become recognizable for their altered QS systems (Stevens et al., 2012).

Darwinian evolution theory was supplemented in the 1960s and 1970s, with the concepts of *kin altruism* (helping one's closest relatives whose genes are very similar to yours) and *reciprocal altruism* (helping those who help you in a similar situation) introduced by Hamilton (1964) and Trivers (1972), respectively. At least the first option seems applicable to the microbial cooperation/defection dilemma, especially because many of the cells of one colony or biofilm are very closely related genetically. Evidence was presented that the cells of some "conditional cheater" strains of the slime mold *Dictyostelium discoideum*, whose fruiting body stalks are composed of dead cells, undergo programmed cell death in pure culture (were cells typically are identical clones) but attempt to avoid this in mixed culture. Actually, they exploit the cells of other strains contained in the mixed culture that altruistically die to enable the formation of fruiting bodies by all the strains involved (Velicer, 2003).

In addition to public goods-producing cooperators and public goods-exploiting defectors (free riders), a microbial population may contain *loners*, the third behavioral type. Loners do not produce public goods and do not use them (Tarnita, 2017). These cells may start a new colony/biofilm.

1.2. COMMUNICATION IN THE MICROBIAL WORLD

> "For many researchers, understanding bacterial communication represents the promise of new treatment modalities for infections. For others, untangling complex networks of quorum sensing regulatory systems is a tantalizing puzzle to solve. And still other groups of researchers hope to apply their knowledge of bacterial communication to address important environmental issues such as bioremediation and sustainability" (Rumbaux, 2018. P. v).

There is a large body of evidence that "… bacteria, like all other living organisms, process and use information about the environment during their life-sustaining activities. *Exchanging information and obtaining it from other living organisms is called communication*" (Nikolaev, 2000, p. 597, *emphasis added – O.A.*). Communication in microorganisms, as well as in any other kinds of biological systems, includes the three main stages (Zhao et al., 2017): (1) detecting a signal, e.g., via its binding with the cognate receptor; (2) recognizing the signal; for instance, a cyclic adenosine monophosphate molecule (cAMP) is interpreted by a myxomycete cell as the "start cell aggregation" instruction; (3) making a decision with regard to the response to the signal; in the aforementioned example with *D. discoideum,* it is cell competence (resulting from cell starvation) that determines the decision. In this respect, communicating cell groups are similar to neuronal networks or their artificial analogs such as perceptrons that contain specialized layers responsible for data perception, information processing, and decision-making, respectively.

Microorganisms including bacteria use contact, distant chemical, and, presumably, distant physical communication.

1.2.1. Contact Communication

Contact communication is based on cell-cell contacts that represent cytoplasmic bridges (plasmodesms), outer membrane fusion sites (in gram-negative bacteria), or peptdoglucan fusion sites (in gram-positive bacteria; Tetz et al., 1990). Presumably, cytoplasmic bridges, or nanotubes, can function as wave conductors to transmit electromagnetic waves (belonging to various wavelength ranges) between bacterial cells (Vysotsky et al., 1991); electromagnetic communication is briefly discussed below (1.2.4).

The cells of the gram-negative bacterium *Myxococcus xanthus* aggregate and subsequently form fruiting bodies under conditions of nutrient deprivation. At the later stages of this process, the cells are densely packed, which enables spore formation. These developmental events are subject to regulation by non-diffusible factor C. Its precursor (p25) is the product of

the *csgA* gene. In starving cells of *M. xanthus*, the secreted protease PopC converts p25 to factor C (Stevens et al., 2012). "…The secretion of PopC is dependent on the stringent response protein RelA, which produces alarmone guanosine tetraphosphate (ppGpp)… The components providing the link between RelA/ppGpp and PopC were identified as PopD and FtsH: ppGpp directs activation of FtsH, an ATP-dependent protease that degrades PopD, which in turn inhibits PopC " (Stevens et al., 2012, p.2132). Factor C induces the expression of the genes that are involved in the maturation of fruiting bodies with spores and interact with transcription factors FruA and MrpC (reviewed, Zhao et al., 2017).

Strain *E. coli* EC93 inhibits the growth of the cultures of other strains of the same species in a mixed culture. The inhibition is based on direct cell–cell contact. Communication involves the CdiA/CdiB two-component system. CdiB is an outer membrane protein that is necessary for the secretion of protein CdiA that remains attached to the cell surface. Upon contacting the target cell, CdiA interacts with its receptor, BamA. The C terminal part of the CdiA molecule (CdiA-CT) is detached by a protease and transported into the target cell where it suppresses metabolic processes (Aoki et al., 2009; Otto, 2010; Zhao et al., 2017).

Intercellular contacts involve a wide variety of surface structures, including microfibrils, cone-shaped protrusions, cell wall evaginates, and glycocalyx (reviewed, Oleskin et al., 2000).

Direct cell–cell contact is a prerequisite for communication via surface organelles such as pili and via the components of the exopolymer matrix that coats bacterial cells, their groups, and the whole colony/biofilm. Aggregation and spore formation in *M. xanthus* depend on type IV pili. Their homologues are formed by the pathogenic bacteria *Ps. aeruginosa* and *Neisseria gonorhoeae*, and they are responsible for socially coordinated cell movements in these species (Will et al., 1998). As for *M. xanthus*, its collective cell behaviors also involve polysaccharide-protein fibrils and the polysacharide O-antigen of the external layer of the outer membrane (Shapiro, 1995; Will et al., 1998). All these cell surface structures are synthesized with the help of *S (social)* genes that are necessary for collective coordinated cell translocation and the formation of multicellular structures.

In contrast, the *A (adventurous)* genes of the myxobacteria are responsible for individual cell motility and enable cells to move away from their colony.

As already mentioned, some bacteria have been established to form membrane nanotubes for transferring macromolecules (proteins, DNA, and RNA) to adjacent cells. Such nanotubes form between the cells of the same species (*Bacillus subtilis)* and those belonging to different species, e.g., between *B. subtilis* and *E. coli* cells (Dubey & Ben-Yehuda, 2011; Zhao et al., 2017). In a similar fashion, networks of intercellular membrane nanotubes connect mammalian cells.

A large number of proteobacteria produce outer membrane vesicles (OMVs). Apart from other functions (virulence factor secretion and immunomodulation), OVMs, similar to nanotubes, are used for transferring chemical agents, including quorum-sensing signals (see below in this subsection), such as quinolone in *Ps. aeruginosa* (Stevens et al., 2012).

The biopolymers of the extracellular matrix in a colony or biofilm form trails that separate from the cells that produce them and guide the migration of "traveler" cells that give rise to new colonies/biofilms.

1.2.2. Distant Chemical Communication among Spatially Separated Cells

Many diffusible chemical signals are implicated in coordinating microbial growth, developmental processes, and the transition between the stages of the life-cycle of a microbial culture (culture ontogeny, Yerusalimsky, 1952). Such signals are referred to as *autoregulatory substances*, or *autoregulators*. They are microbial metabolites that are released by a cell population, or its part, into the medium. Many autoregulators are not utilizable in constructive or energy metabolism but perform major communicative functions and, therefore, influence the physiological state and the reproductive potential of the cells involved (El'-Registan, 1988).

It was established that, during the initial stages of culture growth an *E. coli* culture releases substances (autostimulators) that, when added to

another *E. coli* culture, stimulate its growth; during the later growth stages, the growth deceleration stage and the stationary phase, an *E. coli* culture releases autoinhibitors that suppress the growth of another culture (Vakhitov et al., 2003).

Autoregulatory substances that are produced by a microbial culture and influence the development of other cultures of the same strain also include glutamate that, together with lysine, methionine, and succinate, stimulates, and aspartate that, along with lactate and formate, inhibits the growth of the probiotic strain *E. coli* M-17. Aspartate, in contrast, stimulates the growth of another strain, *E. coli* BL (Vakhitov et al., 2000; Vakhitov & Sitkin, 2014).

Autoregulators are also exemplified by microbially produced factors d_1 (anabiosis factors) that represent alkylhydroxybenzenes (AHBs) and factors d_2 (autolysis factors) that belong to unsaturated fatty acids (El'-Registan, 1988; Plakunov & El'-Registan, 2004). AHBs induce the transition of bacterial cells to the dormancy state and increase their stress resistance. At sufficient concentrations, AHBs suppress the growth of microbial cultures and biofilms (Mart'yanov et al., 2015) and induce cell differentiation processes (El'-Registan et al., 2006). The mechanism of action of AHBs is based on their capacity to modify the structure and activity of cell biopolymers such as proteins and the DNA, to increase the microviscosity of biological membranes, and to influence ion transfer processes and the cell's water balance (Bukharin et al., 2005; El'-Registan et al., 2006). Importantly, AHBs change the activity of the cell effectors of the innate immunity system (Deryabin et al., 2013a, b) and the functional stability of antibodies (Deyabin et al., 2010).

Factors d_2 are unsaturated fatty acids. They uncouple membrane phosphorylation and, at high concentrations, damage cell membranes, resulting in cell death. Some unsaturated fatty acids also operate as quorum sensing signals (DSF factors, see below).

1.2.3. Quorum Sensing Systems

A large number of studies have been conducted on *quorum-sensing (QS)* systems that control, in a cell density-dependent fashion, many important processes in microbial cells and their groups (Fuqua et al., 1994; Gray, 1997; Waters & Bassler, 2005; Khmel', 2006; Tarighi & Taheri, 2011; Stevensetal., 2012; Bassler & Miller, 2013; Hagen, 2015; Kalia, 2015; Leoni & Rampioni, 2018), including bioluminescence, synthesis of antibiotics and enzyme complexes, cell-to-cell transfer of genetic information (transformation and conjugation), cell aggregation, protein secretion, biofilm and gas vesicle formation, sporulation, virulence factor production, etc. "QS is an environmental sensing system that allows bacteria to monitor population density and to connect cell population density with gene expression" (Thornhill & McLean, 2018, p.3-4). Microbial populations estimate the density of their population from the concentration of the QS signal molecules (pheromones, or autoinducers) that are released by each cell in the population. Once the QS signal concentrations reach specific thresholds, respective QS systems are either activated or repressed. Many QS systems function according to the positive feedback (autoinduction) principle (Duan & Surette, 2007).

"Bacteria use quorum sensing to communicate both within and between species. Both species-specific and species-nonspecific autoinducers exist" (Bassler & Miller, 2013, p.495). Some microbially produced substances, e.g., N-acylhomoserine lactones, only operate as QS signals. However, there are also multifunctional compounds, including factor AI-2 (see below) that, apart from operating as an interspecies QS signal, is used as the sink for metabolic waste products. Generally, QS signals form a part of a spectrum of evolutionarily conserved biologically active substances: a large number of them are multifunctional (Vakhitov, 2019).

In terms of interaction between the microbiota and the host, the main subject of this work, it should be emphasized that QS "systems play global regulatory roles in bacterial virulence. They synchronize the expression of multiple virulence factors and they control and modulate bacterial antibiotic

tolerance systems and host defense mechanisms" (Maura et al., 2018, p.227). Some of the main QS signals are shown in Figure 3.

A majority of the QS signals of gram-negative bacteria are N-acylated homoserine lactons (N-AHLs), also called autoinducers-1 (AI-1s). Such QS systems are denoted as *luxI*-*luxR*-type QS systems because they are similar to the prototypical QS system of the marine luminescent bacterium *Vibrio fisheri*. N-AHLs bind to regulatory R proteins, and the resulting complex activates (or, alternatively, inhibits) the transcription of the genes that are responsible for diverse quorum-dependent processes. If the cell density is sufficienty high, bacteria engage in various collective behaviors.

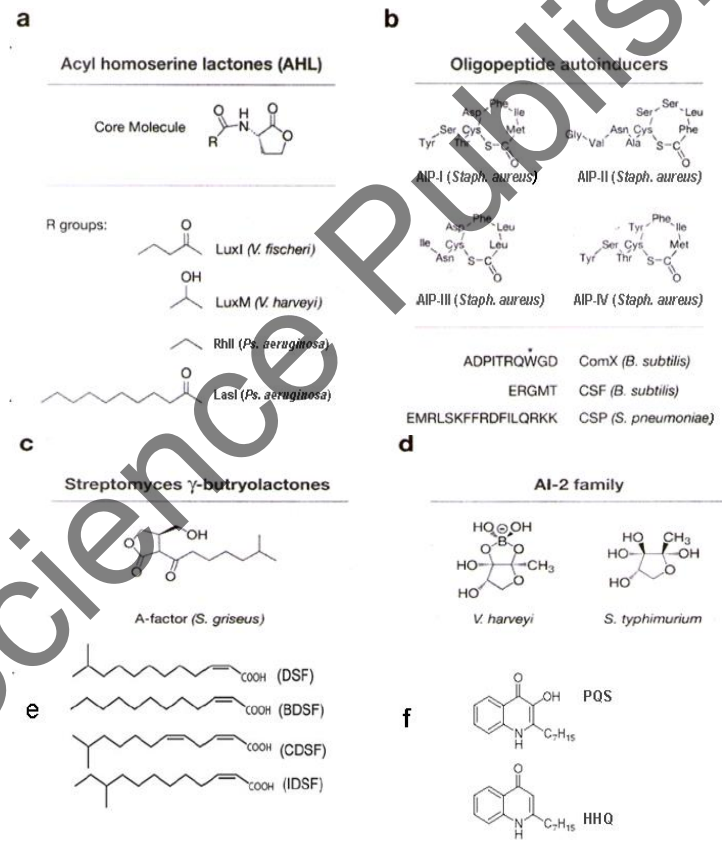

Figure 3. Some types of QS signals: a, N-acylhomoserine lactones (AI-1 signals); b, peptide signals used by gram-positive bacteria; c, γ-butyrolactone of *Streptomyces*; d, AI-2 signals; e, DSFs; f, quinolones.

The prototypical system of *Vibrio fischeri* (Fuqua et al., 1994) enables this bacterium to emit light in concentrated cell populations. They inhabit the light organ of the bobtail squid *Euprymna scolopes*, in which the bacterial cell density may be as high as 10^{10}-10^{11} cells/mL.

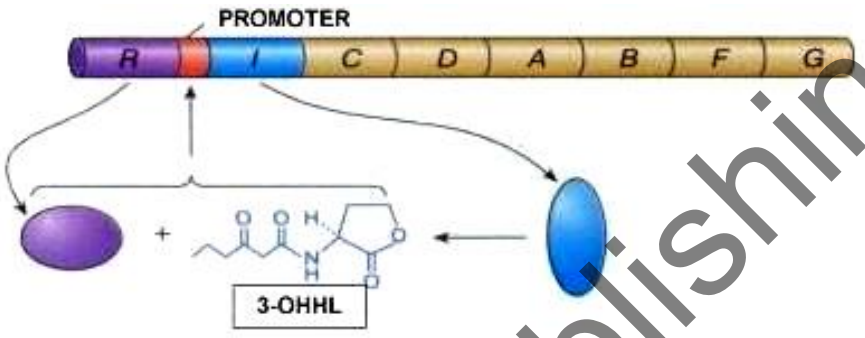

Figure 4. The QS system of *Vibrio fischeri.* The *C, D, A, B, F,* and *G* genes that encode luciferase components are cotranscribed with the *I* gene; its protein product catalyzes the synthesis of the signal (3-OHHL). All these genes are efficiently transcribed provided that the *R* gene product binds to the signal and their complex attaches to the promoter (filled rectangle; according to: Oleskin, 2001).

"Academic lore tells us that while Woody Hastings and his graduate student were trying to isolate and characterize luciferase, the enzyme responsible for the bioluminescence of *V. fischeri*, they noticed that luminescence increased dramatically during the mid- to late exponential phase of bacterial growth … A few years later, Hastings, along with Ken Nealson and Terry Platt, was able to conclude that bacteria produced diffusible "autoinducer" compounds that accumulated in the medium during growth" (Stevens et al., 2012, p.2137).

This QS system includes two main gene complexes. One of them is the *luxICDABEG* operon. The *luxI* gene encodes the protein that is responsible for the synthesis of the QS signal, N-(3-oxohexanoyl)-L-homoserine lactone (3-OHHL). The other genes (*luxA, B, C, D, E,* and *G*) encode the components of the enzymes that are required for bioluminescence. The second gene complex includes the *lux R* gene. Its product, LuxR, binds to 3-OHHL. The LuxR-3-OHHL complex binds to the promoter site of the *luxICDABEG* operon and activates its transcription if the *V. fischeri* cell

density and, accordingly, the signal concentration reach the threshold level. Most other QS systems in gram-negative bacteria function according to similar principles.

N-AHLs contain fatty-acid chains; their length is different, and they have different substituents. Some N-AHLs have aromatic radicals or branched amino acid side chains. For instance, the aromatic radical-containing N-AHL signals (aryl-HSLs) cinnamoyl-HSL and isovaleryl-HSL are produced by *Bradyrhizobium* species utilizing the BtaI and BjaI synthases, respectively (Stevens et al., 2012, p.2133). The binding of N-AHLs to respective R proteins results in conformational changes that enable the HTH domain of the R proteins to bind to the DNA of these QS-controlled genes. This allows the R protein-HSL complex to recruit the RNA polymerase and to activate transcription.

LuxR type proteins contain the acylhomoserine-binding domain at the N terminal and the DNA-binding domain at the C terminal (reviewed, Venturi et al., 2018).

Some bacteria of the genus *Erwinia* (*Erw. carotovora*, *Erw. chrysanthemii*, and others) cause the soft rot of potatoes, chrysanthems, and other plants. They degrade plant cell walls using pectinases and cellulases. These enzymes are important virulence factors in *Erwinia*, and their formation is a quorum-dependent process (Fuqua et al., 1994; Revenchon et al., 1998). At a high population density, the synthesis of these enzymes is so rapid that plant cells are destroyed before their immune system responds to the pathogen. *Erwinia* contains the *expI-expR* system, an analog of the *luxI-luxR* system in *V. fischeri.* Protein ExpI, which is partly homologous to protein LuxI, is necessary for the synthesis of the diffusible communicative signal 3-OHHL (the same signal is used by *V. fischeri*). Since *Erwinia* and *V. fischeri* share the 3-OHHL signal, a plasmid containing all *lux* genes of *V. fischeri* except *luxI* brings about QS-dependent luminescence in *Erw. carotovora* (Revenchon et al., 1998).

Apart from *expI-expR*, *Erw. carotovora* posseses the *carI-carR* gene system. The *carI-carR* system controls the synthesis of the antibiotic carbapenem in a quorum-dependent fashion. Activation of the antibiotic's synthesis at a high population density via the *carI-carR* system helps *Erw.*

carotovora eliminate bacterial competitors that attempt to use the products of plant cell degradation by *Erw. carotovora* exoenzymes (Fuqua et al., 1994; Salmond et al., 1995).

In addition to 3-OHHL, the species *Erwinia chrysanthemii* produces other QS signals (Revenchon et al., 1998). QS systems in this bacterium are subject to regulation of other control systems, some of which depend on cyclic adenosine monophosphate (cAMP) and the cAMP-binding protein CRP; a similar dependence on the cAMP system has been revealed in *V. fischeri*. QS systems actually estimate not only population density but also other environmental factors via respective gene regulatory systems.

V. fisheri and *Erw. carotovora* provide demonstrative examples of cell density-dependent interaction between microorganisms and plant or animal macroorganisms. This interaction may result in establishing parasitic or mutually beneficial (mutualistic) relationships. An additional example is provided by the nodular bacteria of the genus *Rhizobium*. For instance, *Rh. leguminosarum* bv. *viciae* strains are responsible for the formation of nitrogen-binding nodules in the root systems of legumes. Their QS system, *rhiI-rhiR*, promotes the expression of the *rhiABC* genes at a high population density. The protein products of these genes are involved in interactivity between the bacterial symbiont and the cells of the rhizosphere. Interestingly, the closely related species *Rhizobium etli* contains an additional *raiI-raiR* system that is implicated in limiting the nodule number on host plant roots (mutants lacking this system form two times more nodules on bean roots than the wild type; Rosemeyer et al., 1998).

The bacterium *Agrobacterium tumefaciens* forms crown galls in a large number of plant species. The galls represent plant analogs of malignant tumors. The development of crown galls results from the transfer of oncogenic DNA fragments from the bacterium to the plant cell nucleus via specific Ti plasmids. Some of the genes of Ti plasmids induce the synthesis of opines that are utilized as nutrient substrates by *Ag. tumefaciens*. A homologue of *luxI-luxR*, the *traI-traR* gene system, stimulates the spread of Ti plasmids within the bacterial population. Since the *traI-traR* is located on this plasmid, this mechanism conforms with the selfish DNA theory suggested by Richard Dawkins. The plasmid DNA aims to spread in a

bacterial population. As soon as the population becomes "quorate" (sufficiently dense), plasmid-carrying cells are induced to conjugate with other bacterial cells (Greenberg et al. 1996). In addition, the conjugative transfer of Ti plasmids depends on opines. Therefore, efficient interaction between the microbiota and the macroorganism, a plant with an opine-producing tumor, are a prerequisite for carrying out this process. In particular, *traR* transcription is stimulated by factor OccR that is activated by octopine, one of the opines.

Many sponges are inhabited by symbiotic bacteria that use AI1-based QS systems as exemplified by the symbiont *Ruegeria* sp. strain KLH11. In this bacterium, "two genetic loci, designated symbiont *lociA* and *B* (designated *ssa* and *ssb*), were found to encode the LuxR/I homologues SsaR/I and SsbR/I, respectively. SsaI directs synthesis of long-chain AHLs with 3-oxo substitutions, while SsbI directs synthesis of long-chain AHLs with 3-hydroxysubstitutions. SsaI is necessary for the production of AHLs by SsbI, but SsbI, in turn, modulates the levels of SsaI-producedAHLs, suggesting a complex regulatory circuitry with feedback mechanisms. SsaR/I are necessary for swimming motility and production of flagella. Thus, this QS system was hypothesized to play an important role in controlling dispersal of the bacteria within and perhaps even between host sponges" (Stevens et al., 2012, p.2134).

The gram-negative bacterium *Chromobacterium violaceum* synthesizes the violet pigment violacein. This process is subject to regulation by the QS signal N-hexylhomoseine lactone; the QS system is called the *cviI–cviR* system. *C. violaceum* also contains N-decanoylhomoserine lactone (Thornhill & McLean, 2018).

The formation of swarmer cells that promote the spread of a bacterial population and the colonization of various ecological niches (see above) is subject to regulation, in some bacterial species, by *luxI-luxR* type systems. The *swr* gene system stimulates the movement of the swarmer cells of *Serratia liquefaciens* on a solid medium. Presumably, the expression of the QS-dependent *swr* genes results in producing an extracellular surface-active substance (surfactant) that facilitates swarmer migration on the surface of the medium (Givskov et al., 1998).

Some of the systems that use homoserine lactones as QS signals help bacteria eliminate competitors by producing antibiotics (including bacteriocins). For instance, the *phzI-phzR* system regulates the synthesis of antifungal antibiotics in *Pseudomonas aureofaciens* (Salmond et al., 1995).

A large number of tested bacteria contain several QS systems. Their interactivity pattern is complex. In *Vibrio harveyi*, luminescence is subject to regulation by three QS systems. While internal signal transmission processes are carried out consecutively within a single QS system, several QS systems can interact both in a consecutive and a parallel fashion. QS systems may compete or inhibit each other's operation.

The pathogenic bacterium *Ps. aeruginosa* forms biofilms and releases virulence factors (involved in invading the human host and destroying human tissues) under the influence of several consecutive QS systems, including LasI-LasR and RhlI-RhlR (also called VsmI-VsmR)[2]. The functioning of the LasI-LasR system results in activating the RhlI-RhlR system (Waters & Bassler, 2005) via promoting the synthesis of protein RhlR that binds the N-AHL signal (Ganin et al., 2015).

In *Ps. aeruginosa*, the product of the *lasI* gene catalyzes the synthesis of the QS signal N-(3-oxododecanoyl)-L-homoserine lactone that forms a complex with transcription regulator LasR. LasR activates the expression of virulence factors-encoding genes such as *lasB* (elastase), *lasA* (protease), *toxA* (exotoxin A), *aprA* (alkaline protease), and *lasI* (the enzyme responsible for the synthesis of the QS signal). The *lasI-lasR* system also activates the *rhlI-rhlR* system in which the product of the *rhlI* gene catalyzes the synthesis of the signal, N-butanoyl-L-homoserine lactone. This signal forms a complex with transcription regulator RhlR, and this complex activates *las B* and *aprA* expression. In addition, the *rhlI-rhlR* system activates the synthesis of the genes responsible for the synthesis of the surfactant rhamnolipid (that facilitates the migration of *Ps. aeruginosa* cells in a hydrophilic medium) and the pigment pyocianine, as well as of the *rhlI*

[2]An additional QS system of *Ps. aeruginosa* depends on a quinolone signal,MvfR (PqsR), that is implicated in regulating the virulence of this pathogen and its interaction with the protective systems of the host organism (Ganin et al., 2015; Mauro et al., 2018). This system will be briefly discussed below.

gene required for the biosynthesis of the QS signal N-butanoyl-L-homoserine lactone (reviewed, Bassler & Miller, 2013; Fletscher et al., 2018). The same QS system is involved in biofilm formation in *Ps. aeruginosa.*

Yersinia pseudotuberculosis, a parasite of the nematode *Caenorhabditis elegans,* uses two AI-1-dependent QS systems, YpsI-YpsR and YthI-YthR, that control flagellar motility via the master motility regulator FlhDC and the flagella-specific σ factor FliA, respectively (reviewed, Stevens et al., 2012).

A number of structural homologues of QS signals of the N-AHL type inhibit the operation of QS signals. Apparently, their producers use them to suppress competitors by blocking their communication systems (Zhao et al., 2017). An analogous function in interspecies aggression is plausibly performed by an enzyme formed by *B. subtilis.* It inactivates the QS signal of *Erw. carotovora*, which results in decreasing the virulence of this plant pathogen (Bassler & Williams, 2013, p.506). In Chapter four, the application of QS system inhibitors as new-generation antibacterial drugs will be discussed.

Modern-day genomic technology enables us to analyze a large number of bacterial genomes, including those containing QS system homologues. It has been revealed that some systems contain LuxR type, and not LuxI type proteins (solos or orphans, reviewed, Venturi et al., 2018). Extensive data are available on the proteins SdiA of *E. coli* and *Salmonella enterica* that bind the QS signals of other bacteria. In *Ps. aeruginosa,* protein QscR was identified as an orphan homologue of protein LuxR in the classical QS system of *V. fischeri*; *Ps. aeruginosa* lacks a QscR-binding signal. However, QscR binds the QS signal 3-oxododecanoylhomoserine lactone of the QS system of *V. fisheri* and the QS signals of the N-AHL type of *Ps. fluorescens, Burkholderia vietnamiensis,* and *Roseobacter gallaeciensis.* This testifies to an involvement of QscR in interspecies communi-cation. Interestingly, the functioning of the QscR-dependent QS system results in inhibiting the *lasI-lasR* and *rhlI-rhlR* systems in *Ps. aeruginosa* and, therefore, in suppressing biofilm formation in this bacterium and decreasing its antibiotic resistance

(reviewed, Ganin et al., 2015). This potentially enables using QscR-binding foreign QS signals as a new generation of antibacterial drugs.

Of special interest is the presence of *luxI-luxR*-like acylated homoserine lactones in archaeans, including the methanogen *Methanosaeta harundinacea* 6Ac. Its carboxylated N-AHL signal controls the transition from the short-cell to the filamentous phenotype in response to an increase in population density. The transcription of the genes involved in methane formation is concomitantly enhanced, while that of carbon assimilation genes is suppressed. *Meth. harundinacea* 6Ac contains the FilI-FilR QS system, a homologue of the LuxI-LuxR system (Zhao et al., 2017).

Another type of QS signals that is characteristic of a large number of gram-negative bacteria, including *Xanthomonas campestris, Xylella fastidiosa, Lysobacter enzymogenes, Stenotrophomonas maltophilia, Burkholderia cepacia,* and *Ps. aeruginosa,* comprises DSFs (diffusible signal factors). They represent unsaturated fatty acids such as cis-2-dodecenoic, cis-11-methyldodeca-2,5-dienoic, and cis-11-methyl-2-dodecenoic acid. Such QS systems regulate the expression of virulence and antibiotic resistance genes, cell motility and stimulate biofilm dispersal in, e.g., *X. campestris* (Zhao et al., 2017; Zhou & Cai, 2018). In *X. campestris,* the *cis*-11-methyl-dodecenoic acid signal is sensed by "the sensor kinase RpfC and the response regulator RpfG. RpfG has a… receiver domain attached to a HD-GYP domain that functions to degrade the second messenger cyclic di-GMP" (Stevens et al., 2012, p. 2135) that is involved in regulating motility and biofilm formation (see. 1.3.9). Presumably, DSFs represent interspecies signals involved in infection; for instance, they are produced by the opportunistic bacteria *Burkholderia cenopacia* and *Stenotrophomonas maltophila* in the lungs of individuals with cystic fibrosis (a hereditary disease characterized by excessive mucus formation in the lungs). The DSFs influence biofilm formation by *Ps. aeruginosa*, rendering the pathogen more resistant to antimicrobial agents (Stevens et al., 2012).

Actinobacteria of the genus *Streptomyces* use QS systems that regulate antibiotic synthesis, aerial mycelium development, and spore formation. The signals that function in these systems are homoserine γ-butyrolactones (e.g., A factor in *S. griseus*) that bind to the transcription repressor. It loses its

activity once bound to the QS signal. At least 15 homoserine γ-butyrolactones have been identified in *Streptomyces* and other prokaryotes (Biarnes-Carrera et al., 2018).

Apart from the LasI-LasR and RhlI-RhlR QS systems, *Ps. aeruginosa* contains a system that is dependent on 2-heptyl-3-hydroxy-4-quinolone (the *Pseudomonas* quinolone signal, PQS). This QS system is called the PQS system or the Mvf system; it consists of the PQS synthase (PqsI) and the regulatory protein PqsR (also referred to as MvfR). *Ps. aeruginosa* also produces PQS-like compounds such as 2-heptyl-4-hydroxyquinoline (HHQ), 2-nonyl-4-hydroxyquinoline (NHQ), and 2-heptyl-4-quinolone-N-oxide (HQNO). This QS system, along with the LasI-LasR and RhlI-RhlR system, is required for the production of virulence factors (pyocyanine, rhamnolipid, and lectin A). The same QS system is involved in releasing DNA molecules from the cells, which is associated with biofilm formation. Quinolone-type signals are also formed by other bacterial species, including the melioidosis pathogen *Burkholderia pseudomallei* (Fletscher et al., 2018).

The PQS system is packaged into outer membrane vesicles (OMVs) as mentioned above; this QS system also facilitates OMV formation by inserting into the membrane and inducing crenation (bulge formation, Stevens et al., 2012).

In *Ps. aeruginosa*, the PQS system provides a link between the LasI-LasR and RhlI-RhlR systems. PQS formation depends on LasR; at the same time, PQS stimulates the expression of the *rhlI* gene (Bassler & Miller, 2013; Ganin et al., 2015). PQS was revealed in the lungs of individuals with hereditary cystic fibrosis that are predisposed to develop *Ps. aeruginosa*-caused pneumonia; PQS promotes chronic inflammation that facilitates biofilm formation by *Ps. aeruginosa* (Ganin et al., 2015).

QS signals also include S-3-hydroxytridecane-4-on (CAI-1) that is produced by *Vibrio cholerae*[3]. CAI-1 synthesis is catalyzed by enzyme protein CqsA; the sensor is CqsS and the response regulator LuxO. LuxO is phosphorylated (and CqsS functions as a kinase) as long as the *V. cholerae* cell density is below the threshold level. Once it exceeds this level, CqsS

[3]*V. cholerae* virulence is also subject to regulation by the AI-2 signal (see below); the CAI-1 and AI-2 signals synergistically influence gene expression, but the CAI-1 effect prevails.

starts performing a different function: it behaves as a phosphatase that dephosphorylates LuxO. This results in inhibiting virulence and biofilm formation in *V. cholerae* at a high population density. Presumably, the transition from the biofilm to the planktonic lifestyle facilitates the migration of the cholera pathogen from an infected organism to a new host (reviewed, Ganin et al., 2015; Zhao et al., 2017).

Most QS systems of gram-positive bacteria are based on peptide signals that are either linear or contain a thiolactone ring. A peptide QS signal is produced by processing a longer precursor peptide and subsequently releasing it from the cell by means of an ATP-dependent ABC transporter (Bassler & Miller, 2013). Such QS systems are composed of two parts. A sensory histidine kinase binds the signal and phosphorylates the second part, the response regulator. A kinase cascade is initiated, which ultimately results in phosphorylating, and thereby activating, the protein that induces the transcription of the respective DNA operon.

For instance, one of the QS systems of *Staphylococcus aureus* (the Agr system, or SQS2) uses a peptide with a thiolactone ring (AIP, or AgrD). This QS system represses the synthesis of surface and attachment proteins, downregulates biofilm formation in *Staph. aureus* and upregulates the synthesis of toxins and exoenzymes, thereby facilitating infections caused by this dangerous pathogen (Shaw et al., 2007). The corresponding *agr* locus on the bacterial DNA is comprised of two suboperons with divergent promoters P2 and P3. P2 enables the transcription of the *agrBDCA* cluster. Among its protein products, AgrB is a membrane-associated protease that cleaves and excretes a modified octapeptide form of AgrD (AIP). The peptide binds to sensor protein AgrC and regulates the synthesis of toxins, adhesion and colonization factors, proteases, and other agents involved in infection. AIP binding to AgrC results in its phosphorylation, which induces the phosphorylation of AgrA. It activates the P3 promoter of the *agr* operon. The RNA III molecules transcribed[4] encode the hemolysin protein with

[4] The posited other QS system of *Staph. aureus*, referred to as SQS1, is based on the constitutive synthesis of ribosomal protein L2, or RNAIII-activating protein (RAP) that phosphorylates, at a sufficiently high concentration, the target protein (TRAP), which thereupon additionally stimulates the production of RNAIII molecules and, therefore, virulence. The role of SQS1

surfactant properties; they also stimulate the expression of extracellular proteases Aur and Spl (staphopains) that degrade bioflims (Karatan & Watnick, 2009). "Purified staphopains were able to prevent biofilm formation" by *Staph. aureus* (Stevens et al., 2012, p.2138). Analogous AIP-dependent QS systems are characteristic of other *Firmicuta* including coagulase-negative staphylococci, enterococci, clostridia, and listeria (Murray & Williams, 2018).

Staph. aureus strains can be subdivided into four groups (1, 2, 3, and 4) that differ in the amino acid composition of their signal molecules. Of note is the mechanism that is used by *Staph. aureus* to inactivate the QS systems of competitor strains: a "foreign" signal binds to sensor protein AgrC and inhibits its activity, like a faulty key that damages the lock if inserted[5] (Zhao et al., 2017).

Another typical peptide QS system is responsible for conjugative plasmid translocation in *Enterococcus faecalis* and related bacterial species (Fuqua et al., 1994; Nakayama et al., 1998). The peptide QS system facilitates the transfer of the following plasmids: *pAD1* involved in hemolysin synthesis, *pCD1* responsible for bacteriocine formation, and *pCF10* conferring tetracycline resistance on *Ent. faecalis*.

Each of the hexa- or octopeptide QS signals used by *Ent. faecalis* induces the clumping of bacterial cells and their conjugation that enables them to transfer a plasmide from the donor to the recipient cells. Octapeptide cPD1 stimulates the conjugative transfer of the *pPD1* plasmid. The plasmid codes for the QS receptor that is located on the repressor protein of the respective operon. For instance, the *pPD1* plasmid contains the *traA* gene that performs this function (Nakayama et al., 1998). The QS signal interacts with the receptor and inactivates it, inducing the expression of the operon. The *pPD1* plasmid also includes the *traC* gene. Its product is a signal-binding protein that promotes the translocation of the QS peptide across the cell wall; the efficiency of the signal in cell wall-deficient spheroplasts is

was called into question in a work in which no effect of an SQS1-inactivating mutation on virulence was detected (Shaw et al., 2007).

[5] It should be noted that the AIPs of the qroup 1 and group 4 strains differ only in one amino acid. The group 1 signal (AIP1) weakly activates, rather than impairs, the group 4 receptor AgrC and vice versa (Murray & Williams, 2018).

traC-independent (Rosemeyer et al., 1998). Such signals are only intensely synthesized by plasmid-free cells. Signal synthesis is suppressed in donor cells. Moreover, the plasmid encodes an inhibitory protein exemplified by peptide iPD1, the product of the *pPD1* plasmid that inactivates signal peptide *cPD1* (Khokhlov, 1988; Nakayama et al., 1998).

In *B. subtilis*, spore formation efficiently proceeds at a high cell population density or after adding the culture liquid of a concentrated cell population. The process is subject to regulation by a QS system with an oligopeptide signal molecule that is encoded by the *pfrA* gene. Its expression results in formation of the inactive precursor with 41 amino acids. Upon excretion from the cell, the N-terminal amino acid sequence is detached from this peptide and may other signal proteins. The remaining peptide with 19 amino acids is further cleaved by an extacellular protease, resulting in the formation of anactive signal pentapeptide (CSF, PEP5; Perego, 1997).

The CSF-mediated mechanism of spore formation activation in *B. subtilis* has been elucidated. CSF enters the cell via the oligopeptide permease. Once its concentration exceeds a certain threshold, CSF inhibits phosphatase RapA by forming an inactive complex with it. Without the phosphatase, the key sporulation factors, Spo0F and Spo0A, are in the active (phosphorylated) state.

The *rapA* phosphatase gene is co-transribed with the *pfrA* gene; they belong to the same operon. At low cell densities, the protein CSF formed by excreting and processing PfrA is present inside cells at low (sub-threshold) concentrations. Under these conditions, Spo0F and Spo0A are dephosphorylated by RapA, and spore formation does not start. Once the quorum level of cell density is achieved, the PfrA:CSF complex is formed, and the sporulation program is implemented (Mamson et al., 1998: Nakayama et al., 1998). Further research revealed two distinct levels of activation for phosphorylated Spo0A. A low activation level induces matrix production and a higher level results in sporulation (Fujita et al., 2005). It also renders cells insensitive to the Skf and Sdp toxins that are produced by them and kill sensitive cells. This is an analog of animal cannibalism "because dead cells serve as food to delay sporulation when nutrients are scarce" (Mielich-Süss & Lopez, 2015).

Another QS signal, ComX, activates the ComA QS system that turns on the transformation system (DNA transfer from cell to cell), rendering *B. subtilis* competent to transformation. The growth of the *B. subtilis* culture results in increasing the concentration of signal ComX produced by the cells. The signal is recognized by a two-component system that is composed of sensor kinase ComP and regulatory protein ComA. Upon binding the QS signal, ComA is phosphorylated. It activates the transcription of the *comS* gene. The product (protein ComS) protects another protein, ComK, against protease-catalyzed degradation. Protein ComK activates the transcription of the genes that are responsible for DNA transfer between cells (Bassler & Miller, 2013).

Finally, activation of the third QS system results in phosphorylating DegU that promotes the secretion of exporoteases, enabling a subpopulation of cells to behave as "miners". They are involved in producing "public goods", i.e., degrading proteins into nutritive small peptides to be utilized by the whole population (Mielich-Süss & Lopez, 2015).

To sum up, the activation of master regulators Spo0A, DegU, and ComA leads to the development of several different cell subpopulations specializing in cannibalism and sporulation, DNA transformation, and biopolymer degradation, respectively.

The QS signal of *Strepococcus pneumonia* is called the competence-stimulating peptide (CSP). It contains 17 amino acids and results from processing the 41 amino acids-containing precursor, ComC. CSP is recognized by sensor kinase ComD. It causes the phosphorylation of regulatory protein ComE via a cascade of consecutively phosphorylated kinases. As a result, the transcription of the *comX* gene is activated. The product of this gene is an alternative σ factor that is required for the active expression of the genes which are responsible for cell competence, i.e., capacity for DNA transformation, in an analogy to the QS system of *B. subtilis* (Bassler & Miller, 2013).

Both gram-positive and gram-negative bacteria also use furanones as signals. While many homoserine lactones and peptides are species- (or strain-)specific, furanones are recognized as signals by a wide variety of bacterial species and, in all likelihood, are used for interspecies

communication in microbial associations (Waters & Bassler, 2005; Khmel', 2006; Shpakov, 2009; Zhao et al., 2017). There are at least four optical isomers of furanone AI-2 (2-methyl-2,3,4,5-tetrahydroxytetra-hydrofuran), a regulator that seems to be very widely spread in the microbial world. However, *Salmonella enterica* serovar. *Typhimurium* produces a different furanone lacking the boron atom that forms a part of other furanones as organoboron compounds.

AI-2 regulates luminescence in *Vibrio harveyi*, virulence in *Vibrio cholerae* and other enteric pathogens, and spore formation in *Bacilus subtilis* (Waters & Bassler, 2005; Khmel', 2006). Homologues of the *luxS* gene that encode AI-2 synthase were revealed in 537 tested bacterial genomes (Zhao et al., 2017).

The luminescent bacterium *V. harveyi* produces two proteins, LuxP and LuxQ, that are involved in sensing the AI-2 signal. LuxP is a periplasmic protein, and LuxQ is a sensor kinase. LuxP and LuxQ form the LuxPQ complex. At a low *V. harveyi* cell density, LuxQ is in the phosphorylated state; it transfers a phosphate group to protein LuxO via LuxU. The phosphorylated LuxO interacts with factor σ54, promoting the transcription of small regulatory RNA molecules. This results in destabilizing the matrix RNA that is required for the synthesis of transcription regulator LuxR. Therefore, LuxR-dependent genes are not transcribed. At a high *V. harveyi* cell density, AI-2 binds to Lux P and alters the function of the LuxPQ compex: instead of behaving as a kinase, it starts functioning as a phosphatase; it dephosphorylates proteins LuxU and LuxO. The dephosphorylated LuxO fails to promote the synthesis of the small RNA molecules. LuxR-dependent transcription of the luminescence system (luciferase) genes of *V. harveyi* is carried out, enabling bioluminescence. Interestingly, *V. harveyi* also possesses an N-AHL-dependent QS system, which, unlike the prototypical LuxI-LuxR system, is analogous to the two-component systems of gram-positive bacteria. Overall, three types of receptors for the AI-2 signal have been identified. They are termed LuxP (exemplified by the aforementioned *V. harveyi* receptor), LsrB, and RbsB. Importantly, some bacteria that lack all the types of receptors, nonetheless,

specifically respond to AI-2, suggestive of the existence of other, hitherto unidentified, receptor types (Zhao et al., 2017).

A furanone QS signal that is implicated in virulence factor production and biofilm formation in *Ps. aeruginosa* is synthesized in patients with lung cystic fibrosis by normal respiratory tract microbiota, which, therefore, stimulates *Ps. aeruginosa*-dependent infection (Duan et al., 2003). This seems to account for the clinical data that antibiotics that fail to eliminate *Ps. aeruginosa*, nevertheless, ameliorate the symptoms of *Ps. aeruginosa*-caused infection. The antibiotics kill the normal microbiota, so that the pathogen is left without its microbial "friends".

E. coli (both its symbiotic and pathogenic strains), *Klebsiella pneumonia*, *Enterobacter cloacae*, *Shigella spp.*, and *Salmonella spp.* possess QS systems that are based on the AI-3 signal (Sircili et al., 2004; Walters & Sperandio, 2006). AI-3 is an aromatic compound. It binds to histidine kinases QseC and QseE involved in regulating the transcription of the genes which are responsible for the flagellar motility (*flhDC*) and the virulence (*LEE*) of the pathogenic strain *E. coli* O157:H7 (Clarke et al., 2006; Hughes et al., 2009; Shpakov, 2009) that produces "attaching and effacing lesions on the host's intestinal epithelial cells and eventually diarrhea" (Stevens et al., 2012, p.2138). The bacterial receptors bind, along with AI-3, neurochemicals such as catecholamines (Clarke et al., 2006) that produce stimulatory effects on *E.coli* motility and virulence; this subject will be discussed in more detail in Chapter three (subsection 3.1.1) that is concerned with the functions of neurochemicals in microorganisms.

The cells of the virulent *E. coli* strain also contain another QS system, FusK-FusR, which is modulated by fucose; its activation results in attenuating the pathogen's virulence. Presumably, the QS system enables the bacterium to adjust its behavior to the local environmental conditions. While in the gut lumen (high fucose content), *E. coli* does not need virulence factors and, therefore, downregulates their production; conversely, upon attachment to the epithelium (low fucose content), the virulence factors-producing machinery is disinhibited and performs its destructive functions (Stevens et al., 2012).

To sum up, the quorum-dependent regulation of gene expression enables microorganisms to adjust their behavior, taking account of their population density and also of diverse environmental factors. Moreover, QS systems provide for a coordinated expression of functional operons within the framework of a population or, with interspecies signals, of the whole microbial community, which, therefore, is comparable to a multicellular organism (Shapiro, 1988).

QS-like compounds are also produced by eukaryotic cells. Eukaryotes are likely to engage in "bluffing" bacterial cells into aimlessly carrying out costly quorum-dependent processes, even though the cell density is actually too low for the bacteria to be "quorate". This seems to be the reason why halogenated furanones formed by red algae of the genus *Delysea* are efficient antimicrobial agents (Givskov et al., 1998). The furanone of *D. pulchra* suppresses QS system-dependent swarming in *Serratia liquefaciens* and other bacterial species (Bassler & Miller, 2013).

Bacteria produce signals that are recognized by eukaryotes. The cells of a number of bacterial species, including *E. coli*, release an unidentified temperature- and pH-tolerant chemical factor. It induces the [GAR+] phenotype in the yeast *Saccharomyces cerevisiae*, enabling it to utilize various carbohydrates in the presence of glucose by overcoming catabolite repression. The bacteria of the genus *Sulfitobacter* stimulate cell division in diatomic algae by releasing the plant growth hormone auxin (indole-3-acetic acid: Zhao et al., 2017).

Some eukaryotes possess their own analogs of bacterial quorum-sensing systems. Such eukaryotic signal systems have been revealed in a number of yeast species, including *Ceratocystis ulmi*, *Candida albicans,* and *S. cerevisiae*. *S. cerevisiae* produces two QS signals, tryptophol and phenyl ethanol; their structures are similar to the aromatic "backbones" of the neuromediators serotonin and dopamine, respectively (see Chapter three). If the concentrations of yeast-produced signal molecules reach the threshold level, the *FLO11* gene is activated. Its protein product stimulates cell-cell contact formation and prevents the separation of the daughter cell from the mother cell upon budding. As a result, the QS system induces the transition from the yeast (solitary cells) to the pseudomycelium (branched

multicellular filaments) phenotype. This QS system works only under nitrogen limitation in the medium. High nitrogen concentrations repress the genes that are required for synthesizing the signals (Chen & Fink, 2006; Zhao et al., 2017).

The cells of diverse animal species release retinoic acid and also respond to it, i.e., retinoic acid is involved in a positive feed-back loop that is analogous to those characteristic of most bacterial QS systems. Retinoic acid is implicated in regulating cell proliferation, morphogenesis, and cell differentiation during the development of animal individuals (Mangelsdorf et al., 1995).

Presumably, analogs of quorum-sensing systems operate in malignant tumors if they give rise to metastases (Hickson et al., 2009). In an analogy to a microbial colony or biofilm, tumor cells form complex coherent systems (primary tumors) that are composed of functionally differentiated cells. Complex communication systems are at work that transmit "messages" both inside the tumor and between it and the cells of the stroma, of distant organs, and of the immune system (Ben-Jacob et al., 2012).

Interestingly, analogs of quorum-dependent processes were revealed in diverse biosocial systems of multicellular organisms. Such proceses help those systems chose collective behavior strategies in the absence of a central leader/pacemaker.

For instance, shoaling is a process that takes place upon reaching the threshold density of a fish population, in an analogy to quorum sensing-dependent processes in microorganisms. Spawning herrings form dense aggregations once their density increases to 0.2 individual per 1 m^2. These aggregations generate spreading "waves," resulting in the formation of a 20–30 × 3-4 km shoal (Makris et al., 2009).

Of relevance to the further sections of this work is the fact that bacterial QS systems are involved in communication between the microbiota and the host macroorganism. For instance, there are LuxR-type proteins that bind signal molecules produced by the host, a plant (Gonzalez &Venturi, 2013) or an animal (catecholamines behave as homologues of the aforementioned signal AI-3; see Chapter three for details).

Bacterial QS systems may depend on signal molecules containing host-produced components. For instance, the bacterium *Rhodopseudomonas palustris* incorporates plant host-produced *p*-coumarate in its QS signal, *p*-coumaroyl-homoserine lactone (Cooley et al., 2008).

The host organism can specifically respond to bacterial QS signals. Some of them behave as immunomodulators (Ulvestad, 2009). 3-oxo-dodecanoyl-homoserine lactone, a major QS signal of *Ps. aeruginosa*, inhibits tumor necrosis factor-α (TNF-α) and interleukin-12 (IL-12) synthesis by immunocytes and stimulates the production of the proinflammatory γ-interferone as well as interleukin-8 (IL-8); this regulatory effect implicates transcription factor NF-κB and activator protein 2. Cytolysin, the signal that activates the *cyl* operon of *Enterococcus faecalis*, has been revealed to produce toxic effects on neutrophils, macrophages, epithelial cells, and erythrocytes (Kaper & Sperandio, 2005) The same signal affects intestinal epithelial cells, disrupting the function of tight junction proteins and, therefore, increasing the permeability of the intestinal epithelial barrier and facilitating bacterial translocation into the bloodstream. The gram-negative anaerobic rod *Fusobacterium nucleatum* that inhabits the human gut forms protein Fap2. It interacts with host immunocytes. Fap2 binds to the TIGIT receptor of NK (natural killer) cells, preventing them from efficiently eliminating tumor cells. Therefore, this bacterium promotes the development of the intestinal adenocarcinoma (Zhao et al., 2017).

1.2.4. Distant Physical Communication

Electromagnetic and acoustic waves are likely to be involved in distant information transmission. As early as in the 1920's and 1930's, Alexander Gurwitch and co-workers investigated ultraviolet radiation that is emitted by living cells and stimulates cell division, as mentioned in the Introduction section above. For example, the UV radiation produced by *Nadsonia* sp.

yeast stimulated cell proliferation in *Bacillus* sp. cultures (Sewertzowa, 1929).

Recently, data on communication via electrical fields have been presented. They are actually a variation on the "electromagnetic waves-mediated communication" theme, since oscillations in electrical fields are known to produce electromagnetic waves that can carry messages across long distances. Electrical field oscillations that are generated by transmembrane potassium pumps in *Bacillus subtilis* cells can spread within a biofilm formed by this bacterium and synchronize the metabolic activities of its cells (Prindle et al., 2015). Such electrical field oscillations can function as long-range signals and attract bacterial cells that are located outside the biofilm and may belong to the same (*B. subtilis*) or a different (*Ps. aeruginosa*) bacterial species; these cells may be induced to join the electrical signal-producing biofilm (Humphries et al., 2017).

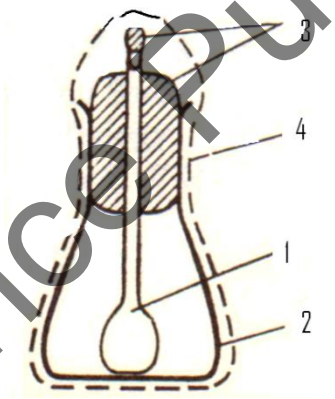

Figure 5. Equipment used to detect distant interaction between bacterial cells. 1, inner flask; 2, outer flask; 3, cotton bung; 4, foil. The culture in the outer flask was supplemented with a stress factor (chloramphenicol, an antibiotic); the culture in the inner flask received the signal from the outer flask, which resulted in accelerating its growth. According to: Nikolaev, 1992, with the author's permission.

In the 1990s, Yuri Nikolaev revealed that a *Vibrio costicola* culture treated with a lethal dose of the antibiotic chloramphenicol produces a signal that stimulates the growth of another culture of the same species that was separated by a quartz glass layer (Nikolaev, 1992, 2000). Several years later,

similar data were presented by Japanese researchers (Matsuhashi et al., 1996a, b). They hypothesized that the signal was an ultrasonic wave. Studies conducted by Nikolaev & Prosser (2000) demonstrated a synergistic effect of the physical and the chemical channels of intercellular communication. This was established in their experiments on the influence of a *Pseudomonas fluorescens* culture on the adhesive properties of another culture of the same species.

1.3. MICROBIAL BIOSOCIAL SYSTEMS

Biosocial systems can be defined *as systems composed of biological individuals or their groups that are characterized by communication, affiliation, and cooperation* among them (Oleskin, 2012). Is this definition applicable to unicellular organisms, particularly to *Prokaryota*? In light of all the above, it is evident that bacterial cells display selective affiliation-like behavior toward genetically related ("ingroup") cells while isolating from "outgroup" cells. They also engage in cooperation and communica-tion. Extracellular biopolymers (the matrix, see below) produced by bacterial cells limit the spread of their low molecular-weight signals: all recipients of a message typically belong to the same biosocial system (a colony or a biofilm) as the message-producing cell(s).

To sum up, the important features of biosocial systems highlighted in the aforementioned definition enable us to draw meaningful comparisons between various animal social groups and the systems composed of microbial cells (colonies, biofilms, flocs, etc.).

A widely debated issue in animal ethology concerns the advantages of the social lifestyle. In primates, even an individual with a low hierarchical rank seems to gain much benefit from staying in a social group that provides protection from many dangers and shares food with the individual (de Waal, 1996; Deryagina & Butovskaya, 2004). In a similar fashion, cells in a microbial colony/biofilm benefit from the social lifestyle: it increases their tolerance to antimicrobial agents, such as antibiotics, surfactants, chloramine, and alkali (Pavlova et al., 2007) and allows using nutrients

much more efficiently. As mentioned above, a major problem faced by many biosocial systems is the existence of defectors (cheaters) that benefit from the goods produced by cooperators without cooperating with them.

The following is a brief description of some aspects of microbial biosocial systems.

1.3.1. Homotypic and Heterotypic Biosocial Systems

Biosocial systems may consist of individuals/groups that belong to the same species (homotypic systems) or, alternatively, to several species (heterotypic systems, or associations). Unicellular organisms form both kinds of systems. Many microbial colonies or biofilms are composed of cells belonging to the same species; however, multispecies associations that may even include both prokaryotes and eukaryotes (e.g., bacteria and yeast) are also quite common in such ecological niches as wastewater treatment facilities, fermented dairy products, and the human/animal gut. Heterotypic biosocial systems form a part of higher-order systems such as consortia and ecosystems. For instance, an animal organism with its microbiota can be considered a consortium.

1.3.2. Hierarchies and Networks

Many biosocial systems include leaders (pacemakers) that control the behavior of other individuals. This *hierarchy* can be more or less rigid; it may be characterized by a completely centralized or a split leadership pattern in which case there may be several leaders (pacemakers). There are also systems that lack a hierarchy (Figure 6). Such systems in human society are currently exemplified by increasingly popular and influential *decentralized network structures*. They have a large number of partial leaders with limited power, competence, and leadership time (Oleskin, 2014). The operation of such nonhierarchical systems depends on cooperative interactions among their members; internal cooperation should override competition among

network members. Otherwise, if competition is more important than cooperation, the whole structure is called a (quasi-) market and not a network structure *sensu stricto* (Oleskin, 2014).

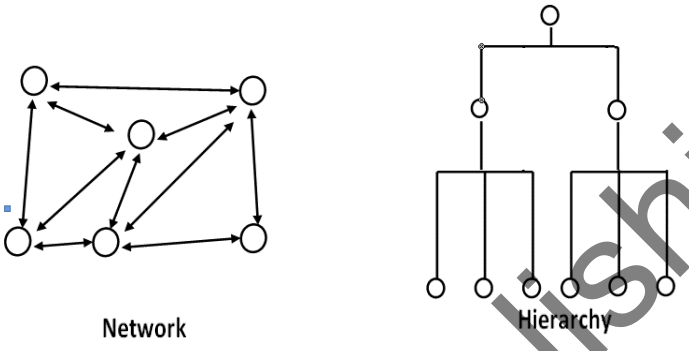

Figure 6. A hierarchy and a network (according to: Oleskin, 2012).

Decentralized networks and hierarchies exist not only in human society, and there are examples of both kinds of structures in living nature.

Hierarchies may be formed by systems composed of unicellular organisms or cells in the tissues of a multicellular organism. In cultivated animal epithelial cells, there are "leader cells" that move faster than other cells during the growth of the cell layer but stay in contact with them (Samoilov & Vasiliev, 2009).

Populations of starving bacteria, e.g., *E. coli* or *B. subtilis*, include two subgroups (Akaizin et al., 1990; Ellermeier et al., 2006; González-Pastor, 2011):

- subgroup 1: autolysing cells that die and release their nutrients into the culture fluid;
- subgroup 2: actively growing cells utilizing these nutrients.

In *B. subtilis*, subgroup 2 consists of cells in which the sporulation regulator gene Spo0A is active; they release two toxins that kill group 1 cells in which Spo0A is inactive. Subgroup 2 cells, in contrast, are immune to their own toxic products (Ellermeier et al., 2006; González-Pastor, 2011).

Thus, some of cells die and enable the relatively few survivors ("the elite") to grow.

Nonetheless, it is the decentralized network organization pattern that is widely spread among unicellular life forms including prokaryotes. Importantly, a lack of a single central pacemaker does not prevent an efficient coordination of the social behavior of the individuals (cells) inside microbial biosocial systems.

Many microorganisms are characterized by multilevel network structures. Their network structures are comprised of smaller network structures which, in their turn, are made up of still smaller networks of cells. In other words, a microbial biosocial system, e.g., a biofilm (see below) or a colony typically represents a *self-similar*, or *fractal*, structure. A colony/biofilm does not directly consist of individual cells. It is made up of their compact groups, or microcolonies, each of them being a smaller network composed of several tens or hundreds of cells. Cells inside a microcolony display coordinated behavior. In the colonies of the motile bacteria of the genus *Proteus*, cells in each small microcolony synchronously migrate over the surface of the nutrient agar. James Shapiro (1995) emphasized that such coordinated movement of cell aggregates (rafts) on the agar surface enables us to consider a whole colony as a multicellular organism. If one cell in a raft accidentally starts moving faster than its neighbors, it stops until thay catch up with it.

In many colonies or biofilms, local compact microcolonies are linked via intermediary cells that establish contact with adjacent cells using cell surface structures such as fimbria or pili. In addition, some bacteria including *E. coli* (Oleskin et al., 1998a, b) form extraordinarily long cells that bridge the gaps between microcolonies. There are literature data on the involvement of biopolymers, such as extracellular DNA molecules, in establishing links between microcolonies within the framework of the coherent higher-order network structure (a colony or a biofilm; Skarlyachan et al., 2018).

The aforementioned swarming behavior (1.1.6) actually implies decentralized network organization of the swarmers: they collectively

"make decisions" and migrate, in the absence of a leader (Ben-Jacob et al., 2016).

A still higher level of network organization is exemplified by interspecies network structures in bacterial associations or mixed communities that also involve representatives of other kingdoms and lack any central leader/pacemaker. Such networks are formed by several (or many) cooperating microbial species, based on the cross-feeding principle: products released by one species are utilized as substrates by other species. "With many species interacting, there is the potential for crossfeeding networks to reach dizzying complexity. One nice example occurs inside an insect, itself an exemplar of animal sociality: a termite. Termite society rests upon the ability to eat wood, and they are helped along by protozoa that break down cellulose… These protozoa in turn rely on bacteria, often spirochetes, that provide both metabolic assistance…, and even motility… Meanwhile, some spirochetes rely on crossfeeding from yet other bacteria in the termite gut…" (Foster, 2010, p. 336).

Microbial networks may also be based on the principle of regulatory, and not substrate, interaction. One of the species involved synthesizes a product that is recognized as a signal by another species within the network structure. This is exemplified by the aforementioned signal AI-2 that is implicated in the mutually beneficial "dialogue" between the representatives of the human microbiota that include, unfortunately, both useful and potentially harmful species and strains (reviewed, Oleskin et al., 2010).

1.3.3. Functional Specialization

Individuals in a biosocial system are not only distinguished by their rank in a hierarchy (if it exists). Many biosocial systems are characterized by functional specialization and structural differentiation of individuals in them. For instance, an ant society includes foragers, soldiers, and brood-rearing workers. Such specialization and differentiation is also evident in the cells of the tissues of multicellular plant and animal organisms. Similar phenomena are characteristic of bacterial colonies and biofilms. In the

bacterial realm, the diversity of cell forms is largely due to the plasticity of the genome, which contains genetic vectors (transposons, episomes, integrons, phages, etc.), although phenotypic variability is possible in prokaryotes even without genome alterations.

Smirnov (2004; see also: Smirnov et al., 1982) singled out cell clusters with distinct properties in microbial cultures, in an analogy to a multicellular organism that contains different tissues and organs. However, organs and tissues mostly represent compact local structures, whereas microbial cell clusters are delocalized and distributed within the whole cell population. Some of the clusters may dominate the population during certain culture development stages. Smirnov suggested several criteria for classifying microbial cell clusters:

- *Cell clusters with different growth and division rates ("short distance runners", "long distance runners", and mixed clusters*). "Short distance runners" rapidly proliferate while high concentrations of nutrients are available, and slowly growing "long distance runners" utilize low nutrient amounts and secure the population's survival during starvation.
- *Cell clusters containing actively dividing, dormant (persister), and autolysing ("altruistic") cells.* The nutrients released by spontaneously bursting "altruistic" cells improve the composition of the medium. Dormant cells (persisters) secure the survival of the whole colony/biofilm dring a starvation period or under stress.
- *Cell clusters that differ in surface potential (ζ potential) magnitude,* which may be correlated with such cell characteristics as division rate, immnunogenicity, and virulence.

Additional cell cluster types include the clusters of tightly attached and loose, planktonic, cells in developing biofilms (Stoodley et al., 2002; Agladze et al., 2005). Generally, "cells encased in biofilms are able to differentiate into subpopulations of phenotypically distinct but genetically identical cells… These are subpopulations of specialized cells that produce or respond to different signals and serve distinct purposes to the overall

community to strategically distribute labor and efficiently minimize energy costs" (Mielich-Süss & Lopez, 2015). Some other examples of functionally specialized cell clusters in microbial biofilms are to be found in the special subsection on biofilms (1.3.9).

Apart from clusters, functionally differentiated cells are also exemplified by *heterocysts* in cyanobacteria. These cells with thickened cell walls specialize in nitrogen binding, which is important for the whole cyanobacterial population. Unlike vegetative cyanobacterial cells, they lack photosystem II in their photosynthetic apparatus and, therefore, do not evolve oxygen from water. It should be noted that a large number of cyanobacteria also form other specialized cell types, such as akinetes, i.e., spore-like dormant cells, and hormogonia, cell groups that promote the spread of the population.

The aforementioned autolysing cell cluster illustrates a widely spread social phenomenon called programmed cell death for the benefit of the whole biosocial system. With respect to animal cells, such a phenomenon is referred to as *apoptosis*. An analogous phenomenon is the programmed death of bacteriophage-infected bacterial cells: they "commit suicide" to prevent the bacteriophage from reproducing and invading other cells (Samuilov et al., 2000).

A number of other similar phenomena are also to be considered in terms of functional specialization; such phenomena increase the heterogeneity of microbial populations.

Heteromorphism is the formation of abnormal cell types, including cells with disrupted division and defective cell walls, as well as cell wall-lacking forms (oval or spherical cells of the spheroplast or protoplast type), filamentous, giant, and miniscule cells such as L forms. Such "monsters" are likely to contribute to the viability of bacterial populations and their adaptation under changeable environmental conditions. L forms can persist in an infected animal organsm for a long time and cause a relapse of the infection once more favorable conditions are created (Vysotsky et al., 1991). Heteromorphism is also characteristic of cyanobacterial populations, especially when they establish a symbiotic relationship with plants. In such symbiotic systems, they form a whole gamut of bizarre structural variants,

including protoplasts and spheroplasts, giant and amophous cells, small microcells, minicells lacking the DNA, and cell wall-deficient elementary bodies (Baulina, 2012).

Phenotypic (phase) dissociation. Dissociants are bacterial cells that belong to the same species but differ in terms of colony shape and structure. Depending on the visual features of their colonies, they are denoted as the R (rough), S (smooth), and M (mucoid) variant; these variants differ in cell wall and matrix structure. The differences in the thickness and chemical composition of the capsule, the cell wall, and the cytoplasmic membrane result in different physiological, biochemical, and structural characteristics of the dissociants, including the influx/efflux rates of chemical substances, resistance to environmental factors, membrane fluidity, and cell morphology. Because of all these differences, dissocciants can occupy different ecological niches and promote the population's spread and survival. Such population heterogeneity is illustrated by the population of the luminescent bacteria *Photobacterium leiognathi* that contain clusters of dark, dim, and brightly shining cells (Medvedeva et al., 2006).

1.3.4. Matrix

Unicellular organisms form structures that function at the level of the whole biosocial system. The biopolymer-composed extracellular *matrix* is a structure that belongs to a whole microbial colony or biofilm. The formation of the matrix results from the merging of the outer layers of the envelopes of bacterial cells, and it incorporates their capsules and extracellular mucus. The microbial matrix contains polysaccharides, e.g., colanic acid and poly-β-1,6-acetylglucoseamine (PNAG) in *E. coli* biofilms. Such polysaccharides as Psl, Pel[6], and alginate are typical of *Ps. aeruginosa* biofilms. The matrix formed by some bacteria may contain teichoic acids, glycoproteins, polyglutamic acid and other peptides, polypeptides including polysaccharide-binding lectins, high molecular weight proteins (LapA in

[6] This cationic polysaccharide contains a large number of glucose residues and enhances the antibiotic resistance of *Ps. aeruginosa* (Skarlyachan et al., 2018).

Pseudomonas putida), surface proteins such as Bap in staphylococci (Aguilar et al., 2015), cell protrusions including fimbria, and extracellular DNA and RNA strands that bind calcium ions and form cross-links between cells (Skarlyachan et al., 2018).

For instance, the rigid extracellular matrix of *B. subtilis* contains exopolysaccharides (EPS) and proteins. Two secreted proteins, TasA and TapA, strengthen the matrix structure. Amyloid protein TasA is secreted into the extracellular space with the help of SipW, where it self-assembles into fibers that are anchored to the cell wall by TapA (reviewed, Mielich-Süss & Lopez, 2015).

DNA molecules form a part of the extracellular matrix because of the programmed death and autolysis of some cells in the bacterial population, which, as mentioned above, can be envisaged as an analog of altruistic behavior (Payne & Boles, 2016). The altruistic cell death with the extrusion of the DNA is based on two mechanisms: (1) the quorum-independent mechanism that maintains the baseline level of the DNA concentration in the matrix; and (2) the quorum-dependent mechanism enabled at a high bacterial cell density (in a "quorate" bacterial culture). An increase in extracellular DNA concentration is characteristic of the late logarithmic growth phase when the cell density is sufficiently high (Skarlyachan et al., 2018). It was reported that DNAse treatment leads to the dispersal of young *Ps. aeruginosa* biofilms (Harmsen et al., 2010). Aggregated proteins such as, e.g., pilin IV (Jurcisek & Bakaletz, 2007) and β-toxin (in *Staphylococcus. aureus*, Payne & Boles, 2016) as well as polysaccharide Psl in *Ps. aeruginosa* (Payne & Boles, 2016) attach to extracellular DNA strands.

The synthesis of matrix components that perform useful functions for the benefit of the whole biosocial sysem is envisaged in the literature as costly cooperation (Aguilar et al., 2015), i.e., an energy investment made by all cells except cheaters (free riders). "*Ps. aeruginosa* induces the synthesis of matrix components in response to environmental signals sensed by the sensor kinase/response regulators LadS, RetS, and GacS" (Harmsen et al., 2010, p.255).

Important matrix components that fulfill informational functions are the lipooligosaccharides of many bacterial species. The microbial matrix also

contains fibrillar components. Some components, as exemplified by sialic acids, are common to the bacterial extracellular matrix and that of animal tissues. The matrix composition varies depending on the bacterial species and strain and on cultivation conditions (Payne & Boles, 2016). Some microorganisms that inhabit multicellular organisms contain host-produced components in their matrix.

"The matrix plays a role in numerous processes including attachment, cell-to-cell interconnection, interactions between subpopulations, tolerance, and exchange of genetic material" (Harmsen et al., 2010, p.255). It performs a number of important functions, including the following:

- *Structural function.* The matrix coats the entire microbial network structure, represents both its external and internal "backbone", and separates it into a large number of small compartments where dense subnetworks (cell clusters or microcolonies) are located. This promotes local interaction between the cells inside each of the compartments.
- *Integrating function.* Since all compartments are interconnected within the matrix, all subnetworks are integrated into one coherent entity, i.e., the colony or the biofilm.
- *Adhesive function.* The matrix is involved in the attachment of bacterial cells to a surface, which is an important stage of biofilm formation. The polysaccharide-containing matrix of oral streptococci promotes their adherence to the teeth and the gum (Botvinko, 1985). Hydrophobic lipids and amphiphilic surfactant molecules also form a part of the matrix. By changing the surface tension on the vapor-water interface, they help bacteria adhere to hydrophobic surfaces such as polytetrafluoroethylene and polystyrene (Zhou & Cai, 2018).
- *Protective function*. The matrix protects colony/biofilm cells from dehydration, heating, hydrolytic enzymes, antibiotics, and other detrimental factors; the matrix formed by pathogenic bacteria masks their antigens otherwise targeted by the host's immune system (reviewed, Oleskin, 2009; Oleskin et al., 2010). Under stress (lack

of oxygen, heating, nonlethal antibiotic doses, etc.), elevated amounts of matrix components including PNAG are produced (Payne & Boles, 2016). *Ps. aeruginosa* mutants that overproduce the matrix component alginate are more resistant to the antibiotic tobramycin than the wild type (Harmsen et al., 2010). The matrix masks the Tol-like receptors-recognizable pathogen-associated molecular patterns (PAMPs) contained in the cell wall peptidoglucan or flagellin. Enriching the matrix in DNA[7] increases the resistance of the bacterial biofilm to antibiotics and other antimicrobial agents, and degrading the DNA with DNase makes the biofilm more vulnerable (Martins et al., 2010). This is partly due to the binding of the DNA to positively charged antimicrobial agents such as aminoglucosides and peptides. Alginate promotes the elimination of destructive radical oxygen species and slows down the complement-dependent response of the host immune system (Skarlyachan et al., 2018).

- *Transfer-stimulating function with respect to various chemical substances*. The matrix often contains water channels and possesses hydrophilic properties, which facilitates the distribution of various chemical factors, including ions, and also energy exchange within the colony/biofilm. Extracellular substances excreted by bacteria as exemplified by surfactin, viscosin and emulsan produced by *Serratia marcescens*, disperse hydrophobic substances, promoting their utilization (Zhou & Cai, 2018).
- *Competitor-eliminating function.* The matrix and its mucous components can block the supply of oxygen and nutrients to competitor cells, while improving the delivery of these resources to the matrix producer population (Foster, 2010).
- *Communicative function.* Signal chemicals, including pheromones used by quorum-sensing systems, are excreted by cells into the matrix; hydrophilic matrix components promote their spread within a microbial network structure. The matrix forms a diffusion barrier

[7] The DNA also facilitates biofilm formation; experimental degradation of extracellular DNA results in decreasing biofilm thickness by approximately 40% (Skarlyachan et al., 2018).

that enhances the efficiency of communication, rendering it target-oriented (Aguilar et al., 2015), in an analogy to the localized paracrine system of multicelular organisms that only delivers chemical signals to adjacent cells.

- *Synchronizing function.* By helping signals spread inside a colony/biofilm, the matrix also facilitates the synchronization of the processes that are carried out by partly autonomous subnetworks (cell clusters, microcolonies).

A continuous matrix layer coats multispecies microbal associations (heterotypic biosocial systems) in nature. Matrix components are directly implicated in interspecies interaction. The interaction of lactic-acid bacteria (*Lactobacillus brevis*) and yeast (*Saccharomyces delbrückii*) within the framework of the symbiotic community of the originally Caucasian beverage kefir is facilitated by the production of the exopolysaccharide kefiran by the lactobacilli (Botvinko, 1985).

Apart from the matrix, a large number of microbial colonies or biofilms contain specialized structures that do not belong to any single cell and represent functional "organs" of the whole biosocial systems.

1.3.5. Air- or Liquid-Filled Channels (Tubes) in the Extracellular Matrix

These channels distribute nutrients and, in aerobic bacteria, oxygen; they also help excrete metabolic waste products. Such channels may be surrounded by compact bacterial microcolonies that benefit from their "coastal" location; the channels can be spanned by long strands composed of matrix-coated bacterial cells and, in some bacteria, e.g., in *Haemophilus influenza*, by extracellular DNA molecules (Jurcisek & Bakaletz, 2007). Similar channels can be used by migrating cells that typically spread as small L forms. Such migration via channels is characteristic of bacteria that form a part of the animal (human) symbiotic microbiota (Pavlova et al., 1990a, b).

1.3.6. Hemosomes

These structures were revealed in the colonies of the bacteria of the genus *Alkaligenes*. They are covered with a membrane and contain extracellular hemoproteins (Duda et al., 1995, 1996). Presumably, these colony-level organelles are involved in oxygen transfer in microbial colonies/biofilms, in an analogy to the respiratory system of animals.

1.3.7. Colony Membrane

Such a membrane covers the colonies of various gram-positive and gram-negative bacteria, similar to the epidermal layer of the skin of an animal. The membrane is additionally fortified by polymer components that are located both on its outer and its inner side (Tetz et al., 1993), so that it actually forms a part of the matrix.

1.3.8. Unitary Developmental Program of the Microbial Biosocial System ("Culture Ontogeny" according to Yerusalimsky, 1952)

Since microbial biosocial systems are integral entities, their development can be envisaged as a life-cycle that follows a single colony-level temporal pattern and rhythm. The stages of the life-cycle of a microbial batch culture that were singled out in Yerusalimsky's seminal work in the 1950s, are in principle still considered valid by the global microbiological community:

- *Embryonic period (lag phase):* no appreciable culture growth takes place.
- *Youth period (exponential phase):* the culture actively grows.

- *Senescence period (stationary phase):* culture growth slows down and stops; vegetative cells are rare or lacking, the culture predominantly consists of unviable cells and dormant forms.

The development (ontogeny in Yerusalimsky's usage) of a microbial culture can be interpreted in terms of "anticipatory reflection". This term was coined by Smirnov et al. (1982) with respect to the development of microbial populations. It was revealed by them that the first developmental stage (the lag phase) in the life cycle of a bacterial population includes the formation of several "anticipatory" cell subgroups whose features are similar to those of the subsequent developmental stages of the same population. One cell subgroup in the lag phase population is analogous to the cells characteristic of the culture's senescence (i.e., stationary) stage. There are subgroups whose cells actively grow as if the population had already reached the logarithmic growth phase. It seems that they "'rehearse' the behavioral program carried out… in the main logarithmic growth phase" (Smirnov et al., 1982). Similar data were obtained by Gusev & Bobrova (1989), who suggested that, during the lag phase, the culture releases growth-regulating substances, including growth stimulators and inhibitors. Growth inhibitors were independently detected and characterized as d_1 factors by El'-Registan et al. (1979), see above (1.3.2).

The life cycle of a microbial culture manifests itself in a formation of concentric circles (cell concentration areas) on the agar surface (Budrene, 1985; Shapiro, 1988; Budrene & Berg, 1991, 2002; Mittal et al., 2003).

1.3.9. Biofilms

Biofilms are *"matrix-enclosed microbial accretions that adhere to biological or non-biological surfaces"* (Hall-Stoodley et al., 2004, p.95) that are mostly formed at interphase boundaries. Microbial biofilms are structurally heterogenous even if they contain cells of a single bacterial species because they include cells with different phenotypes. However, biofilms may include representatives of many different species, genera, and

even kingdoms or empires of life (Nikolaev & Plakunov, 2007). For instance, the film of a methanogenic association is composed of cells of eubacteria and archaeans. Apart from prokaryotes, biofilms may be composed of fungal or protozoan cells (Vidyasagar, 2016).

Many biofilms are characterized by functional differentiation of the cell types they contain and coordinated behavior that enables the biofilm to develop as a single coherent entity with its life-cycle (ontogeny). Like a multcelullar organism, a biofilm can reproduce and regenerate after injury (Sumina, 2006; Karatan & Watnick, 2009). In spite of their diversity, all microbial biofilms exhibit the following typical features (Nikolaev & Plakunov, 2007):

- *Spatial organization*, i.e., the formation of two- and three-dimensional structures in a biofilm, exemplified by local cell aggregates (microcolonies), cavities (pores and channels), lipid membrane vesicles, the outer cover of the biofilm including the biofilm-enveloping lipid bilayer (Tetz et al., 2004), and the biofilm's functional "organs" such as the O_2-transferring hemosomes of *Alcaligenes* sp. (Duda et al., 1995, 1996, 1998) and fruiting bodies with maturating spores (in myxobacteria) or their analogs (in bacilli).
- *Metabolic organization* implying the existence of a directed metabolite flow in a biofilm.
- *Extracellular biopolymer matrix* that is responsible for maintaining the structural integrity of a biofilm, protecting microbial cells from deleterious environmental factors, masking the cells' surface antigens to prevent their recognition by host immune cells, and creating a hydrophilic environment to promote the spread of metabolites and signal molecules within the biofilm; these matrix features were considered in more detail above.
- *Adherence to a phase boundary* such as a solid/liquid, solid/air, liquid/air, or liquid/liquid boundary.

Biofilms are comparable to human-made buildings: the matrix to the construction material(s), and the bacterial cells to the residents (Zhou & Cai, 2018).

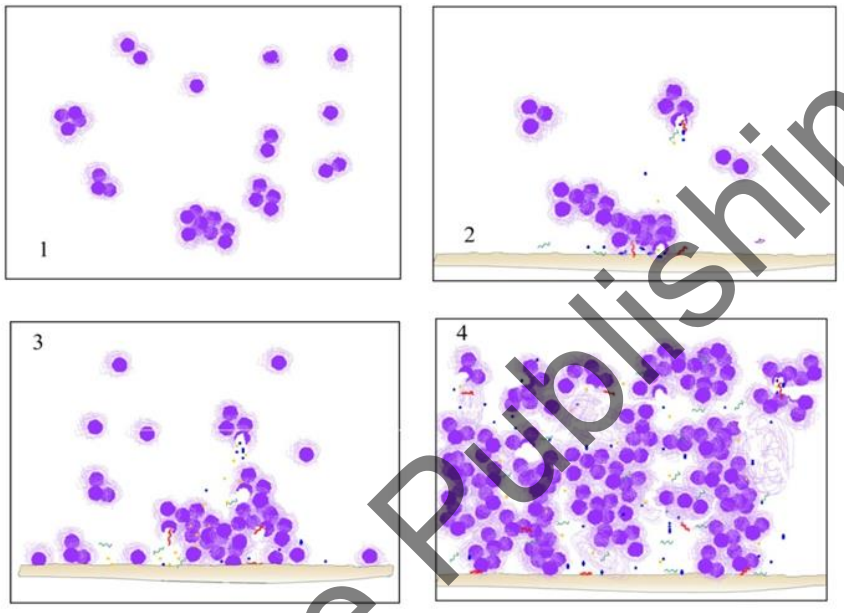

Figure 7. Stages of biofilm formation by *Staphylococcus epidermidis* 33 in the human oral cavity (a scheme). The Figure demonstrates the consecutive stages of the transition from a planktonic lifestyle (1) via the attachment of primary colonizers (2) and extracellular matrix synthesis (2, 3) to the formation of three-dimensional pillar- and mushroom-like structures (4). Balls, *Staph. epidermidis* cells; Pale halos around them, matrix elements; Spirals, extracellular DNA and RNA; Dots, proteins and peptides including enzymes and quorum-sensing autoinducers. The picture is a gift from Dr. Vladimir P. Korobov.

The typical structure of a biofilm is formed stepwise (the stages of development of a biofilm in the oral cavity are schematically represented in Figure 7). Initially, a *transient attachment* of microbial cells (primary colonizers) occurs, which is due to their interaction with the substratum involving flagella, pili, fimbria, and the proteins of the outer membrane (in gram-negative bacteria). “Transport of *Ps. aeruginosa* bacteria to a surface before attachment is assumed to involve diffusive, convective, and active flagellum-driven transport” (Harmsen et al., 2010, p.253).

This stage is followed by the *permanent attachment* of microbial cells to the surface. For example, motile bacterial cells first attach with one of their poles by means of flagella to a substratum; thereupon, one of their sides contacts the surface and is anchored there. At this stage, the microstructural features of the substrate surface play an important role. For instance, nano- and microscale surface roughness promotes bacterial adhesion, providing more area for cell attachment (Renner & Weibel, 2011).

Subsequently, microbial cells *spread* on the substratum colonized by them. This is accompanied by the formation of local cell aggregates, small microcolonies (which confers fractal properties, see 1.3.2 above, on the whole biofilm structure), and the intracellular matrix with characteristic cavities and the biofilm cover (Tetz et al., 1993, 2004; Pavlova et al., 2007; Zhou & Cai, 2018).

The development of a majority of biofilms includes the stage characterized by the *attachment of new microbial cells (secondary colonizers)* to the substratum-anchored cells, which results in the formation of multilayer biofilms. Cells attach to other cells and the substrate, and the attachment process largely depends on the matrix components with adhesive properties such as alginate, the linear anionic polysaccharide of *Ps. aeruginosa* (Skarlyachan et al., 2018).

Biofilm maturation is also often associated with the formation of wrinkles on its surface. They result from local cell death, the formation of empty spaces, and the shriveling of the matrix. Wrinkles increase the surface:volume ratio, promote oxygen supply to aerobic biofilm cells, and facilitate the development of a decentralized network of liquid channels that accelerate liquid distribution within the biofilm (in an analogy to a circulatory system in a multicellular organism). In an aging biofilm of spore-forming bacteria, e.g., *B. subtilis*, wrinkles develop into protrusions that serve as sporulation sites (Mielich-Süss & Lopez, 2015).

Eventually, a single- or multiple-species biofilm with a developed structure is formed; this biofilm can display a lamellar structural pattern, contain mushroom- or pillar-shaped formations, and display a variety of other "architectural features" that are due to cell specialization, communication, and a complex spatio-temporal organization pattern that is

"similar to those described for more sophisticated multicellular organisms" (Mielich-Süss & Lopez, 2015).

Biofilm growth and development is envisaged, in the literature, as a dynamic process that depends on the complex interplay among various physical (nutrient transfer, cell detachment from solid surfaces, shearing force, etc.) and biochemical (microbial cell growth, substrate utilization, etc.) factors. This interplay influences the biofilm's architecture. Empty internal spaces and mushroom-like structures are peculiar to biofilms whose growth is limited by the nutrient transfer rate. If biomass accumulation or shear force are the limiting factors, the biofilm is more compact and flat (Mattei et al., 2018).

Metabolic stress caused by nutrient limitation or toxic product accumulation promotes the expansion of biofilms. Such stress in *Ps. aeruginosa* may result from a lack of carbon, phosphorus, iron, or oxygen or accumulation of the metabolic inhibitor (uncoupler) carbonylcyanidechlorophenylhydrazone (CCCP). It stimulates flagellar motility, enabling outward migration of biofilm cells (reviewed, Zhou & Cai, 2018).

Of paramount importance is cell-cell cooperation during the biofilm formation and spreading process. Cooperation was interpreted above as contributing to the collective good within a distinct group of microbial cells (see 1.1.5). Cooperative interaction was observed in mixed biofilms formed by two or more bacterial species, in which it influences bacterial cell distribution and the biomass yield produced (Burmølle et al., 2014). Such mixed biofilms, e.g., those formed by *Pseudomonas putida* SB5 and *Chryseobacterium* sp. SB9, grow faster than the single-species biofilms of each of the partners, due to the synergistic effect of cooperation caused by metabolite exchange between them (reviewed, Zhou & Cai, 2018). Cooperation among oral bacteria is a prerequisite for the degradation of salivary mucins such as MUC5B carried out by a complex combination of proteases and glycosidases that cannot be produced by any single representative of the oral microbiota (Wickström et al., 2009).

Cooperation is facilitated by the structural and functional differentiation of the cells in multispecies as well as single-species biofilms. The biofilms

of *Pseudomonas fluorescens* include morphotypes M (mucoid) and D (dry, wrinkly). The two morphotypes stimulate each other's propagation ("division of labor"). Presumably, M reduces the tension of the solid surface, and D promotes the biofilm's spread (Kim et al., 2016). Of direct ecological relevance are the data on *Burkholderia cenopacia*: its biofilm includes the smooth (S), ruffled (R), and wrinkly (W) cell types and represents "a resilient symbiotic food web wherein the generalist S variant achieves high biomass by superior growth but attaches preferentially to the biofilm produced by R and W cells, which in turn profit from secreted metabolites" (Poltak & Cooper, 2011, quoted according to: Martin et al., 2016). The authors cited (Martin et al. 2016) conducted their own studies on *Ps. fluorescens* and *B. cenopacia* biofilms that provided further evidence of advanced cooperative interaction in these biofilms.

Like other cooperative acitivities, biofilm formation is vulnerable to cheating. For instance, *Ps. fluorescens* biofilm mats are based on a matrix that is made up of cellulose-like polymers (CLPs). Such biofilms colonize the air-liquid interface. Mutant cells fail to form CLPs but are able to invade and exploit biofilm-forming cooperators. However, upon reaching a high frequency, these cheaters disrupt the biofilm structure, rendering it unable to float on the liquid medium surface; such a biofilm ultimately loses viability (Velicer, 2003).

Chemical communication is actively involved in biofilm formation, spread, and dispersal. Quorum-sensing systems regulate various stages of a biofilm's lifecycle. Some of the relevant genes were revealed to be activated upon transition from the planktonic to the sessile lifestyle (in a biofilm). In *Ps. aeruginosa*, they stimulate the production of the extracellular matrix in biofilms, provided that the cell density is sufficiently high. If a biofilm is shared by several different strains, a matrix production-promoting QS system gives a competitive advantage to the strain in which it operates. However, at a lower cell density the QS system does not work, and this allows the cells to save the energy otherwise spent on synthesizing matrix components (reviewed, Zhou & Cai, 2018). In many microbial species, the structural and functional cell specialization depends on the operation of

quorum-sensing systems based on master regulators exemplified by Spo0A, DegU, and ComA in *B. subtilis* (see above, 1.2.3).

As mentioned above, *Ps. aeruginosa* possesses the LasI-LasR and RhlI-RhlR quorum-sensing systems. A mutant with an impaired LasI-LasR system as well as a double mutant lacking both systems forms abnormal thin flat biofilms that are destroyed by the detergent sodium dodecyl sulfate (SDS)[8]. The wild-type strain and a mutant with an impaired RhlI-RhlR system forms SDS-resistant three-dimensional biofilms with mushroom- or pillar-like structures separated by water-filled cavities. From these data it is evident that only the LasI-LasR quorum-sensing system is essential for biofilm formation in *Ps. aeruginosa* (Davies et al., 1998; Aguilar et al., 2015).

However, the RhlI-RhlR QS system is necessary for bacterial motility involved in forming flat biofilms, particularly under iron limitation conditions. A mutant lacking this QS system forms microcolonies instead of biofilms in an iron-depleted medium (Harmsen et al., 2010).

In the gram-negative bacterium *Pantoea stewartii,* the EsaI-EsaR QS system is responsible for the synthesis of stewartin, an acidic polymer with glucose, galactose, and glucuronic acid residues. Stewartin is present in mature biofilms and facilitates their adherence to abiotic surfaces (Aguilar et al., 2015).

Apart from chemical signals, physical factors such as electrical fields (see 1.2.4) seem to be involved in communication among cells within a biofilm (Prindle et al., 2015). The potassium efflux from metabolically stressed, glutamate-deficient *B. subtilis* cells in the interior of a biofilm (that is caused by the operation of the YugO K^+ channel), results in depolarizing the membranes of other cells in the same biofilm and even of bacterial cells outside its boundaries (see: Humphries et al., 2017). This decelerates the membrane potential-dependent influx of glutamate ions and, therefore, slows down metabolic processes in these cells. Hence, electrical communication results in reducing competition between biofilm cells for

[8] The double mutant of *Ps. aeruginosa* is also more sensitive to tobramicin, a clinically important antibiotic (reviewed, Ganin et al., 2015).

glutamate and other substrates and synchronizing the metabolic activities of the cells of a bacterial biofilm (Prindle et al., 2015).

The final stage of a biofilm's life-cycle involves its dispersal; microbial cells return to the planktonic mode of existence. This involves the detachment of the cells from the substratum and the separation of cell aggregates from the biofilm. Solitary cells can exit the film and start seeking new "accommodations" (Vidyasagar, 2016). Cells also detach from solid substrate surfaces (Davies, 2011). This is frequently accompanied by the synthesis of surfactants and enzymes, e.g., dispersin B and DNase, that degrade the matrix components (adhesins and extracellular DNA molecules, respectively) directly involved in the adherence of microbial cells to the substrate and to other cells. For instance, the oral cavity-inhabiting bacterium *Actinobacillus actinomycetemcomitans* produces an enzyme that degrades adhesin PNAG (Itoh et al., 2005; Romeo, 2006).

Biofilm dispersal also involves the suppression of *de novo* adhesin synthesis. Detailed studies conducted with the opportunistic pathogen *Ps. aeruginosa* that may inhabit various niches in the human organism have demonstrated that biofilm dispersal involves an endogenous prophage and the death of a part of the biofilm cells. This is associated with the transition of the remaining viable cells to the planktonic lifestyle. As a result, the surface-adherent microcolonies in biofilms undergo disintegration and become hollow shell-like structures. The whole phenomenon is termed "seeding dispersal" in the literature (Romeo, 2006).

As mentioned above, biofilm dispersal involves QS systems with unsaturated fatty acids, e.g., cis-2-decenoic acid, as signals. The same signal substances counteract the formation of new biofilms, e.g., in *Xanthomonas campestris* and *Ps. aeruginosa* (Aguilar et al., 2015). In *X. campestris,* a high cell density in the biofilm causes enhanced production of the enzyme endo-β-1,4-mannase, which results in degrading xanthan, a biofilm matrix component.

In the dangerous enteric pathogen *Vibrio cholerae*, quorum sensing is implicated in inhibiting matrix production and dispersing the biofilm at high cell densities. This enables a part of the population to separate from the matrix and seek a new ecological niche. After attaching to a new substrate,

V. cholerae cells lose their flagella and form a new biofilm (Zhou & Cai, 2018).

Biofilm dispersal and the transition of microbial cells to the planktonic lifestyle result in a considerable increase in the cells' sensitivity to various agents, including antibiotics, detergents, disinfectants, bcteriophages, immune cells, and predatory bacteria. Therefore, biofilm-degrading enzymes such as proteases that cleave, e.g., the biofilms of *Staph. aureus* (Payne & Boles, 2016), or dispersin B that degrades PNAG in the biofilm matrix (Kaplan, 2014) are regarded as potentially efficient drugs for treating or preventing infections that are caused by biofilm-forming pathogens.

Owing to the diversity of biofilm types (the same species can form structurally different biofilms, depending on its cultivation conditions and the genetic peculiarities of the given strain), different variants of the above stages can occur. Moreover, the biofilm life-cycle may lack some of these stages. Many representatives of the genus *Citrobacter* and some *Ps. aeruginosa* strains form matrix-embedded monolayer biofilms, i.e., no attachment of secondary colonizers to the surface-adherent primary colonizers occurs (Karatan & Watnick, 2009). Multilayer biofilms (mats) formed by associations of photosynthetic or sulphate-reducing microorganisms are often characterized by a lamellar structure lacking the mushroom- or pillar-like formations that are peculiar to a large number of other biofilms, e.g., those formed by oral streptococci.

The biofilm formation process is influenced by environmental factors and regulatory agents formed by microbial cells. Cultivation conditions (pH, temperature, nutrient substrate concentration, pO_2, osmolarity, surface hydrophilicity/hydrophobicity degree, shear force, etc.) produce their effects on microbial biofilms. For instance, nutrient limitation results in enhanced biofilm formation by *Salmonella enterica* var. *Typhimurium.*This process involves the operation of stationary-phase factor RpoS (Gerstel & Romling, 2003). In contrast, the development of *Vibrio cholerae* biofilms is enhanced in a nutrient-rich medium, and RpoS represses the genes involved in biofilm formation (Yildiz et al., 2004). It was established that biofilm formation in pathogenic and non-pathogenic *E. coli* strains is dependent upon their cultivation conditions including medium composition. Most tested *E. coli*

strains failed to form biofilms on the rich Luria-Bertani medium but formed them on a minimal medium and on diluted porcine intestinal mucus (Reisner et al., 2006).

Biofilm formation and matrix production are costly processes in terms of energy. The question to raise is why so many microorganisms form biofilms. The data obtained suggested that biofilm formation is promoted by factors that cause stress in microorganisms. This is exemplified by the fact that the addition of subinhibitory concentrations of antibiotics, such as the aminoglycoside tobramycin, induces biofilm formation in pathogenic *E. coli* strains (Hoffman et al., 2005). In a similar fashion, low doses of antibiotics with a β-lactame ring stimulate biofilm formation in *S. aureus* (Jacubovics et al., 2013).

Biofilms protect microorganisms under unfavorable conditions. "Bacterial biofilms can be likened to protective domiciles, such as nests or hives" (Velicer, 2003, p.330). For instance, marine bacteria in biofilms and structurally similar microbial mats "maintain the osmotic balance and resist the outside high-pressure environment" (Zhou & Cai, 2018) by activating matrix production.

The biofilms that are formed by microorganisms in the host macroorganism (see Chapter two for details) enhance the microorganisms' resistance to antibiotics (Foster, 2010); their lethal concentrations in biofilms are hundreds or even thousands of times higher than those killing plantonic cells of the same species (Mathur et al., 2018)[9]. Biofilms also prevent immune cells from attacking microorganisms. This is largely due to the aforementioned protective function of the matrix (see 1.3.6).

The resistance of biofilms to antibiotics and a wide spectrum of other drugs depends, apart from the matrix, on tolerant persister cells. Of note is also the production of drug-degrading enzymes and drug efflux pumps. Recently, genetic information drift and involvement of mobile elements such as plasmids have been suggested as an additional drug resistance mechanism. Quorum sensing systems are implicated in regulating the

[9] Nonetheless, a large number of bacterial biofilms are comparatively susceptible to the effects of baceriocins (antimicrobial peptides), including clinically impotant lantobiotics such as nisin and lantothionine (Mathur et al., 2018).

operation of drug resistance plasmids (Chen et al., 2016; Zhou & Cai, 2018). The high resistance of biofilms to antibiotics enables some bacteria to survive in the gastro-intestinal (GI) tract even during intense antibiotic therapy. This helps restoring the original intestinal microbiota after the termination of antibiotic treatment. As for the resistance of biofilms to the immune system, suffice it to mention that the biofilm protects *Streptococcus pneumoniae* cells from the oxidative burst caused by polynuclear leucocytes (Jacubovics et al., 2013).

However, there are other important reasons why microorganisms form biofilms (Jefferson, 2004):

- *Sequestration to a nutrient-rich medium*, the colonization of a favorable ecological niche. "A biofilm at an air-water interface has good access to oxygen and light…, and attachment to solid surfaces can yield similar advantages, particularly given that cells will often attach reversibly and swim off if they end up in a bad spot" (Foster, 2010; P.341). This strategy is also exemplified by biofilm formation in the GI tract; importantly, different species and strains, e. g. pathogenic and non-pathogenic *E. coli* strains, can compete for resources available in various areas of the GI tract (Bansal et al., 2007). Microbial cells in a biofilm engage in cooperation, and they can, therefore, more successfully adapt to environmental challenges (Aguilar et al., 2015).
- *Using the advantages of the social lifestyle*, including the functional specialization of microbial cells in metabolic terms; the biofilm lifestyle is unfavorable for non-cooperating cheaters that do not contribute to matrix synthesis and the production ofother public goods (Aguilar et al., 2015). This is largely due to the spatially structured environment that is provided by a biofilm. This environment favors cooperation and communication. In a microbial biofilm, "the secretors <of enzymes, nutrients, regulatory substances, and other products used by the whole microbial biosocial system – O.A.> have the primary access to the substances produced, allowing the public good producers to easily outnumber

the nonproducers" (Martin et al., 2016, p.2565). In contrast, "homogeneous, well-mixed environments may select against 'social' genotypes that secrete costly metabolites, so-called 'public goods', and rather favor fast-reproducing selfish individuals" (Ibid.). In a methanogenic microbial association, bacterial hydrolysers convert organic polymers into monomeric products that are accessible to the acidogenic and acetogenic microbiota. In turn, they supply "raw materials" for methane production by archeans. The advantages of the biofilm lifestyle also include protection from predatory protozoans and the facilitation of genetic information exchange (Romeo, 2006). Biofilms promote efficient cell-cell communication and acquisition and redistribution of nutrients and regulatory substances among the cells; this enables the bacteria in a biofilm to efficiently cope with environmental challenges (Aguilar et al., 2015; Zhou & Cai, 2018). The protective extracellular matrix helps biofilms survive under extreme environmetntal conditions, e.g., in hot-water springs with very low or very high pH values and on the surface of glaciers.

- *Biofilms as the default mode of existence*, as the normal lifestyle of most microorganisms; over 90% of environmental microorganisms form biofilms (Zhou & Cai, 2018). The existence of microbial cells in suspensions (the planktonic lifestyle) represents, in these terms, a temporary adaptation aimed at searching for a new suitable habitat for biofilm formation or just an *in vitro* artifact.

Despite the aforementioned benefits, biofilm formation causes problems for the microbial cells involved. Many biofilm-embedded cells are spatially separated from important nutrients or enzymes; harmful waste products accumulate in biofilms; cells become immobilized in the biofilm matrix and cannot escape from detrimental environmental factors (Davies, 2011).

In the literature, possible strategies of coping with these problems are outlined. In response to waste product accumulation and nutrient depletion in some areas of a biofilm, a significant part of the cells produces dormant forms including spores. Nutrient supply in biofilms is facilitated by the

cross-feeding strategy: adjacent cells exchange their products. Liquid-filled channels in the matrix also promote the transport of various substances within a biofilm. Certainly, the most radical strategy of solving biofilm-caused problems is reversion to the planktonic lifestyle (seeding dispersal). Of relevance is the fact that cell detachment from biofilms is stimulated by transferring them to nutrient-deficient media (Davies, 2011).

Biofilm development and dispersal are subject to control by a number of intra- and intercellular regulators. Microorganisms have special gene blocks involved in the planktonic cells-biofilm interconversion, including genes responsible for the adherence of microbial cells to substrata and to other cells, such as the *algC* gene required for the synthesis of alginate, a matrix component in *Ps. aeruginosa*, and the *wcaB* gene involved in colanic acid synthesis in *E. coli*. Biofilm formation in *E. coli* implicates the expression of genes that are involved in the production of bacterial cell surface structures, such as the *csgA* gene required for the formation of curli fibers (reviewed, Jefferson, 2004).

Gene expression during biofilm formation is influenced by a variety of intracellular regulatory factors. Important functions are performed, particularly in gram-negative bacteria, by cyclic diguanylate monophosphate (c-di-GMP). Accordingly, the regulation of the activities of the c-di-GMP-synthesizing enzyme diguanylate cyclase (DGC) and of the c-di-GMP-degrading phosphodiesterase A (PDEA) are essential for the operation of the intracellular network of regulatory agents involved in biofilm formation/dispersal.

"It is now widely accepted that cyclic di-GMP (c-di-GMP) signaling, first described to control extracellular cellulose biosynthesis in *Gluconacetobacterxylinus…*, is involved in the modulation of matrix components, control of autoaggregation of planktonic cells, and biofilm formation in several microorganisms.." (Morgan et al., 2006, p.7335). c-di-GMP operates as a multilevel intracellular regulator that influences transcription, translation, and post-translational protein modification and activity modulation. c-di-GMP is involved in producing and modifying matrix components, cell aggregation in microbial suspensions, and a wide variety of other processes that are implicated in biofilm formation

(Skarlyachan et al., 2018). In *Ps. aeruginosa*, "the transmembrane protein PelD binds c-di-GMP, and… there is a strict correlation between c-di-GMP binding and the synthesis of the Pel polysaccharide that forms a part of the matrix". Of relevance is also the fact that the membrane-bound protein Alg44, which is essential for the biosynthesis of the antibiotic resistane-enhancing matrix component alginate, includes a c-di-GMP-binding PilZ domain (Harmsen et al., 2010, p.255). During *Ps. aeruginosa*-caused infections, c-di-GMP is involved in the transition from the acute to the chronic infection stage, which is due to its impact on matrix polysaccharide synthesis. Excessive c-di-GMP production renders the cells less virulent and more antibiotic-tolerant; they form small colonies and are capable of long-term persistence in the infected region of the organism (Skarlyachan et al., 2018).

The effects of c-di-GMP may vary depending on its concentration. "High c-di-GMP concentrations have been shown in *Salmonella enterica* serovar *Typhimurium* to stimulate biofilm formation and EPS production (and thus, adhesiveness) but to suppress motility, while low concentrations inhibited biofilm formation, repressed the production of EPS, and stimulated swimming and swarming motilities" (Morgan et al., 2006, p.7335).

As already mentioned, primary bacterial colonizers attach to a new surface; they may either detach or remain fixed, and their fate depends, in *Ps. aeruginosa,* on the attachment regulator SadB (it is the fixed cells that give rise to a new biofilm). Synthesis of SadB is regulated by the intracellular level of c-di-GMP (Harmsen et al., 2010).

The intracellular c-di-GMP concentration decreases in response to environmental stimuli such as sudden changes in the nutrient concentration (in *Ps. aeruginosa,* this can be an increase in glutamate concentration) and oxygen depletion in the interior of the biofilm. The decrease in c-di-GMP concentration results, in a large number of bacterial species, in biofilm dispersal (Camilli & Bassler, 2006; Karatan & Watnick, 2009). The influence of environmental factors on the c-di-GMP pool is mediated by the chemotaxis protein BdlA that contains two PAS (Per-Arnt-Sint) domains involved in receiving a variety of extracellular signals including nitric oxide (Barraud et al., 2009a).

The functional differentiaton of cells in microbial biosocial systems that was discussed above in terms of "cell cluster differentiation" is probably characteristic of most biofilms. In *B. subtilis* biofilms, cells are subdivided into (i) actively migrating cells including swarmers; (ii) matrix component producers; (iii) surfactant-synthesizing cells; (iv) spore-forming cells; and (v) cells that are competent for DNA transformation. Different cell types prevail during different biofm development stages. At an early stage (primary colonization), the culture is dominated by highly motile cells, and at later stages by matrix producers. At still later stages, spore formers tend to prevail in the biofilm. The change of the dominant functional cell type is largely due to the functioning of QS systems. Sensory kinase KinD that forms a part of a QS system behaves as a phosphatase at low bacterial cell density. It detaches a phosphate group from regulatory protein Spo0A. Its dephosphorylated form activates matrix synthesis and suppresses spore formation. If the threshold cell concentration is exceeded, KinD exhibits kinase activity. It converts Spo0A into its phosphorylated form in which it stimulates spore formation (reviewed, Aguilar et al., 2015).

Biofilms are of paramount practical importance. A large number of biotechnological processes are carried out by means of microbial biofilms, as exemplified by the traditional French technology of producing vinegar with *Acetomonas* biofilms that are grown on woodchips. A thick multispecies biofilm containing bacterial and yeast cells is the producer of a useful beverage with medicinal properties, the "tea fungus" (kombucha, Yurkevich & Kutyshenko, 2002). Biofilms find application in bioremediation projects, including the removal of oil spills in the ocean and degradation of soil pollutants. Biofilms overgrow plant roots and cover the mucosa of the human/animal intestines; this "extracorporal organ" fulfills a number of important functions (see Chapter two below).

However, microbial biofilms can also do much harm. They cause the destruction of various materials and constructions (biofouling). A serious threat is posed by the biofilms of pathogenic microorganisms (see 4.2.3 below).

1.3.10. Scenarios of Biosocial System Formation

Two different scenarios are widely used by living nature for creating biosocial systems composed of liing organisms.

- *Nondisjunction,* i.e., biosocial system formation as a result of retaining close physical contacts among the offspring of an individual or, alternatively, between the offspring and the parental individual.
- *Secondary aggregation* of originally independent individuals.

Both scenarios work not only in animals. In the realm of unicellular organisms, the first scenario is referred to as incomplete *cell division;* it is also termed "the non-divisional mechanism" that is based on "staying together" behavior (Tarnita, 2017). Secondary cohesion of formerly independent cells occurs in the bacterial world. Although *Myxococcus xanthus* bacteria exist as solitary rod-shaped cells, they also form organism-like closely knit biosocial systems that are capable of cell-cell communication, coordinated collective movement, and the formation of multicellular structures (Chavira et al., 2007). To reiterate, starving myxobacteria form large swarms and thereupon fruiting bodies with ripening spores.

To sum up, the above data enable us to conclude that "… bacteria form complex communities that can collectively forage and engage in goal-directed movements and other activities that are coordinated by secreted extracellular signal substances termed autoregulators" (El'-Registan, 2005, p.14).

1.3.11. Are Microbial Biosocial Systems Analogs of True Multicellular Organisms?

Microbial colonies, biofilms, flocs, and other systems composed of many cells seem to be comparable to multicellular organisms (Shapiro,

1988, 1995) in light of the recent data that were considered above. Such systems contain functionally differentiated cells or cell clusters, undergo a collective life-cycle, and regenerate after injuries. In some microbial biosocial systems, cells can move in unison over agar surfaces. Microbial collective systems exhibit a large number of other quasi-organismic features. For instance, contacts between bacterial cells are structurally similar to those between tissue cells in multicellular organisms (Shapiro, 1988; Tetz et al., 1990; Smirnov, 2004; Sumina, 2006).

Nonetheless, there are important differences between microbial biosocial systems and true multicellular forms of life. The development of microbial systems is to a much greater exent dependent on environmental factors and to a lesser extent preprogrammed. There are bifurcation points in a bacterial culture's lifecycle, and the choice made by the system is strongly influenced by environmental factors. It is well known since the 1950's (Yerusalimsky, 1952) that clostridia do not form spores while cultivated on a nutrient-rich medium. Under nitrogen limitation, prespores are actively formed. If carbon supply is also limited, prespores irreversibly convert into spores.

It has been suggested that bacterial biofilm is a "city of microbes" rather than a single multicellular organism: cell differentiation in most biofilms is not as advanced and irreversible as in a true organism (Nikolaev & Plakunov, 2007).

The issue whether and to what extent microbial biosocial systems can be compared to multicellular organisms is difficult to resolve, in the authors' opinion, because microbial systems had emerged on the Earth long before multicellular forms of life came into being. Therefore, they lie at an evolutionary branching point from which two evolutionary trajectories diverge.

One of them leads to true multicellular organisms, and. therefore, microbial communicative signals are comparable to intraorganismic informational molecules (hormones, neurochemicals, etc.); the whole colony or biofilm is comparable to an organism.

The other trajectory is based on retaining the individuality of each microbial cell as a self-contained entity; from this viewpoint, microbial

signals are analogs of pheromones used by independent individuals (e.g., animals or plants) to communicate messages; the whole colony or biofilm (etc.) can be likened to an organized group of animals, e.g., to an ant society.

Likewise, there are two different interpretations of ant biosocial systems in the literature: (i) an ant society is a "superorganism", with individual ants as analogs of functionally differentiated "cells" within its framework (Hölldobler & Wilson, 2009, 2010); (ii) an ant society is a biosocial system that is similar to a flock of birds or a troop of apes (Zakharov, 2005).

Of much interest is also the question whether a microbial biosocial system is comparable to a nervous system, given the idea that a microbial colony is capable of "collective decision-making" (Ben-Jacob et al., 2016). Of relevance are recent data on the microbial production of substances that perform neurochemical functions in animals and humans (Tsavkelova et al., 2000; Özogul, 2004; Özogul & Özogul, 2005, 2007; Shishov et al., 2009; Malikina et al., 2010; Özogul et al., 2012; Oleskin et al., 2010, 2014a, b). Elongated cells in the colonies of many bacteria (hypothetical "wave conductors" facilitating electromagnetic communication between different colony parts, Vysotsky et al., 1991, and see above, 1.2.4) are comparable to the long axons of nervous cells that also transmit electrical impulses. The extracellular matrix is an analog of the glia which integrates nervous cells into a single coherent entity, and the outer membrane coating bacterial colonies is similar to the brain tunic (meninx). The analogy between a microbial colony and the nervous system was emphasized by Mark Lyte (1993).

Of general biological interest are the processes that are involved in the development of multicellularity in biological evolution. Importantly, true multicellularity implies the development of functionally differentiated cell tissues; microbial colonies, biofilms or flocs contain differentiated cell clusters but typically lack compact, segregated tissues (Smirnov, 2004). To some extent, the microbial mats that emerged on the Earth in the Archean era met the criteria of true multicellularity because they were composed of several structurally and functionally differentiated cell layers such as, e.g., the layer of photosynthesizing cells, the supporting layer, and the layer of heterotrophic cells.

In general terms, the transition to multicellularity represents a multistage process of the development of "collective individuality" (Panov, 2001), the stages of its development ranging from simple biofilms and mats to true mulcellular organisms with highly differentiated tissues. This process can be considered in ethological terms as a result of combining different kinds of social behavior including (see above, 1.1): (i) cooperation among cells that caused formerly independent units to merge into an adaptable whole system (Ulvestad, 2009); (ii) affiliation within the framework of the new emergent entity, so that cells selectively interact with other "ingroup" cells; and (iii) isolation with possible aggression against those cells that are recognized as "foreign" (it is pertinent that the self-nonself discrimination is essential for the operation of the immune system).

In summary, microorganisms represent, in light of recent data, complex living organisms that are capable of intense sophisticated communication and endowed with sufficiently advanced social organization that is especially conspicuous in biopolymer matrix-cemented biofilms. Microbial signals, particularly those used by quorum sensing systems, play an important role in communication among microbial cells as well as in the dialogue between the microbiota and the host organism.

Chapter 2

THE MICROBIOTA-HOST SYSTEM: BIDIRECTIONAL COMMUNICATION

"All diseases begin in the gut"

(Hippocrates)

Symbiotic microorganisms inhabit various niches on and in an animal organism. Microorganisms grow on the skin (and their maximum concentrations are detected between the fingers, on the foot soles, in the inguinal folds and the armpits, and on the scalp), on the eye conjunctiva, and on the mucosa of the upper airways and the urogenital system. Normally, the microbiota of each region of the human body performs a number of vital functions.

One of them is the *barrier function.* For instance, the symbiotic microbiota of the airways normally prevents their invasion by pathogenic microorganisms. The barrier function is also fulfilled by the vaginal microbiota. Representatives of the genus *Lactobacillus* consume the carbohydrates of the cells shed by the vaginal epithelium and form lactic acid that suppresses the growth of other microorganisms, including potential pathogens such as *Gardnerella vaginalis* that can cause infections including bacterial vaginosis. Among lactobacilli, *Lact. crispatus* seems to be the most efficient protector from pathogens; the commercial preparation Lactin-V, a

probiotic (see 2.6 below), has been developed from it for the purpose of treating vaginosis (Humphries, 2017).

Naturally, the same functions are characteristic of the skin microbiota that is regarded as "the first layer of defense against infectious microorganisms and toxic agents" (Edmonds-Wilson et al., 2015, p.4); disruption of the barrier poses the threat of developing serious skin problems.

The symbiotic microbiota also contributes to the development (the *developmental function*) and the maintenance of the normal physiological state (the *homeostatic function*) of various organs and systems of the organism including the airways (Man et al., 2017).

In addition, the microbiota performs the *immunomodulatory function.* It can be illustrated by the skin microbiota that significantly influences the operation of the human or animal immune system (reviewed, Liang et al., 2018).

2.1. GASTRO-INTESTINAL (GI) MICROBIOTA

This book mostly focuses on the microorganisms of the gastrointestinal (GI) tract that account for about 90% of the whole resident microbiota of the human organism. Much attention was given to the GI microbiota in the literature in the last decades; of special interest in this context is the distal part of the GI tract, i.e., the colon that harbors a particularly dense microbial population. The gut is the largest digestive, immune, and endocrine organ that also contains the enteric nervous system, which is partly independent of the brain (Shenderov, 1998, 2001, 2008, 2014; Liang et al., 2018).

2.1.1. Abundance and Diversity of the GI Microbiota

While the presence of resident microorganisms in the small intestine is a debatable issue (Sharkey & Savidge, 2014), the microbial cell concentration in the large intestine may be as high as $10^{12}/cm^3$, and the total

cell number is at least 10^{14} cells, which exceeds the human cell number in an adult human individual (Figure 8). The total nucleotide number in the DNA of the human microbiota (the total *microbiome*) is ~150 times higher than that of the human DNA (Shenderov, 2014; Parashar & Udayabanu, 2016; Shenderov et al., 2017). The metagenome of the human GI microbiota, i.e., the total genome of the microbiome, contains over 1 million genes (Boddu & Divakar, 2018). Microbiome genes impact the nutritional and metabolic processes in the host organism; they influence the efficiency of drugs used to treat a diseased host organism (Rees et al., 2018). The microbiome is envisaged as our "second genome" that is comparable, in terms of its impact on human health, to the human organism's own genome (Shenderov, 2016; Shenderov et al., 2017; Herd et al., 2018).

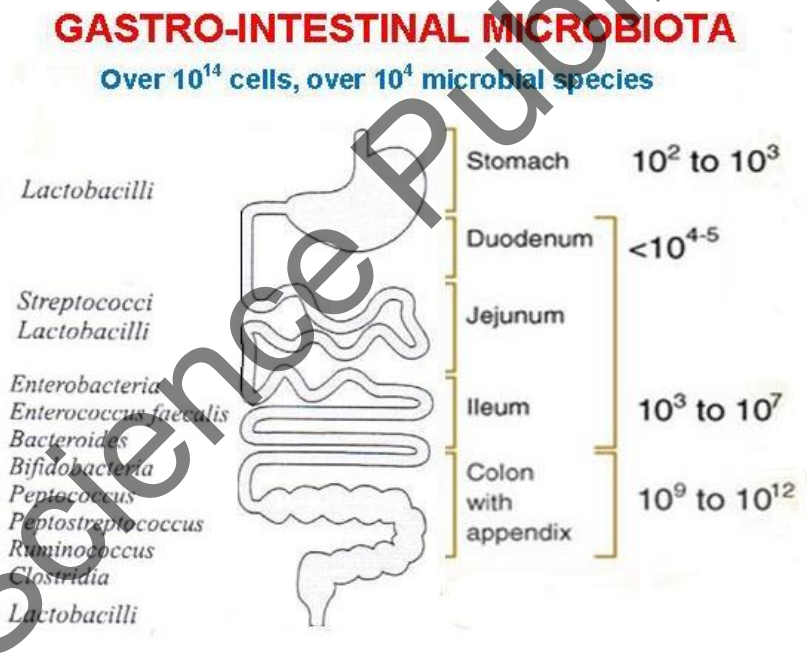

Figure 8. Abundance and composition of the microbiota in various GI tract parts.

The microbial *metabolome*, i.e., all low molecular weight (< 1500 Da) metabolites of microbial origin that are present in the GI tract, contains over 2.5 million different molecules, including about 1 million proteins and 300

thousand lipids (Shenderov et al., 2017). The total weight of the microbial biomass is 1,5–2 kg, i.e., it equals or exceeds the weight of such organs as the liver and the brain.

The total number of microbial species that are detectable in the GI tract may exceed ten thousand (estimatedly, there are 15,000–36,000 species, Huang et al., 2019). However, only ~1500 species are cultivable. Among the predominantly occurring 160–300 bacterial species, only 18 species are invariably present in all tested individuals, 57in 90%, and 75 in 50% of them. The most abundant microorganisms belong to the *Cytophaga-Flavobacterium-Bacteroides* (phylum *Bacteroidetes*) and *Clostridium-Lactobacillus-Enterococccus* (phylum *Firmicutes*) groups, each of them accounting for 30-40% of all detectable microorganisms in the colon. Less abundant but still sufficiently numerous microorganisms include *Actinobacterium* (especially *Bifidobacterium*), *Proteobacterium, Fusobacterium, Verrucobacterium,* and *Cyanobacterium.* In many human individuals, the colon harbors a significant number of methanogenic and methan-oxidizing archeans (Clarke et al., 2014; Shenderov, 2014; Shenderov et al., 2017; Logan et al., 2016; van de Wouw et al., 2017: Westfall et al., 2017; Rinninella et al., 2019).

2.1.2. Microbial Biofilms in the GI Tract: Interaction with the Host Organism

GI microorganisms can exist as planktonic cells in the intestinal lumen or as biofilms in the mucus layer overlying the epithelium, mucus within intestinal crypts, and the surface of mucosal epithelial cells (Kaper & Sperandio, 2005). Apart from microbial cells, GI biofilms contain the matrix (see 1.3.4) that includes microbial expolysaccharides and other biopolymers as well as host-produced components such as goblet cells-released mucin. Microbial biofilms line the most part of the intestinal mucosa, including that of the colon, the cecum, and the vermiform appendix.

The GI microbiota directly interacts with the barrier layer of the mucosal epithelium (“the firewall”); epithelial cells form tight junctions. This layer

regulates the entry of ions and organic molecules into the submucosal layer of the GI tract and subsequently into the bloodstream (Shenderov, 2014; Sharkey & Savidge, 2014).

2.1.3. Ontogenetic Development of the Microbiota

Until recently, it was widely accepted that the microbiota invades the GI tract and other niches of the organism during childbirth. However, the assumption that the fetus and the placenta are completely germ-free is currently called into question. It is admitted that microorganisms may penetrate into the fetal organism (Dinan et al., 2015; Sampson & Mazmanian, 2015; Stinson et al., 2019). The maternal microbiota that enters the fetus via the placenta may migrate from adjacent organs such as the vagina and the colon or, alternatively, be transferred via the bloodstream from distant body regions, including even the oral cavity. In fact, the placental microbiota is similar to that of the periodontal membrane, containing the characteristic bacterial species *Fusobacterium nucleatum* (Gur et al., 2015). If placenta-invading microbes cause inflammation, this may result in preterm birth (Gur et al., 2015).

Shortly after birth, a neonate's microbiota is composed of a limited number of species; it is dominated by proteobacteria and actinobacteria (Dinan et al., 2015). However, there are data that bifidobacteria also belong to the predominant bacteria in the intestines of newborns from Venezuela, Malawi, and the USA (Yatsunenko et al., 2012).

In the postnatal period, the GI microbiota that has entered the newborn's organism during birth and breastfeeding undergoes a rapid evolution that largely depends on the feeding regimen. The organism is invaded by primary colonizers such as enterobacteria, streptococci, and staphylococci. They consume oxygen in the gut, promoting the development of the anaerobic microbiota including *Clostridia*, *Bacteroides*, *Lactobacillus*, and *Bifidobacteria* (Dinan et al., 2015).

Natural (vaginal) delivery results in the predominance of vagina-specific species such as *Lactobacillus*, *Prevotella*, *Sneathia*, and

Bifidobacterium in the primary microbiota (Rinninella et al., 2019). Clostridia from the mother's skin are characteristic of infants born via *cesarian delivery* (Liang et al., 2018). Such infants are characterized by an impoverished microbiota, and this presumably is one of the reasons why they subsequently (by the age of seven) may develop serious problems including asthma, juvenile arthritis, inflammatory bowel disease (IBD), and obesity (Rinninella et al., 2019). The microbiota of such neonates is dominated by staphylococci and corynebacteria. The differences between infants that are born via vaginal and cesarian delivery persist until the age of two and concern not only the intestinal but also the skin and naso-pharyngeal microbiota (Osadchiy et al., 2019).

The impact of cesarian delivery on the newborn's microbiota may be due, apart from the unusual delivery technique, to the serious stress to which the maternal organism is exposed during the operation. The stress-induced increase in catecholamine and glucocorticoid levels in the maternal organism may affect the breast milk microbiota composition (Gur et al., 2015).

Preterm newborns suffer from serious problems that are caused by their immature internal organs, a long stay at hospital, and rectal feeding. They are characterized by decreased intestinal contents of *Bifidobacterium* and *Bacteroides* and increased levels of opportunistic and pathogenic enterobacteria (Rinninella et al., 2019).

Breastfeeding increases the probability that bifidobacteria and lactobacilli will dominate the GI microbiota. Breast milk lactoferrin facilitates the colonization of the neonatal gut by useful bacteria (Rinninella et al., 2019); the development of the GI microbiota is also stimulated by milk oligosaccharides (Osadchiy et al., 2019).

Formula feeding modifies the primary microbiota, which is characterized by the presence of clostridia and bifidobacteria, especially if the newborn is supplied with prebiotics such as galacto- and fructooligosaccharides; the GI microbiota also contains enterobacteria including *Escherichia coli* and enterococci (Dinan et al., 2015; Liang et al., 2018; Rinninella et al., 2019). Antibiotic administration in infancy results in a decrease in *Bifidobacterium* and *Bacteroides* contents (Liang et al., 2018).

Interaction between the maternal microbiota and the fetal or neonatal organism seems to account for the data that introducing *E. coli* cells into the organism of pregnant or lactating female rats resulted in an increase in intestinal wall permeability, systemic inflammation, and obesity in their pups (Gur et al., 2015; Stinson et al., 2019).

Of note in this context is the hypothesis proposed by Barker & Osmond (1986). According to this hypothesis, negative factors that affect the fetus or the neonate, including malnutrition, undernourish-ment, stress, and pathogen invasion, bring about long-term physiological alterations and increase the risk of developing serious health problems in adulthood. The nervous system is particularly susceptible to these factors that pose the risk of the development of such psychological disorders and syndromes as schizophrenia, autism, depression, and anxiety. In light of all the above, there seems to be an important relationship between the state of the microbiota in infancy and the physiological and psychological status of an individual (Gur et al., 2015).

The stability and the diversity of the intestinal microbiota tend to increase during the first 1000 days of a child's life (Logan et al., 2016). At the age of 3 to 5, the GI microbiota is similar to that of adults in terms of species composition (Yatsunenko et al., 2012; Logan et al., 2016); *Firmicutes* and *Bacteroidetes* species tend to dominate the microbiota. By the age of 12-14, the gut microbiota becomes completely "adult" with respect to its qualitative and quantitative composition (Dinan et al., 2015; Shenderov et al., 2017). Of considerable importance is the age-related dynamics of the ratio between the phyla *Firmicutes* and *Bacteroidetes.* The ratio is 0.4 in the GI tract of an infant, it increases to 10.9 in that of an adult and decreases to 0.6 with aging (Shenderov, 2014; Shenderov et al., 2017). If the microbiota of an adult remains immature, "neonatal", this poses the threat of the development of nervous system problems and, accordingly, psychological disorders (van de Wouw et al., 2017).

Nonetheless, "although early life events – including the mode of birth, type of feeding and complementary diet… have strong effects on the microbiota, it does retain some degree of flexibility and can be modulated

through exposure to a variety of environmental factors" (Kolodziejczyk et al., 2019, p.742).

2.1.4. Interindividual Differences: The Impact of the Diet

The composition of the symbiotic microbiota varies with different individuals, as far as the lowest taxonomic levels are concerned, including microbial genera, species, and especially strains. The microbiota is under the influence of the diet, the genotype, the epigenotype, and the state of the immune and the antioxidant system. Importantly, the influence of environmental factors tends to override that of hereditary factors (Shenderov, 2008; Shenderov et al., 2016; 2017; Herd et al., 2018; Rotschild, 2018). Genetically unrelated individuals are characterized by a similar micobiota composition after living together for a long time (Liang et al., 2018). For instance, the microbiota of the husband is similar to that of the wife provided that they live together. The intestinal microbiotas of identical (monozygous) twins do not bear greater similarity than those of fraternal (dizigous) twins (Yatsunenko et al., 2012). In general, comparison of human individual microbiotas reveals that "dietary changes can account for up to 57% of gut microbiota changes, whereas genes account for no more than 12%" (Clark & March, 2016).

Apart from the shared environment and diet, the nongenetic similarity of the microbiota of cohabiting individuals may be due to direct microbiota transfer between them. This was demonstrated in studies with baboon troops (Herd et al., 2018). Interestingly, interindividual microbiota differences in bees reflect their status differences in the social hierarchy, because the status influences the bees' diet and stress level (Herd et al., 2018). In human society, interindividual microbiota differences tend to be more manifest in children than in adults (Yatsunenko et al., 2012).

Taking account of the impact of the diet and the regimen (which is under the influence of cultural factors) on the microbiota, we can subdivide the population of the planet into several major subpopulations that are

characterized by different predominant microorganisms in the GI tract and live in the following regions (Shenderov, 2008):

- Tropical and subtropical areas
- Deserts
- Mountains
- Polar and circumpolar area
- West Europe and North America (including all those preferring a western-type diet).

The predominance of either carbohydrates or proteins and animal lipids in the diet results in the prevalence of either *Prevotella* or *Bacteroides* species in the intestinal microbiota (Wu et al., 2011; van de Wouw et al., 2017). The difference between the GI microbiota of Venezuela Indians and indigenous Malawi inhabitants (Africa) is relatively insignificant, since the common feature of both subpopulations is that they prefer a traditional lifestyle and diet. However, the GI microbiota of both subpopulations is considerably different from that of the inhabitants of the large cities of the USA. The preponderance of proteins in the diet of the American urban subpopulation, in contrast to the plant carbohydrate-rich diet of the inhabitants of Venezuela and Malawi, seems to account for these data. Therefore, the microbiota of urban Americans is enriched in microorganisms that produce α-amylase to degrade starch (Yatsunenko et al., 2012).

The intestinal microbiota of Hadza hunters in Africa is rich in plant fiber-degrading bacteria. In contrast to Italians that were also tested in the same work, the Hadza microbiota is dominated by *Proteobacteria* and *Spirochaetes* that very seldom occur in the Italian microbiota. Hadza hunters lack *Actinobacteria* that significantly contribute to the typical GI microbiota of Italians (Rinninella et al., 2019). The microbiota of seaweed-consuming Japanese is enriched in enzymes that are specific for marine bacteria (van de Wouw et al., 2017).

Of note in this context are seasonal diet changes that can significantly impact the GI microbiota. “In the community of Hadza hunter-gatherers in Tanzania, more frequent berry foraging and honey consumption in the wet

season result in significantly lower abundances of the phylum *Bacteroidetes* (particularly of the family *Prevotellaceae*) than in the dry season, when hunting becomes the dominant activity" (Kolodziejczyk et al., 2019, p. 744).

Traditional food in different regions of the world contains different ingredients, and, therefore, the GI microbiota of the inhabitants of Northern Europe (that predominantly contains bifidobacteria) differs from that of the people from Southern Europe (that is chiefly dominated by bacteroids and lactobacilli; Rinninella et al., 2019).

Nevertheless, despite the differences among the traditional diets of geographically remote regions, they are mainly characterized by a high fiber content and a large amount of plant ingredients (vegetables and fruit), seafood or its equivalents such as walrus meat in the Inuit diet, lean meat, wholemeal bread, nuts, and legumes. Importantly, in model studies with germ-free (GF) mice colonized by human microorganisms, "shifting from a low-fat, plant polysaccharide-rich diet to a high-fat, high-sugar diet alters the microbial community structure and metabolic pathways within a single day" (Kolodziejczyk et al., 2019, p.745).

The Western-type diet is rich in refined sugar and fat-containing additives, posing the risk of inflammatory diseases that are widely spread in the present-day world and often accompanied by psychological problems (Shenderov, 2008; Shenderov et al., 2016; Sarris et al., 2015; Logan et al., 2016). For instance, there is evidence that "migration from a non-Western country to a Western country is associated with immediate loss of gut microbiome diversity and functions and increasing obesity over the next generations in humans" (Lynch et al., 2019, p. 656).

A healthy diet is associated with a decreased depression and suicidal behavior risk (Sarris et al., 2015), which directly points to the effect of the diet on the brain and behavior, an area of interest to be revisited in section 4.2.

A typical urban resident's GI microbiota in many regions of the West contains few fiber-degrading bacteria but is rich in animal proteins- and lipids-decomposing bacteria. Increased consumption of fibers is expected to improve the microbiota composition, increase its diversity, and decrease the risk of inflammatory diseases and pathological symptoms (biomarkers) such

as an elevated blood lipid level and increased insulin resistance, a factor contributing to the development of metabolic syndrome and obesity. The negative influence of westernized diets can be mitigated by supplementing them with phytochemical (plant) compounds and ω-3-unaturated fatty acids (Shenderov, 2008; Logan et al., 2016).

Such natural polysaccharides as β-glucan, arabinoglucan, and digestion-resistant starch are examples of microbiota-accesible carbohydrates (MACs). Their lack in the organism results from using the western diet for a long time and may cause detrimental alterations in the GI microbiota, which affect also the offspring's microbiota (Shenderov, 2001; Sonnenburg et al., 2016; Liang et al., 2018). To reiterate, such host-indigestible microbial growth stimulators are referred to as *prebiotics* (Shenderov, 2001; 2014).

Daily physical exercise increases the GI microbiota diversity and enriches the microbiota in *Firmicutes (Clostridiales, Roseburia, Lachnospiraceae, Erysipelotrichaceae)* that produce valuable short-chain faty acids (SCFAs) (Rinninella et al., 2019).

It should be noted that the influence of genetic factors on the GI microbiota composition is sufficiently important, despite the very strong impact of the diet and other environmental factors. There is evidence that "when the microbiomes of different species of non-human primates or small mammals are analysed, the strongest determinant of differences in the microbiome was evolutionary distance rather than diet, indicating that major differences in gut niches exist, due to genetic factors between these organisms" (Kolodziejczyk et al., 2019, p.744).

Despite all individual differences in the GI microbiota, it includes the "core microbiota" that is common to most human individuals and contains *Firmicutes* and *Bacteroidetes* (Shenderov et al., 2017; Dinan et al., 2015).

2.1.5. Classifying Humans into Bacteriotypes

Sequencing colon content samples of several hundred humans within the framework of the MetaHIT Consortium (Metagenomics of the Human Intestinal Tract Consortium) project enabled classifying people into three

bacteriotypes, depending on the predominance of *Prevotella, Bacteroides, or Ruminococcus*in the colon (Arumugamet al., 2011; Clarke et al., 2014). Importantly, the bacteriotype was not determined by the tested individuals' gender, age, nationality, or the height/weght ratio (Dinan et al., 2015).

However, the bacteriotype (alternatively termed the *enterotype*) of an individual is under the influence of the diet that is typical of his/her region and local cultural traditions. For instance, African traditional diets (based on millet, sorgo, and vegetables) result in the predominance of the *Prevotella* bacteriotype, while the modern European diet favors the *Bacteroides* bacteriotype (Rinninella et al., 2019). The bacteriotype-specific microbiota tends to restore itself after temporary alterations caused by antibiotics or unusual food.

The validity of the above classification is still open to question. The alternative suggestion is that there is a continuum of human individuals in terms of the ratio between the three types of bacteria, and that this ratio changes to some extent during an individual's lifespan, despite its relative stability.

Account should be taken of more traditional classifications of human types. One of them is based on classifying individuals into four temperaments, and the issue is how the three bacteriotypes can be combined with these four temperaments, namely, the sanguine, the phlegmatic, the choleric, and the melancholic.

Of more relevance seems to be a more recent classification suggested by Helen Fischer (reviewed, Brown et al., 2013). She singles out four neurochemical types of people whose brain is dominated by four different neurochemical systems. These four systems in the brain depend on serotonin (sociable people), dopamine and norepinephrine (creative people), oxytocin plus estrogen (empathetic people), and testosterone (assertive, strong-willed people). Since not only human cells but also microbes produce most of the listed chemicals (see Chapter three), an interesting idea to be explored in the future is to attempt to correlate these four neurochemical types with the three bacterial types, bearing in mind the neurochemical impact of bacteria-produced substances. One could also consider, within the microbial and neurochemical context, other psychological scales, such as the classical

Kretschmer scale in which people are subdivided into schizothymic and cyclothymic subtypes.

The suggestion concerning a relationship between personality types and the microbiota is in line with the data that the degree of extroversion (communicability and sociability) in 1-1.5 years-old children is positively correlated with the diversity of their microbiota (Liang et al., 2018). In adult individuals, a high neuroticism (anxiety, timidity) level and a low responisibility (vigilance, diligence) level are associated with a *Gammaproteobacteria*- and *Proteobacteria*-enriched microbiota. In contrast, a high responsibility level is correlated with the abundance of bacteria that produce butyrate, one of the SCFAs (Kim et al., 2017). In studies with animal models, microbiota transplantation from one individual to another results in the recipient developing donor-characteristic behavioral traits. GI microbiota transplantation from obese mice to mice raised in the absence of any GI microbiota (germ-free, or GF, mice) results in overeating and obesity in the recipients (Osadchiy et al., 2019; see 2.4.1).

The hypothetical relationship between individual bacteriotypes and psychological features could be due to microbialy produced neuroactive substances that impact brain neurochemistry; they can influence the brain directly, by crossing the gut-blood and the blood-brain barrier (BBB), or indirectly, via enteric nervous cells and immune cells (to be discussed in Chapter three).

2.1.6. Relative Stability of the Individual Microbiota

It should be re-emphasized that, in spite of the effects of environmental stress factors, the GI microbiota of an individual is widely accepted to be relatively stable during the most part of the life cycle (Shenderov, 2014; O'Mahony et al., 2015). Nonetheless, there are also data that the qualitative and quantitative composition of the lactobacilli and bifidobacteria in the GI microbiota may significantly change during the course of a single year (Bercik et al., 2012).

2.1.7. Aging and the Microbiota

According to the literature, aging exerts a predominantly negative influence on the GI microbiota. Its composition is impoverished after the age of 65; the phyla *Firmicutes* and *Bacteroidetes* tend to prevail in the microbiota after this age (Dinan et al., 2015). The *Firmicutes:Bacteroidetes* ratio tends to decrease, finally approaching that of infants. Aging also results in a decrease in the total numbers of anaerobes, bifidobacteria, and lactobacilli. In contrast, the numbers of potential (opportunistic) pathogens such as enterobacteria, clostridia, and staphylococci, as well as the yeast *Candida*, tend to increase. The amounts of microbially synthesized butyrate and other SCFAs decrease, and the concentrations of ammonia and phenolic compounds increase (Shenderov, 2016; Shenderov et al., 2016, 2017; Westfall et al., 2017; Liang et al., 2018).

Aging induces or stimulates the production of toxic substances, including endotoxins produced by gram-negative bacteria and muramyl peptides that are synthesized by gram-positive bacteria. Overloading the host organism with these microbial products results in chronic inflammation, disrupted cell proliferation and apoptosis processes, host genome instability, epigenetically induced gene expression alterations (e.g., a decrease in histone acetylation and an increase in the phosphorylation degree of hippocampal genes involved in learning and memorization), changes in intra- and intercellular signaling pathways, the development of oxidative stress, and radical oxygen species (ROS) accumulation (Shenderov et al., 2016, 2017; Westfall et al., 2017).

Interestingly, centenarians predominantly do not exhibit these negative microbiota-related phenomena; their microbiota significantly differs from that of other aged people (Westfall et al., 2017). This corroborates a prophetic idea suggested by the Nobel Prize winner Ilya Mechnikov (also known as Élia Metchnikoff), a Russian microbiologist and immunologist, over a century ago: beneficial microorganisms are an important prerequisite for longevity.

It should be noted that, apart from the *resident microbiota* that is present in the host organism during the whole lifespan, the host contains the

transient microbiota. It is exemplified by some probiotics that are introduced into the host organism but only remain in it for a short time (Shenderov, 2001; Bercik et al., 2012).

2.2. MICROBIOTA AS A SPECIAL MICROBIAL ORGAN: THE IMPACT OF STRESS

2.2.1. Main Functions of the Microbiota

In the animal (human) organism, the microbiota is directly or indirectly involved in most of the physiological processes performed by the host; the "microbial organ" is implicated in metabolic, behavioral, and communicative activities in which the host organism engages. The following are some of the most important functions of the microbiota (Shenderov, 2001, 2008, 2014, 2016; Ulvestad, 2009; Montiel-Castro et al., 2013; Dinan et al., 2015; Clark & March, 2016; Oleskin et al., 2016;Shenderov et al., 2017; van de Wouw et al., 2017; Kolodziejczyk et al., 2019; Long-Smith et al., 2020):

- Protection against colonization by pathogenic microorganisms (production of antimicrobial agents and competition with pathogens for nutrients) and prevention of pathogen translocation into host tissues by stimulating the barrier ("firewall") function of the intestinal epithelium, impeding pathogen attachment to epithelial cells, and inducing the synthesis of secreted immunoglobulin A;
- Detoxification of harmful food-borne, microbially produced, or endogenous chemicals, including toxins and carcinogens;
- Involvement in nutrient digestion including protein, lipid, and carbohydrate metabolism, the utilization of host gut-indigestible components such as complex polysaccharides, and the recycling of fatty acids and other organic molecules;

- Enrichment of nutrients with microbial products including choline, short-chain fatty acids (SCFAs), biogenic amines (BAs), and vitamins (including, notably, vitamin K2 and folate) that are used as nutrients and/or signals by the host organism;
- Involvement in the metabolism of liver-produced bile acids, i.e., in their deconjugation (detachment of glycin and taurine molecules) in the duodenum, reabsorption in the distal part of the small intestine, and conversion to secondary bile acids in the colon;
- Energy supply for the host organism: the per diem contribution of symbiotic microorganisms of the human gut to its total energy level is comparable to that of daily consumed food;
- Regulation of intestinal motility (peristalsis), gas composition and temperature, pH, and redox potential levels as related to oxidation stress level (Clark & March, 2016), influence on many other physiological characteristics of the GI tract that are required for maintaining the constancy of the internal environment of the host organism;
- Regulation of blood vessel development and the maintenance of their functional state in the intestines;
- Modulation of visceral perception;
- Involvement in the ontogenetic development of the immune system and the regulation of its activity (including the intensity of the mucosal immune response, Kolodziejczyk et al., 2019) by producing various signal molecules that help "fine-tune" the interaction of the immune system and its potential targets;
- Genetic information exchange with the host organism (one of the reasons why many host and microbiota proteins are homologous); storage by the microbiota of valuable genetic information, which increases the stability of the metagenome of the whole host–microbiota system;
- Impact on epigenetic regulatory processes;
- Influence on host behavior, which is partly due to microbial production of neurochemicals and their precursors that can enter the

brain or exert their influence via the peripheral nervous system that subsequently influences the brain.

2.2.2. Microbiota as the Microbial Organ

The following aspects of the interaction between the microbiota and the host organism, including the nervous system, enable considering the microbiota as a special multifunctional organ (Lyte, 2010, 2011, 2013a, b; 2016; Oleskin et al., 2016):

1. The host nervous, immune, and other systems significantly influence the microbial organ.
2. In its turn, the microbial organ exerts an influence on the maintenance of the organism's adequate functional state and its neural, psychological, and metabolic homeostasis in health and disease.
3. The GI microbiota produces effects on other organs in the human body and responds to substances secreted by other organs.

The human microbiota responds to changes in the human individual's physiological and even psychological state, including various pathological processes and stress. Stress activates the hypothalamus-pituitary-adrenal axis and the autonomous nervous system. This results in altering intestinal motility, increasing epithelial barrier permeability for microorganisms, and releasing neuropeptides and other biologically active substances into the intestinal lumen. All these effects influence the intestinal microbiota (Sharkey & Savidge, 2014).

The micriobial organ is sometimes also dubbed "the second liver", because of its weight (1-2 kgkG) and multifunctional role. Microorganisms account for up to 60% of the dry feces weight (Siadat & Badi, 2019).

Recently, a large number of studies have been conducted on the impact of the diet and regimen on microbiota composition and, therefore, on the microbiota influence on the GI tract and the immune, endocrine, and nervous

system of the host organism. Suffice it to mention that enriching the diet with honey stimulates the growth of useful lactobacilli and bifidobacteria in GI tract, while suppressing that of potential pathogens (Parashar & Udayabanu, 2016).

2.2.3. Dysfunction of the Microbial Organ (Dysbiosis) and Its Pathological Consequences

Ilya Mechnikov rightfully asserted in the early 20th century that the *normal* microbiota of the GI tract is not necessarily *optimal*. The microbiota of a quite healthy individual still contains microorganisms that can aggravate stress and cause infection. A typical example is provided by the potential pathogen *Helicobacter pylori* that may cause gastric and duodenal ulcer if the host organism is under stress (Murrison, 2001). GI microbiota disruption (*dysbiosis*) under the influence of external and internal factors may result in disturbing the homeostasis of the GI tract and the whole organism. It manifests itself in a decrease in the numbers of useful microorganisms and impoverishment of taxonomic diversity of the microbiota, which is frequently accompanied by an increase in the number of opportunistic bacteria (potential pathogens) predominantly belonging to proteobacteria (Shenderov, 1998; El Aidy et al., 2015; Logan et al., 2016).

A disrupted microbiota can induce local inflammation in the intestine. For instance, the opportunistic pathogen *Clostridioides difficile* (formerly known as *Clostridium difficile*) can cause an intense inflammatory response of the immune system and the destruction of the intestinal epithelium (Shenderov, 1998; Sharkey & Savidge, 2014; Lynch et al., 2019). Intestinal dysbiosis negatively influences the state of the whole organism, affecting the brain and behavior.

At the local level, dysbiosis is frequently associated with inflammatory processes in the abdominal region. *Primary sclerosing cholangitis (PSC)*, i.e., chronic bile duct inflammation, is associated with "reduced bacterial diversity compared with healthy individuals as controls, and some studies

found that the genus *Veilonella* showed increased abundance" (Lynch et al., 2019, p.657).

Massive or prolonged administration of antibiotics, antiseptics, antihistamine drugs, tranquilizers, and antidepressants, as well as consumption of food containing heavy metal salts, pesticides, and other toxic substances results in exceeding the limits within which the microbiota–host system can maintain its homeostasis and, therefore, disrupting the microbiota. The after-effects of the antibiotic amoxycillin still manifest themselves 6 months after its administration; clindamicin causes changes in the species composition of GI bacteria of the genus *Bacteroides* that may persist for two years. Antibiotic treatment of healthy NIH Swiss mice results in decreasing the numbers of lactobacilli and bacteroids and increasing those of enterobacteria. This is accompanied by low intensity inflammation in the gut (Shenderov, 1998, 2014; Bercik et al., 2012).

Risk factors destabilizing human microbiota also include irregular meals, insufficient nutrition, starvation, carbohydrates- and lipids-high and fiber-low diets; cesarian section, formula feeding; alcohol consumption, low physical activity, circadian rhythm disruption; space flights with their specific factors ranging from ionizing radiation to long-term isolation; migration from one geographic region or climate zone to another; surgical operations; and bacterial, fungal, viral, and parasitic infections (Shenderov, 2014; Shenderov et al., 2017; Rook et al., 2017; Liang et al., 2018; Long-Smith et al., 2020). "Currently, humans, particularly those in industrialized countries, are living in an environment to which they have not adaptively evolved" (Ganci et al., 2019).

A negative influence on the microbiota is exerted by food additives, including preservatives such as sodium sorbate, sodium benzoate, and others (Liang et al., 2018). Emulsifiers in the diet, e.g., polysorbate 80 and carboxymethylcellulose, alter the composition of GI microbiota and induces intestinal inflammation (Chassaing et al., 2017) "that promotes development of chronic colitis in susceptible mice and metabolic syndrome in wild-type mice via mediation of the gut microbiota" (Lynch et al., 2019, p.656).

Widely used commercial low-calorie sugar substitutes (sweeteners), including saccharin, aspartame, sucralose, and neotane, also affect the GI

microbiota; they may cause the development of glucose intolerance, metabolic syndrome, and obesity (Shenderov, 2008, 2014; Bian et al., 2017; Chi et al., 2018). Some psychopharmacological drugs such as, e.g., risperidone, that are used to alleviate the symptoms of schizophrenia and bipolar disorder (manic depression) cause GI microbiota disruption and a weight increase to the point of developing obesity (van de Wouw et al., 2017).

Predominantly, abolishing the action of the microbiota-disrupting factors, e.g., antibiotics, enables a partial or even complete restoration of the original microbiota composition. It seems likely that the normal microbiota is stored in intestinal areas that are protected from stress factors. Presumably, the vermiform appendix is one of such areas.

Some attention is given in the literature to the "hygiene hypothesis" concerning the negative consequences of excessive hygiene that is chacteristic of the developed countries. Currently, people more often wash their hands, clean their teeth, and wash laundry, which necessitates using large amounts of chemicals on a daily basis. This predisposes the people to immune system dysregulation which increases the organism's vulnerability to infections and malignant tumors as well as to autoimmune diseases (lupus erythematosis, glomerulonephritis, etc.) in which the unbalanced immune system erroneously recognizes the organism's own cells as foreign (Weinersmith & Earley, 2016; Johnson & Foster, 2018).

Indesputably, the height:weight ratio is affected by GI microbiota disruption. Dysbioses may result both in a pathological appetite increase (bulimia, binge-eating disorder) often associated with obesity and, alternatively, in anorexia (lack of appetite) and emaciation, including severe dystrophy (kwashiorkor) in Third World country children. Both extreme condictions, obesity and anorexia with emaciation, are characterized by an impoverished taxonomic diversity of GI microbiota (van de Wouw et al., 2017). Radical measures for normalizing the microbiota of kwashiorkor children, such as antibiotic treatment, increase their survival chances (van de Wouw et al., 2017).

The important role of the GI microbiota is highlighted by the data that germ-free (aseptically raised) mice become obese after transplanting the GI

microbiota of obese mice to them. Conversely, mice lose weight if their GI tract is colonized by the microbiota of underweight mice with a surgical stomach bypass (Clarke et al., 2014). Germ-free (GF) mice have a decreased weight despite an increased appetite. Their weight increases after transplanting the microbiota of obese humans and further decreases after introducing the microbiota of kwashiorkor children (van de Wouw et al., 2017).

Dysbiosis frequently results in disrupting the barrier function of the intestinal wall. In the normal gut, only small amounts of chemical agents or microorganisms pass the tight junctions (*zonula occludens*) between intestinal epithelial cells, and these low concentrations of potentially harmful agents help develop and activate the adaptive immune system.

The *leaky gut syndrome* enables the intrusion of large amounts of microbial cells and metabolites into the bloodstream, which often results in inflammatory responses. Such an increase in intestinal barrier permeability may be caused by stress, alcoholism, intake of heavy metals, or malnutirion (Liang et al., 2018). Under the influence of these factors, the lipopolysaccharides (LPSs) of gram-negative bacteria enter the bloodstream and induce chronic low-intensity inflammation. This state is referred to as *metabolic endotoxemia.* It poses the risk of the development of type 1 diabetes, obesity, allergic processes, and psychological disorders (Logan et al., 2016). Even relatively low concentrations of bacterial LPSs in the blood (0.5-1 ng per 1 kg of weight) can cause systemic inflammation. Such LPS concentrations affect the brain, which manifests itself in the LPS-dependent activation of brain immune cells (microglia), emotional and cognitive disorders, general indisposition, and depression (Logan et al., 2016).

Administration of amipicillin and neomycin decreases the bacterial LPS content in the cecum and mitigates metabolic endotoxemia in obese mice; these data highlight the involvement of the gut microbiota in the development of metabolic endotoxemia (van de Wouw et al., 2017). To reiterate, tradional diets and intense interaction with microbiota in infancy lower the risk of systemic inflammatory problems. This is consistent with a decrease in C-reactive protein level under the influence of these benefical factors (Logan et al., 2016).

Local (GI tract-affecting) and systemic diseases associated with GI dysbiosis include inflammatory bowel disease (IBD), Crohn's disease, nonalcoholic fatty liver disease (NAFLD), colon cancer and other malignant tumors, neurodegenerative disorders (Alzheimer's, Parkinson's, and Huntington's disease, lateral amyotrophic sclerosis, and Friedreich's ataxia), metabolic syndrome (aterosclerosis, type II diabetes, and obesity), autoimmune diseases (multiple sclerosis, type I diabetes, and lupus), psychological disorders [10] with behavioral symptomes (autism, schizophrenia, depression, chronic fatigue syndrome, and post-traumatic problems), locomotive system diseases (fibromyalgia, skeletal muscle hypertrophy and atrophy, non-specific arthritis and arthrosis), urolithiasis, cholelithiasis, bronchial asthma, atopic dermatitis, psoriasis, periodontal and gum inflammation, vaginal infection (vaginosis), menstrual cycle disruption, infertility, preterm delivery, cachexia (emaciation), transplant-against-host response, and opportunistic infections (not necessarily associated with the GI tract; Shenderov, 1998, 2014; Shenderov et al., 2017; Herd et al., 2018; Lynch et al., 2019).

"Importantly, specific microorganisms have been identified as pathobionts in disease pathogenesis and resistance and/or response to treatment, including *Fusobacterium nucleatum* in colorectal tumorigenesis and chemoresistance, low-abundance *Faecalibacterium prausnitzii* (a short-chain fatty acid producer) in IBD, *Akkermansia muciniphila* in controlling diabetes and obesity and *Proteus mirabilis* as a potential major player in Crohn's disease pathogenesis in animal and human studies" (Lynch et al., 2019, p.656).

2.2.4. Stress and the Microbial Organ

Any physical or psychological stress that is too severe for the organism to cope with (*distress* according to Hans Selye, 1956) impacts all systems of the human (animal) organism, and especially the neurohormonal

[10] The part of the list containing neurological and psychological disorders will be revisited in subsection 2.4.5.

hypothalamus–pituitary–adrenal (HPA) axis that produces catecholamines including norepinephrine (Chrousos, 2009). Apart from the organism per se, stress affects the microbiota.

Experimental stress caused by separating infant macaques from their mothers was accompanied by a significant increase in *Shigella* and *Campylobacter* levels and a decrease in *Lactobacillus* content. The macaques that displayed the most manifest behavioral symptoms of stress were characterized by the lowest *Lactobacillus* content (Hawrelak & Myers, 2004; Gur et al., 2015). Maternal separation also alters the GI microbiota composition in rat pups, while increasing the permeability of the colon mucosa; bacteria more actively adhere to it and translocate into the spleen tissue (Gur et al., 2015; Parashar & Udayabanu, 2016).

Prolonged social stress affects the GI barrier. The corticotropin-releasing hormone (CRH) produced by the hypothalamus in response to stress is involved in GI barrier disruption (Lyte, 2011) which takes place while the glucocorticoid concentration increases. In this situation, bacterial translocation from the gut lumen induces inflammation with an increase in the concentration of proinflammatory cytokines that are produced by innate and adaptive immune cells (Ivashkin & Ivashkin, 2018).

Factors released under stress, such as adrenocorticotropic hormone (ACTH) and glucocorticoids, promote the adhesion of bacteria, including the pathogenic strain *E.coli* O157:H7, to the colon mucosa (Lyte, 2011). A similar effect is produced by norepinephrine that drastically stimulates the growth of potential pathogens (Lyte, 1993, 2010, 2011, 2014, 2016; Lyte & Ernst, 1992, 1993; Lyte & Freestone, 2009). The stress caused by immobilizing a mouse increases intestinal permeability by decreasing the amount of protein ZO-1 (*zonula occludens*) that is implicated in forming tight junctions between cells. The stress effect is prevented by the glucocorticoid antagonist mifepristone. Stress facilitates microbial translocation from the lumen into the intestinal wall and thereupon into the bloodstream. This results in GI microbiota alterations: a decrease in the concentration of beneficial microorganisms including bacteroids and an increase in that of opportunistic bacteria such as clostridia (Parashar & Udayabanu, 2016). Chronic social stress in mice defeated by conspecifics

causes a decrease in microbial content and impoverishment of GI microbiota species diversity (Bharwani et al., 2016). A decrease in lactobacillus number also occurred in the organism of mice that were placed in cages without litter, food, and water (Gur et al., 2015).

Stress brings about a shift in the *Firmicutes:Bacteroidetes* ratio; a similar shift occurs under pathological conditions associated with obesity (metabolic syndrome) (Bharwani et al., 2016). Accumulation of opportunistic pathogens and a decrease in *Bifidobacterium* and *Lactobacillus* content of the intestinal microbiota was detected in astronauts during a long-term space flight; this was attributable to the social stress that developed in the closed space (Valyshev & Gilmutdinova, 2006). The *Lactobacillus* content also decreased in the GI tract of students during exams (Gur et al., 2015).

Prolonged starvation results in serious stress that implicates intestinal motility dysregulation and, as a result, massive microbial colonization of the small intestine that normally contains low microbial cell concentrations (Sharkey& Savidge, 2014). Stress-induced inflammation in the GI tract is an additional important stress factor; this is consistent with an increase in the numbers of anaerobic clostridia, *E. coli*, and streptococci and a decrease in those of lactobacilli and bifidobacteria (Hemarajata & Versalovic, 2013).

Stress may both suppress and stimulate the operation of the immune system. There is evidence that stressed animals are more susceptible to microbial infections, e.g., those caused by *Yersinia enterocolitica*. Nonetheless, social conflict-induced stress in mice considerably increases the capacity of phagocytes to destroy bacteria (Lyte, 2011).

Data have been presented that pregnancy-associated stress in the maternal organism (whose degree can be estimated from the increase in salivary cortisol concentration) influences the child's GI microbiota during the first 110 days of life; the *Proteobacterium* cell number is increased and the concentration of useful bacteria is decreased (Zijlmans et al., 2015).

2.3. Bilateral Communication in the Microbiota: Host System in Terms of Network Organization

The GI microbiota produces a large number of low molecular weight substances that function as effectors, cofactors, and/or signal molecules. Such factors are implicated in regulating the rate and intensity of a wide variety of physiological, metabolic, and behavioral processes (Shenderov, 2011, 2013b; Shenderov et al., 2017); they considerably influence the concentrations of various metabolites in the blood of mammals (Wikoff et al., 2009; Dinan et al., 2015).

Regulation of intracellular processes, cell-cell communication, and the "dialogue" within the microbiota-organism system involve a large number of signal molecules (amino acids, biogenic amines, short-chain fatty acids, serpines, sirtuins, lectins, and many others). "The symbiotic microbiota is a source of a plethora of endogenous… signal molecules that promote health and, nevertheless, pose the risk of development of human health problems from birth to old age" (Shenderov et al., 2017). Bidirectional communication between the host organism and the microbiota is very intense; the metaphorical statement can be made that "they supervise us" and, at the same time, "we supervise them" (Lyte, 2013b).

Depending on their site of action, signal molecules can be subdivided into autocrine signals that exert an effect on the producer cells; paracrine signals that typically influence adjacent cells; and endocrine signals that are produced by endocrine glands and involved in regulating many cell metabolic processes within the whole organism. Microbially produced low molecular weight compounds are meaningful "words" that can be used to communicate messages between the microbiota and the host organism (Shenderov, 2011). In the following, some important signal molecules ("words") used both by the microbiota and the host will be discussed.

Recently, much evidence has been presented that microbial signal molecules strongly influence the physiology and the viability of various prokaryotic organisms (i.e., they perform the signal function within the

microbial community) and modulate the operation of the nervous and the immune system of the host organism.

Microorganisms produce a wide variety of hormones and especially neurochemicals; they also modify host-produced signal molecules and influence their production. For instance, microorganisms regulate the levels of the female hormones estrogens in the organism because they contain β-glucuronidase that activates conjugated (inactive) estrogens by deconjugating them (Markle et al., 2013). The microbiota can also regulate the liberation of hormones in the thyroid gland.

The peptide ghrelin causes a feeling of hunger and anxiety; it also facilitates information memorization. The concentration of this peptide in the blood serum varies depending on GI microbiota composition. This concentration decreases with an increase in the numbers of lactobacilli and bifidobacteria in the GI tract, which, therefore, mitigate the feeling of hunger. In contrast, the ghrelin concentration increases with an increase in the numbers of *Bacteroides* and *Prevotella*. The concentration of peptide YY is also under the influence of the GI microbiota; peptide YY stimulates the memory system and increases anxiety and the excitability of nervous cells (Parashar & Udayabanu, 2016).

Since communication in the microbiota-host system is bidirectional, host-produced chemical factors exert a strong specific influence on the growth-related characteristics and physiological, biochemical, genetic, and epigenetic features of microorganisms. Some otherwise silent genes of symbiotic bacteria are activated if they are introduced into the GI tract. In *Lactobacillus plantarum* WSFS 1, the expression of 72 genes is induced in the host gut. Bidirectional communication between the microbiota and the host organism is highlighted by the fact that also 400 host genes are activated in the presence of the bacterium *Lactobacillus* GG (Shenderov, 2011; Shenderov et al., 2017). Special emphasis should be placed on neurochemicals-mediated bidirectional communication: neurochemicals are produced both by the host and the microbiota, and the two main components of the coherent microbiota-host system rely on them (as well as other evolutionarily conserved signal molecules) while exchanging information.

As pointed out in Chapter one (1.3.2), microbial associations are mainly characterized by decentralized network organization that enables behavior coordination without a central leader (pacemaker). Even though we chiefly describe the interrelationship between the two partners, the microbiota and the host, as *bidirectional*, this interaction can rightfully be envisioned as *multidirectional* if account is taken of (i) the large number of different microbal agents that constitute the GI microbiota and (ii) the existence of several important systems within the host organism, including the nervous and the immune systems (considered in special sections below). The overall interactivity pattern is "multidirectional in the sense that each component of this extensive communication network has the ability to moderate and manipulate the function of the other systems involved" (Ganci et al., 2019).

Coordination in this highly complex supersystem is chiefly based on contact and distant cell-cell interaction. The network structure of the microbial consortium that inhabits the microecological niches of the human organism constantly interacts with this organism. Overall, the human (animal) organism functions under the combined influence of

- The *hierarchical structure* dominated by the central nervous system (CNS)[11] that controls the activities of various organs and tissues by generating impulses and chemical regulatory substances produced by the CNS per se (neurohormones) or by CNS-regulated endocrine glands (hormones);
- The complex system composed of *network structures* that includes: (1) human cells that form local or global (distributed within the organism)[12] chemical signal-releasing networks and (2) symbiotic microorganisms inhabiting various niches in the organism, especially the GI tract.

It should be re-emphasized that, under normal conditions, constructive interaction between the hierarchy and the networks improves the physical

[11] The CNS combines hierarchical and network structures in its internal organization.

[12] Such distributed and, nevertheless, coherent cell network structures are exemplified by the network of immune cells that release important regulatory substances.

and mental health of humans; it is an important prerequisite for their adequate social behavior. For instance, the GI microbiota forms a part of the"first defense line" of the human organism (Verbrugge et al., 2012) together with the other components of the intestinal "firewall", i.e., the mucosa, the epithelium, the lamina propra, etc.

Nonetheless, recent research in the field of the currently popular network science (see Oleskin, 2014) has also revealed the potential harmful properties of network structures. Under pathological conditions caused, e.g., by dysbiosis, the interaction within the microbiota-host system may become destructive. This potential destructive impact of the microbiota is linked to the following features of network structures in general and, more specifically, of the networked microbial consortium of the GI tract (Oleskin, 2014):

1. *Networks tend to ignore boundaries between social structures and actively communicate with "outsiders".* Signal substances (such as signal AI-2 mentioned above, 1.2.3) released by the normal microbiota of the mouth and the pharynx stimulate the growth and virulence of pathogens such as *Ps. aeruginosa*.
2. *Networks' excessive growth negatively influences structures interacting with them.* Excessive growth of even useful microorganisms may be harmful to the organism (although their insufficient growth may also cause problems because of the "vacancies" that can be used by unwanted intruders). Such excessive growth makes the host organism spend energy, substrate, and immune system resources on low-intensity chronic inflammation caused by unbalanced microbial growth. Besides, excessive growth of some human gut inhabitants including the widespread intestinal symbiont *E. coli* is likely to increase the activity of the dopamine-using (dopaminergic) systems of the brain because they release the precursor *L*-3,4-dihydroxypenylalanine (DOPA; Shishov et al., 2009) that converts into catecholamine neurotransmitters in the bloodstream and the brain. Excessive activity of the dopamine-using system manifests itself in

hypersociability and euphoria and may promote the development of schizophrenia (Dubynin et al., 2010).

3. *Networks forming part of a network-hierarchy tandem do not necessarily follow the rhythm set by the hierarchical partner in their behavior, threatening to disrupt this rhythm*. The symbiotic microbiota of the human organism normally functions in unison with the biorhythms of the host organism, and any discrepancy between the rhythms/tempos of the two structures in the tandem poses the threat of serious problems in terms of physical and mental health. Since networks generally tend to ignore the rhythm of their hierarchical partners, the human organism-microbiota tandem is extremely fragile and the concordance between the rhythms of its components can be easily disrupted by seemingly insignificant external factors such as a wrong diet, stress, or usually non-problematic low levels of toxic substances in the environment.
4. *Networks attempt to perform regulatory functions instead of the hierarchy*. The dominant role in the host organism is normally played by its own main controlling organ, the brain. In pathological situations, a "netocracy" (Bard & Söderqvist, 2002) can be established, and the activities of the brain are heavily influenced by microbial networks with their neurochemicals. This may account for the fact that a number of nervous system problems and psychological disorders are associated with disruptions in the functioning of the GI microbiota (see below). Even in healthy humans (or animals), the microbiota attempts to assume control over the functioning and behavior of the host organism. As mentioned above, bacteria presumably induce the host to prefer food that contains nutrients required by them. Pregnancy-associated changes in eating habits may be directly caused by alterations in the microbiota composition that occur due to hormonal changes.
5. *Networks are more complex in organizational terms than hierarchies.* Extreme organizational complexity is characteristic of the decentralized structures that are formed by human symbiotic microorganisms. This causes serious problems in terms of the

prevention or therapy of infections. Most drugs produce multiple effects on the microbiota, which, to reiterate, includes not only symbionts but also (potential) pathogens; these two subtypes of microbiota constantly communicate and actually form a part of one complex, coherent interorganismic network structure. An extremely difficult task, therefore, is to make the positive effects of a drug (such as an antibiotic) outweigh its negative effects. The antibiotic may not kill the pathogen but, instead, eliminate useful microbes that could otherwise restrict the pathogen's reproduction and migration inside the organism.

2.4. MICROBIOTA AND THE HOST'S NERVOUS SYSTEM

Within the framework of the multidirectional microbiota-host signaling system, microbial metabolites can modify the functioning of the nervous system via metabolic, epigenetic, and neuroendocirne mechanisms. We depend on myriads of essential neurochemical factors produced by microorganisms (Dinan et al., 2015). For instance, the serotonin-dependent (serotonergic) brain system that is responsible for many aspects of emotional behavior does not develop to the mature state without the microbiota (Clarke et al., 2013).

The effects of microbial neurochemicals on nervous cells can be both direct and indirect. Chapter three contains literature data and the authors' own findings that demonstrate the capacity of microbial, endogenous, and nutritional SCFAs, biogenic amines, amino acids, peptides, and other neuroactive substances to interact with various cell receptors of prokaryotic and eukaryotic organisms. This results in modifying the microecological, immune, and nervous system of the host organism. In particular, biogenic amines including catecholamines, serotonin, hisamine, acetylcholine, and, presumably, agmatine and some other substances, fulfil a wide variety of hormonal and neurochemical functions in the human organism (reviewed, Oleskin et al., 2016, 2017a, b; Oleskin & Shenderov, 2019). "The microbiota and the brain communicate with each other via various routes including the

immune system, tryptophan metabolism, the vagus nerve, and the enteric nervous system" (Cryan et al., 2019, p.1877); this communication will be considered in more detail in this subsection.

It is evident that the normal functioning of the brain depends, apart from the organism per se, on the symbiotic microbiota. Since bacteria are capable of both recognizing and synthesizing neuromediators and hormones, we should agree with Wenner (2008) who stressed that "we have some evidence now that shows that if you mess around with the gut microbes, you mess around with brain chemistry in major ways". The important role of the interaction between the gut mictobiota and the mammalian brain has been demonstrated in a large number of recent works (Asano et al., 2012; Norris et al., 2013; Lyte, 2013a, b; Stilling et al., 2014; Cryan et al., 2019). The hypothesis has been suggested that humans would not develop advanced cognitive capacities in the absence of the microbiota (Montiel-Castro et al., 2013).

2.4.1. Studies with Germ-Free Animals

Comparative studies with *germ-free (GF)* and, in contrast, with microbiota-containing animals provided extensive data on the role of the GI microbiota in terms of metabolic, trophic, and protective mechanisms that directly impact the nervous system and especially the brain and, therefore, influence host behavior (Verbrugge et al., 2012; Shenderov, 2014; O'Mahony et al., 2015). For instance, studies with GF mice revealed that the density of their neural networks is decreased and they contain comparatively few nervous cells per one ganglion (Ivashkin & Ivashkin, 2018).

An increased *anxiety* level was detected in GF rats that spend less time than conventional rats sniffing unknown conspecifics; they less frequently visit the central area of the experimental chamber, preferring its safer corner areas (Crumeyrolle-Arias et al., 2014; Foster et al., 2016).

The aforementioned data are not entirely consistent with recent studies with mice. The results of an open field (OF) test revealed that the exploratory behavior of GF mice is stimulated and their anxiety level is decreased. GF

mice are less depressive than their microbiota-containing conspecifics. Anxiety- and depression-like behavior was only characteristic of "former GF" mice after transplanting the microbiota of humans with severe depression (but not that of humans with the normal psychological status) to them (Foster et al., 2016; Luo et al., 2018).

GF mice display an increased behavioral response to stress caused by immobilization (Dinan et al., 2015). GF mice are socially inactive, they avoid interacting with familiar or new conspecifics. These data highlight the role of the GI microbiota in the development of adequate social behavior. Colonizing GF mice at an early age with GI microorganisms improves their social behavior (Desbonnet et al., 2014).

GF mice have disrupted cognitive processes, including the memorization of new objects (working memory dysfunction, see Dinan et al., 2015). GF mice are likely to engage in repetitive stereotyped behavior such as self-grooming (in an analogy to the stereotyped behavior of people with mental problems including autistic spectrum disorders). Gut colonization with the normal microbiota normalizes some of the behaviors of GF mice but fails to ameliorate a cognitive symptome: they do not recognize familiar conspecifics (Sudo et al., 2004; Desbonnet et al., 2014; Sampson & Mazmanian, 2015; Parashar & Udayabanu, 2016).

Many behavioral peculiarities of such "former GF" mice resemble those of the microbiota donors. GF mice belonging to the BALB/c line characterized by low exploratory activity and shyness start displaying intense exploratory behavior after colonization with the GI microbiota of NIH Swiss mice that are distinguished by high exploratory activitiy. GF mice belonging to the NIH Swiss line display BALB/c mice-specific behavior if colonized by their microbiota (Bercik et al., 2012; Foster et al., 2016; Parashar & Udayabanu, 2016).

In GF mice, the blood-brain barrier (BBB) is weakened because the expression of the genes responsible for tight junctions between barrier-forming glial cells is decreased. The barrier can be restored by GI colonization with useful bacteria such as *Clostridium tyrobutyricum* or *Bacteroides thetaiotaomicrobium* or by administering butyrate that is produced by these bacteria.

The brain of GF mice is characterized by decreased levels of the brain-derived neurotrophic factor (BDNF) that stimulates the growth and differentiation of neurons and the formation of synapses between them (Sudo et al., 2004). GF mice exhibit abnormally high levels of adrenocorticotropichormone (ACTH) and corticosterone in the blood[13] and low serum concentrations of dopamine, γ-aminobutyric acid (GABA), and serotonin (Sudo et al., 2004). The activity of the genes involved in the synthesis of gluccocorticoid receptors (*Sec22a5, Aqp1, Stat5a, Ampd3*, and others) is increased in the hippocampus of their brain (Luo et al., 2018). The level of the neurotransmitter histamine is decreased in the hypothalamus of GF mice (van de Wouw et al., 2017).

In the striatum of their brain, the rate of metabolism of neurotransmitters such as norepinephrine, dopamine, and serotonin is increased, which is correlated with increased locomotive activity (Sudo et al., 2004; Sampson & Mazmanian, 2015). The hippocampal levels of serotonin and its metabolite 5-hydroxyindoleacetic acid (5-HIAA) are elevated. These levels are normalized after introducing the normal microbiota into the organism of a pup but not of an adult mouse (Parashar & Udayabanu, 2016).

The blood corticosterone level is increased in GF rats; their hippocampus contains decreased amounts of mRNA that encodes the glucocorticoid receptor (Crumeyrolle-Arias et al., 2014; Luo et al., 2018).

The normalization of cortisol and serotonin levels in GF mice is attained by colonizing the GI tract with the beneficial probiotic bacteria *Bifidobacterium infantis* (Sudo et al., 2004). Importantly, SCFAs of microbial origin also normalize the serum serotonin level by inducing serotonin release by the enterochromaffine cells of the intestinal epithelium. Therefore, such SCFAs function as metabiotics (see below).

The microbiota also exerts a more direct influence on the concentrations of serotonin and other neurochemicals: it produces them or their BBB-crossing precursors, e.g., the serotonin precursor tryptophan.

[13]However, the data of a recent study indicate that the levels of ACTH and cortisol are only increased in the mouse blood serum during acute stress, and their baseline levels are not significantly different from those in GI microbiota-colonized mice (Luo et al., 2018).

A lack of microbiota produces different effects on male and female mice. A decrease in brain BDNF level is characteristic of GF males but not females. Unlike GF females, GF males release an abnormally large amount of cortisol into the bloodstream in response to stress (Gur et al., 2015).

Chronic administration of an antibiotic combination to animals, starting from an early age, results in significantly impoverishing the microbiota's taxonomic diversity and decreasing its total content in the GI tract; antibiotic-treated animals display neurochemical and behavioral features that are comparable to those of GF animals (Desbonnet et al., 2015).

2.4.2. Role of the Enteric Nervous System (ENS)

The intestinal microbiota directly interacts with the ENS, a semi-autonomous part of the nervous system. The ENS contains at least about 0.5 million neurons, i.e., it includes more neurons than all peripheral ganglia taken together (Rao & Gershon, 2016). The ENS also contains auxiliary cells such as astroglia (enteroglia) that form a diffusion barrier between intestinal capillaries and the ENS ganglia. Glial cells perform a protective function vis-à-vis ENS neurons and provide them with nutrients (Sharkey & Savidge, 2014). The ENS is structurally similar to the central nervous system (CNS), and it uses virtually all types of neurochemicals that function in the CNS (Rao & Gershon, 2016). Unlike the other parts of the peripheral nervous system, ENS can operate without CNS control, even despite a chronic vegetative state of the brain in which most of its parts are inactive (Liang et al., 2018). ENS regulates the secretory activity of the gut, its motility, and the activity of the gut immune system; it helps maintain the mucosa in the functional state. An additional important function of the ENS is the regulation of permeability of the gut wall barrier for chemical factors and microbial cells.

To reiterate, the microbiota directly interacts with the ENS and regulates its activity by means of microbially produced neuroactive compounds. The impact of the microbiota on the ENS does not only boil down to regulating neuronal activity; it is also implicated in guiding the development of the

intestinal population of glial cells and maintaining their homostasis (Ivashkin & Ivashkin, 2018).

The important role of the microbiota for the normal functioning of the ENS is highlighted by the fact that the ENS of GF mice is characterized by a decreased capacity to respond to external stimuli; this capacity is restored in a mouse if the intestine is colonized with a probiotic strain of *Lactobacillus reuteri* (Parashar & Udayabanu, 2016).

The GI tract is innervated by the sympathetic and the parasympathetic nervous systems that directly communicate with the GI microbiota (Sharkey & Savidge, 2014).

2.4.3. Microbiota-Gut-Brain Axis: Multidirectional Communication. The Microbiota Manipulates Host Behavior

Taking into account recent data on the role of the GI microbiota in terms of human health, it has been suggested that the widely used term *gut-brain axis* should be replaced with the more inclusive term *microbiota-gut-brain axis* (Shenderov, 2008; Oleskin & Shenderov, 2013; Lyte, 2010, 2011, 2013a, b; 2016; Stilling et al., 2014). The microbiota-gut-brain axis includes the intestinal microbiota, the ENS, the parasympathetic and sympathetic nervous system, and the CNS which interact with the endocrine and the immune system via chemical communication involving cytokines, neuropeptides, and numerous other signal molecules.

The microbiota directly influences the activities of the nervous system and especially the brain, including microglia, i.e., brain immune cells, as well as BBB permeability, nervous cell maturation (neurogenesis), and neurochemicals' production and release.

Since microbiota-host interaction is multidirectional, of paramount importance is also the impact of the brain and the whole nervous system on the GI tract and microbiota. This is particularly obvious if the brain is injured. For instance, stroke results in changing the microbiota composition, e.g., in the cecum where the strongest effect is produced on the bacteria of the families *Peptococcaceae* and *Prevotellaceae* (Westfall et al., 2017).

Norris et al. (2013) assume that the dietary preferences of the host are partly dependent on the requirements of the GI microbiota. It is pertinent that ingestion of the protein components of *E. coli* cells increases the secretion of appetite-inhibiting peptides such as glucagon-like peptide 1 and pancreatic peptide YY (Mazzoli & Pessione, 2016).

There is much evidence that the microbiota strongly influences the host's social behavior (Libersart et al., 2009; Adamo & Webster, 2013), which also concerns acts of altruism (Lewin-Epstein et al., 2017). It was suggested that microorganisms manipulate the host to increase their viability and promote their spread in the host population (Rohrscheib & Brownlie, 2013; Lewin-Epstein et al., 2017). Therefore, social behavior is more likely to occur whenever it facilitates microbial transfer from host to host. Such transfer takes place during food exchange (e.g., blood exchange between vampire bats), mouth-to-mouth feeding of children by their parents or siblings in primitive societies, and cohabitation (Lewin-Epstein et al., 2017).

Pathogenic microorganisms can modify the host organism's response to infection to increase their own survival and migration chances. Intestinal infection is associated with release of interleukin-1β in the intestine that influences the hypothalamus via *nervus vagus*, resulting in appetite decrease (anorexia). However, *Salmonella enterica* serovar. *Typhimurium* produces protein SlrP that blocks interleukin-1b release in the gut of an infected mouse and prevents the loss of appetite. Therefore, the mouse is expected to live for a longer time than a mouse infected by the SlrP-lacking *Salm. enterica* serovar. *Typhimurium* mutant. A mutant-infected mouse suffers from starvation, which promotes bacterial translocation to the liver, spleen, and mesenterial lymph nodes. The wild-type pathogen prolongs the lifespan of the host by preventing the appetite loss and, therefore, facilitates the release of bacterial cells with the feces and accelerates their spread in the host population (Rao et al., 2017).

As for the symbiotic microbiota, its manipulative influence on the host may be aimed at securing the microbiota's survival and well-being by promoting the host's health. Presumably, host evolution tends to make the host organism more dependent on the microbiota and, in addition, to increase

its capacity to monitor the microbiota's state and regulate it (Johnson & Foster, 2018).

Importantly, the effects of microbial chemical components such as the polysaccharides of the bacterial cell capsule and SCFAs on intestinal motility and the operation of the host nervous system are supplemented by their impact on the immune system (Rooks & Garrett, 2016; Johnson & Foster, 2018: see section 2.5 below), promoting the development of the host's tolerance to them. This microbial strategy results in preventing or attenuating the inflammatory response of the host immune system. Moreover, the beneficial (symbiotic) microbiota mitigates pathogen-induced inflammation. *Helicobacter hepaticus*-induced severe colitis in mice does not develop if a probiotic *Bacteroides fragilis* strain is administered together with the pathogenic bacterium (Ayres, 2016).

Actually, the boundary between the pathogenic, opportunistic, and symbiotic microbiota is somewhat arbitrary because all the kinds of microbiota share the goal of maximizing survival and propagation chances. Depending on the situation, their strategy may be beneficial or detrimental for the host. The symbiotic bacterium *Bacteroides thetaiotaomicron* produces no harmful effect on wild-type mice but causes colon inflammation in genetically modified mice that do not produce normal concentrations of anti-inflammatory factors (IL-10 and TGF-β; Ayres, 2016).

The objection raised to the above host behavior manipulation hypothesis is that such manipulation is costly and, therefore, manipulators are likely to be outcompeted by other microbial cells that only spend energy on their own growth and development. It is more probable that it is the host that is interested in, and, therefore, creates favourable conditions for, the survival and spread of symbiotic microorganisms (Johnson & Foster, 2018).

In line with this suggestion, the host develops tolerance to its normal (commensal) microbiota, preventing immune responses to it. Within the GI tract, intestinal epithelial cells tend to downregulate the expression of Toll-like receptors (TLRs) that otherwise recognize typical molecular patterns on microbial cells and elicit inflammatory reactions to commensal bacteria (Green & Brown, 2016).

The microbiota-host relationship can be envisaged as cooperation that was defined above as interaction between two or more individuals or groups for the purpose of solving a problem or carrying out a task (see 1.1.5). The host provides a niche and nutrients for the microbiota, whereas the microbiota positively influences the development and functioning of the host organism (Shenderov, 1998; Ulvestad, 2009; Liangetal., 2018).

The symbiotic microbiota can modify emotions associated with choosing and consuming food (Lyte, 2013b; van de Wouw et al., 2017) by regulating the operation of the dopamine system of the brain and affecting the hunger/satiety balance that involves neuropeptides (ghrelin, glucagon-like peptide, insulin, leptin, and peptide YY); their synthesis is under the influence of microbially produced SCFAs.

Normally, the symbiotic micriobiota supplies the host organism with neurologically important substances such as vitamins B, biotin, ubiquinone, plasmalogens, and others. One of the reasons why aging poses the risk of developing dementia and Alzheimer's disease is that the concentration of plasmalogens [14] that protect unsaturated fatty acids from oxidation and regulate their metabolism tends to decrease in the aging organism. Many representatives of the genera *Eubacterium*, *Bifidobacterium*, *Propionibacterium*, and *Clostridium* normally contribute to plasmalogen production (Shenderov et al., 2016).

The lipopolysaccharides (LPSs) of gram-negative bacteria influence human mood and cognition, either via the immune system-produced interleukins and other brain-affecting factors or more directly, by binding to Toll-like receptors (TLRs) on brain glial cells (McCusker & Kelly, 2013). Systemic administration of LPSs in model animals results in inflammation in the nervous system and neurodegenerative processes; such model animals are used in research on neurodegenerative diseases (Liang et al., 2018).

The gut microbiota influences the anxiety level in mice and rats; it also affects sexual partner choice, sociability (attachment to a social group), and olfactory signal recognition (Norris et al., 2013). The opportunistic bacteria *Citrobacter rodentium* and *Campylobacter jejunii* activate the solitary tract

[14] Plasmalogens are phosphiolipids that contain alcohol residues connected to glycerol via ether bonds.

nucleus and the lateral parabrachial nucleus in the brainstem via afferent pathways in the vagal nerve. As a result, anxiety-like behavior is stimulated and the cognitive behavior of mice is suppressed; the behavior of the mice is normalized by subsequent introduction of a probiotic bacteria-containing cocktail into their GI tract (Bercik et al., 2012; Cryan & Dinan, 2012; Parashar & Udayabany, 2016). The effects of the pathogens are attributable to the activation of *c-fos* gene expression in the brain that takes place in the presence of even low concentrations of their cells (Lyte, 2014).

The gastric and duodenal ulcer pathogen *Helicobacter pylori* changes the feeding behavior of model animals: they eat more frequently, each time ingesting low amounts of food (presumably, eating becomes painful in this situation). In the neurons of the archuate nucleus of the brain, the synthesis of propiomelanocortine, the precursor of several peptide hormones, is inhibited, whereas the formation of the proinflammatory cytokine TNF-α in the hypothalamus is stimulated. The BBB becomes more permeable (Bercik et al., 2012).

Feeding behavior depends on signal mechanisms that enable the perception of the five different tastes (sweet, salty, sour, bitter, and umami). Modification of these signal mechanisms by the microbiota may cause the host to preferentially consume specific kinds of food, This may form a part of the manipulative strategy of the microbiota. GF mice have an increased number of sweet taste receptors in the gut and a decreased number of fat food taste receptors in the mouth, which obviously affects the dietary preferences of these mice as contrasted with microbiota-colonized mice (van de Wouw et al., 2017).

Microbiota modifies the physiological and behavioral activities of the host that are involved in feeding behavior, including the operation of the olfactory system, food seeking and food intake, food-related obssesive and compulsive behavior (including an irresistible motivation to obey an eating ritual or to obtain the desired food), and even social aspects of food consumption. For instance, close contact between people during a meal may promote the spread of microorganisms in the human population. The impact of the microbiota on olfaction forms a part of its effect on social behavior. In fact, interaction among people is significantly stimulated by odorous

substances (pheromones); their production depends on the microbiota of the organism, with the skin microbiota playing a particularly important role. The microbiota impact is especially important with respect to mate choice (McFall-Ngai et al., 2013).

Inflammation processes in the gut, including inflammatory bowel disease (IBD) and Crohn's disease that are associated with changes in GI microbiota composition, result in anxiety and depression (Lyte, 2013a, b). Under the influence of dysbiosis and the accumulation of potential pathogens in the GI tract, an immune response develops. Cytokines and other proinflammatory mediators are released, affecting both the central and the peripheral nervous system, especially the ENS.

In contrast to the large intestine, the small intestine is normally characterized by a low concentration of microbial cells, and the development of an abundant microbiota in it is a pathological (dysbiotic) phenomenon. Microbiota-produced D-lactate impacts the brain, which results in behavioral and cognitive disorders (Parashar & Udayabanu, 2016).

The following are additional examples of the manipulative influence of the microbiota (inclusive of the viruses) on host behavior: (i) aggressive behavior of rabies virus-infected mammals: (ii) attractiveness of the cat urine odor for *Toxoplasma gondii*-infected rats and mice; (iii) infertility of IIV-6/CrIV virus-infected crickets whose attempts to mate other crickets help the virus spread in the cricket population (Sampson & Mazmanian, 2015); and (iv) abnormally intense sexual behavior and increased responses to pheromones in *Wolbachia pipientis*-colonized fruitflies (Rohrscheib & Brownlie, 2013).

Communication within the microbiota-gut-brain axis can proceed in the direction from the brain via nervous pathways and chemical factors (including neurochemicals in the capacity of neurohormones) to the GI tract and further to the microbiota. Importantly, the human microbiota responds not only to various exogenous factors but also to changes in the physical and neuropsychological state of the host (Stilling et al., 2014). For example, anger and fear cause a transient increase in the number of *Bacteroides theaiotaomicron* cells within the bacterial population of human feces (Hawrelak & Myers, 2004).

If the brain makes the decision to prefer specific kinds of food, this inevitably influences the GI microbiota. Studies were conducted with mice in which they were regularly eating ground beef. The mice were characterized by an increased taxonomic diversity of the microbiota. Their short-term and long-term memory improved, the food search tempo slowed down, and anxious behavior was mitigated (Bercik et al., 2012).

2.4.4. Communication Channels Connecting the GI Microbiota and the Central Nervous System

"Microbiota-gut-brain axis signaling can occur via several pathways, including via the immune system, recruitment of host neurochemical signaling, direct enteric nervous system routes and the vagus nerve, and the production of bacterial metabolites" (Long-Smith et al., 2020, p.17.1). The following factors are of paramount importance:

1. *Microbiota-produced neuroactive compounds* that can cross the barrier between the gut wall and the bloodstream or the lymphatic system as well as the BBB and directly interact with the brain. Such microbial products are exemplified by *L*-3,4-dihydroxypehylalanine (DOPA) and γ-aminobutyric acid (GABA), and their brain effects will be discussed in Chapter three. Microorganisms also produce neurochemicals that do not cross the gut-blood barrier[15], e.g., serotonin, dopamine, and norepinephrine (Oleskin et al., 2010, 2016, 2017a, b; Oleskin & Shenderov, 2016; Shenderov et al., 2017; Lyte, 2016). Such neuroactive compounds exert a local effect, affecting the ENS that can systemically influence the whole organism;
2. *GI tract-innervating nervus vagus* with afferent and efferent pathways (Dinan et al., 2015; Sampson & Mazmanian, 2015). This

[15]Such neuroactive substances may cross the gut-blood barrier and the BBB under stress that impairs these barriers.

nerve forms a part of the organism's major regulatory systems that are implicated in the parasympathetic regulation of the functions of the heart, the bronchi and the GI tract. The density of sensory terminals of *n. vagus* is very high in all organs and tissues, and they supply the brain with spatially structured information concerning their activities (Ivashkin & Ivashkin, 2018). The microbiota affects *n. vagus* activity. Importantly, this nerve connects the ENS and the brain and sends, via afferent pathways, messages to the brain concerning the GI homeostatic state, including the feelings of fullness, satiety, and sickness. It is partly due to *n. vagus* that the microbiota influences behavior and mood. Opportunistic and potentially pathogenic bacteria such as *Citrobacter rodentium* and *Campylobacter jejunii* activate the transfer of stress-induced impulses along *n. vagus*. If the nerve is severed, this prevents many microbial effects, including *Lactobacillus rhamnosus* JB-1-induced activation of synthesis of the $GABA_B$ receptor to GABA in the cingulated gyrus of the mouse brain (Parashar & Udayabanu, 2016);

3. *Immune system* that mediates some of the microbiota-produced effects on the CNS (see 2.5 for details).
4. *Hypothalamus-pitiutary-adrenal system (HPA system)* that is directly involved in the GI microbiota impact on the human organism and its CNS. Modification of the HPA system by harmful microbial compounds predisposes human individuals to depression, anxiety, the bipolar disorder, and emotional burnout and chronic fatigue syndromes. The HPA system is implicated in the effects of microbiota-disrupting factors (diet alteration, antibiotic, psychosocial stress, etc.) on an infant's nervous system. Subsequently, this results in psychological disorders in conformity with the Barker hypothesis (Barker & Osmond, 1986). Restoring the gut microbiota, especially by means of probiotics, decreases the risk of the development of mental problems. GF mice are distinguished by an abnormally intense response of the HPA system to stress, which is alleviated by administering the probiotic *Bifidobacterium infantis* to them. The HPA-dependent stress response is additionally

intensified by colonizing the GI tract of GF mice with pathogenic *E. coli* strains (Liang et al., 2018).

There is evidence that, apart from the hypothalamus (dubbed the brain-visceral organs "interface"), other brain parts including the prefrontal areas of the brain cortex and the amygdala, are affected by microorganisms. The amygdala is directly involved in visceral pain perception, social behavior, and emotional responses. During the course of an individual life cycle, childhood and old age are the critical periods in which the microbiota drastically changes and dysbiosis may develop. The same periods are characterized by the maximum risk of developing amygdala problems that manifest themselves in irritated bowel syndrome (IBS) and mental disorders (schizophrenia, autism, and others; Cowan et al., 2017).

The oral microbiota affects the brain and behavior via the facial and the trigeminal nerve. Under pathological conditions, the microbiota contributes to the progression of neurodegenerative diseases (Liang et al., 2018).

2.4.5. Mental Disorders and the GI Micriobiota

Recently, much evidence has been presented in the literature that nervous and mental diseases are linked to GI microbiota disruption. Dysbiosis, apart from manifest GI symptoms, is often accompanied by brain problems. The list of nervous and mental disorders associated with GI dysbiosis includes obsessive compulsive disorder, posttraumatic stress, the state of panic, bipolar disorder (manic depressive psychosis), schizophrenia, autistic spectrum disorders (ASDs, including autism proper and Asperger syndrome), attention deficit hyperactivity disorder (ADHD), Alzheimer's and Parkinson's disease, drug addiction, multiple sclerosis, hepatic encephalopathy, and migraine (Shenderov et al., 2016; Liang et al., 2018).

Of significant importance are recent studies on the microbiota role in the development of *depression,* a dangerous mental disorder that drastically decreases work performance and may result in suicide attempts. The World Health Organization (2012) considered the promotion of depression-

preventing measures as a priority goal. GF rats colonized with the fecal microbiota of depressive humans acquire depression-like behavioral and physiological features: they ignore agreeable stimuli, display anxious behavior, and exhibit alterations in the metabolism of tryphophan, a serotonin precursor (Kelly et al., 2016). Transplantation of the microbiota of humans with irritable bowel syndrome with diarrhea (IBS-D) to GF mice caused mental symptoms such as anxiety-like behavior. IBS-D patients' microbiota-colonized mice (but not those with the microbiota of healthy humans) were also characterized by accelerated GI tract transit, gut barrier disruption, and immune system activation (de Palma et al., 2017).

Children with autism, a mental disorder that inhibits interaction with the environment, disrupts social communication, decreases cognitive capacities, and stimulates repetitive stereotyped behavior, are characterized by increased numbers of bacteria belonging to the genera *Clostridium, Desufovibrio,* and *Bacteroidetes* in their stools. These bacteria produce large amounts of SCFAs, especially propionic acid that is involved in the development of ASDs (MacFabe, 2012). There is evidence that species *Anaerofustis stercohominis, Anaerotruncus colihominis, Clostridium bolteae,* and *Cetobacterium someria* are typical of the GI tract of autistic people (Valyshev & Gilmutdinova, 2006). Apart from intestinal dysbiosis and cognitive symptoms, autistic individuals often suffer from constipation, esophagitis, gut barrier disruption, carbohydrate digestion problems, and abnormal proliferation of the lymphoid tissue of the small intestine (MacFabe, 2012; Sampson & Mazmanyan, 2015). Children diagnosed with ASDs suffer from GI problems 3-4 times more often than those without them (Rao & Gershon, 2016). Long-term hospitalization of patients with GI symptoms may result in the emergence or aggravation of autistic symptoms (MacFabe, 2012).

The data on the role of the GI microbiota in the development of autism seem to account for the fact that reasonable[16] administration of antibiotics to autistic subjects causes at least a short-term mental state improvement

[16] Obviously, if an antibiotic suppresses not the autism-promoting but the useful probiotic microbiota, this should stimulate autistic symptoms or even cause them; clinical data support this suggestion (MacFabe, 2012).

(Bercik et al., 2012). The current global increase in the frequency of mental disorders is attributable to the changed GI microbiota composition. As mentioned above, this seems to be due to diet and lifestyle alterations, a widespread use of disinfectants, and the stress factors that affect both the host and the microbiota. It is meaningful that there are more individuals with ASDs in the Somali diaspora in developed countries than among the Somalis that stay in their homeland in Africa (MacFabe, 2012). Of note is also the fact that administering 4-ethylphenylsulfate, a chemical that is released by the GI microbiota and accumulates in the blood serum of mice with autism-like disorder, to healthy mice results in the development of autism-like symptoms in them (Sampson & Mazmanian, 2015).

Depression, schizophrenia, and a large number of other mental disorders are frequently accompanied by GI symptoms, in support of the suggestion that the GI microbiota impacts the brain in health and disease. Since schizophrenia is closely linked to microbiota-gut-brain axis disruption, improving the functioning of the axis by normalizing the microbiota is expected to ameliorate the mental state of schizophrenics (Liang et al., 2018).

It has recently been suggested that microbiota alterations in aging individuals (see subsection 2.1.8) may contribute to the progression of inflammatory processes in the brain that cause dementia and Alzheimer's disease as well as diabetes that negatively influences the brain (Dinan et al., 2015).

Many neurodegenerative disorders, including Alzheimer's and Parkinson's disease, are associated with intestinal problems and dysbiosis. Degenerative processes are primarily located in the ENS and subsequently spread to the brain; improving the state of the microbiota-gut-brain axis by normalizing the microbiota composition produces a therapeutic effect in this situation. In the human organism, the dysbiotic microbiota or its metabolites induce the formation of amyloid, a protein that causes degenerative processes in the brain tissue. Specific target-oriented probiotics can prevent or inhibit these processes (Shenderov et al., 2016; Westfall et al., 2017; Giau et al., 2018; Liang et al., 2018; Sobol, 2018).

Alzheimer's disease is characterized by the presence of the antigens of *Treponema socranskii* and *T. pectinovorum* cells in the brain cortex and subcortical structures such as the pons and the hippocampus (Hawrelak & Myers, 2004). Hepatic encephalopathy, a condition in which a dysfunctional liver (e.g., during liver cirrhosis) causes brain damage, is accompanied by an unbalanced microbiota with an increased *Veillonella* content (Parashar & Udayabanu, 2016). According to Lyte, intestinal bacteria produce ligands to benzadipine receptors in individuals with liver dysfunction. Upon translocating to the brain, these microbial products activate the GABA-dependent system, which promotes the development of brain damage in patients with hepatic encephelopathy (Lyte, 2013b).

As for individuals diagnosed with Parkinson's disease, they are characterized by an abnormally high level of the pathological bacterial metabolite indican that is indicative of serious GI dysbiosis. The sigmoid colon of individuals with Parkinson's disease exhibits a significant decrease in the numbers of butyrate-producing bacteria (*Roseburia* and *Faecalibacterium* spp.) with an anti-inflammatory effect and an increase in the numbers of *Ralstonia* that promote inflammation (Westfall et al., 2017).

Alzheimer's and Parkinson's disease as well as lateral amyotrophic sclerosis are associated with the degereneration of the frontal and temporal lobes of the brain and corresponding neurological and mental symptoms. The development of these diseases involves amyloid production; amyloids cause neuronal protein misfolding and promote nervous tissue inflammation and oxidative stress. There is evidence that various microorganisms (*B. subtilis, E. coli, Klebsiella pneumoniae, Mycobac-terium tuberculosis, Salmonella enterica, Staph. aureus,* and *Streptococcus mutans)* are implicated in producing amyloids (Friedland, 2015; Giau et al., 2018; Sobol, 2018).

The similarity of the CNS and the ENS is one of the reasons why *comobidities*, i.e., diseases involving both parts of the nervous system, can develop. Factors that cause CNS function disruption may also result in ENS dysfunction and, therefore, GI dysbiosis. GI symptoms frequently occur during nervous and mental diseases, including ASDs, lateral amyotrophic

sclerosis, spongiform encephalopathy, and Parkinson's and Alzheimer's disease; these GI problems are associated with ENS dysfunction.

To reiterate, many primarily somatic diseases that affect GI microbiota are frequently accompanied by neurological and mental symptoms. The neural pathways that connect the ENS and the CNS can promote the expansion of pathological processes, including those caused by microorganisms (Rao & Gershon, 2016). Unfortunately, despite the data on comorbidities affecting both the mental state and the GI tract and microbiota of a patient, "the functioning of a client's gut is seldom considered <by a psychiatrist- O.A.>…; thus, important diagnostic and treatment options may be missed" (Ganci et al., 2019).

To sum up, serious microbiota-gut-brain axis disruption pose the risk of developing neurodegenerative diseases. This condition is often due to the use of antibiotics, antihistamine drugs[17], and antidepressants as well as to alcohol consumption and drug addiction in the present-day world (Shenderov et al., 2016).

The microbiota significantly influences the host brain and behavior. It can be considered a major evolutionary factor that contributes to the development of social behavior; the microbiota impacts the neural and psychological mechanisms of affiliation (behavior aimed at approaching and remaining near conspecifics, see 1.1.4 above), responses to the social behavior of others, mate choice, and sexual behavior (Dinan et al., 2015).

2.5. Microbiota and the Immune System

As mentioned above, the microbiota's influence on the operation of the nervous system is also mediated by the immune system. The microbiota produces effects on both the innate and the adaptive immune system, including the cellular (T lymphocyte-dependent) and the humoral (B

[17]Of relevance is the fact that histamine (whose function is suppressed by antihistamine drugs) stimulates the growth of some human microsymbionts includuing *E. coli* (see 3.3.1).

lymphocyte- and immunoglobulin-dependent) mechanisms of immune responses.

2.5.1. Gut-Associated Lymphatic Tissue (GALT) and the Enteroendocrine System

The GI microbiota directly interacts with intestinal immune cells located in the GALT that contains up to 70–80% of all active immune cells in the organism (Mazzoli & Pessione, 2016; Liang et al., 2018). Intestinal immune cell responses to the microbiota also involve the enteroendocrine cells (EECs) of the mucosa. They account for less than 1% of all gut epithelial cells but, nonetheless, constitute the largest endocrine organ of the organism. Over 20 types of EECs have been identified; they produce different regulatory peptides (glucagon-like peptides, GLP, pancreatic peptide PYY, cholecystokinin, secretin, etc.). EECs contain G protein-coupled receptors as well as receptors binding microbial metabolites such as glutamate, other amino acids, long-chain fatty acids, and SCFAs (Mazzoli & Pessione, 2016).

2.5.2. Impact of the Microbiota on the Development and Functioning of the Immune System

Involvement of the symbiotic microbiota in the development and maturation of the innate and adaptive immune system (Hevia et al., 2015) includes the regulation of the number and activity of various types of T (especially CD^{4+}) and B lymphocytes. Under normal conditions, the microbiota stimulates the activity of anti-inflammatory T regulatory (T_{reg}) cells and suppresses that of proinflammatory Th1 and Th17 cells, limits the production of immunoglobulins IgE, and, therefore, decreases the risk of allergic processes (Rees et al., 2018).

Evolution enabled the species-specific co-adaptation of the host organism with its immune system and the microbial organ. For instance, the

maturation of mouse immune cells is promoted by the mouse microbiota but not by the rat or human microbiota (Liang et al., 2018).

2.5.3. Role of the Perinatal Microbiota: The Old Friends Hypothesis

Recently, increasing attention has been given to the hypothesis that the perinatal (taking place at birth) colonization of the GI tract by symbiotic microorganisms decreases the risk of infectious diseases during the whole lifespan because they prevent GI colonization by potential pathogens and stimulate the protective mechanisms of the immune system. Importantly, the symbiotic microbiota can not only increase but also decrease immune system activity if it becomes abnormally high, and this seems to account for the beneficial microbiota's capacity to lower the incidence of autoimmune problems (Belkaid & Hand, 2014; El Aidy et al., 2015).

Presumably, the microbiota influences the development of the immune system even in the prenatal period, if the aforementioned hypothesis that maternal microorganisms translocate via the placenta (2.1) is valid. This is in line with the fact that females with a richer microbiota give birth to children with a decreased risk of allergic diseases, according to the data on village residents in Austria; the maternal microbiota is enriched because of constant contact with cows, straw, and unpasteurized milk (Riedler et al., 2000). Experiments with animals indicate that introduction of the symbiont *Lactobacillus rhamnosus* attenuates allergic responses in infants (Logan et al., 2016). In mice, tolerance (lack of immune response to symbiotic microorganisms) develops within 10-20 days after birth, and this time is necessary for the formation of GAPs (goblet cell-associated antigen passages) that enable microbial translocation to the submucosal lamina propria of the gut. The interaction of GAPs-crossing microbial cells and their components with gut immunocytes in the perinatal period is a prerequisite for the development of immune system tolerance to the Old Friends (Knoop et al., 2017).

It is emphasized in the literature that sufficiently intense interaction with primary microbial colonizers is necessary for the proper maturation of dendritic cells that convert to dendritic regulatory cells (DC_{reg}s). In their turn, DC_{reg}s induce the conversion of T lymphocytes into T_{reg}s. They are involved in developing immune system tolerance to the Old Fiends among microorganisms as well as to the host's own cells that should normally cause no immune response (Liang et al., 2018). T_{reg}s also produce signal molecules such as interleukin-10 (IL-10) that attenuate abnormally intense immune responses damaging the host organism and the symbiotic microbiota. Overall, the immune system can distinguish useful microorganisms from pathogens and exhibit tolerance to the components of its own cells and innocuous inhabitants, as a result of its education by useful microbiota at the beginning of the life-cycle (Liang et al., 2018). Apart from IL-10, the microbiota promotes the production by T lymphocytes of adequate amounts of other anti-inflammatory factors including transforming growth factors TGF-α and TGF-β. These anti-inflammatory factors normally are in balance with such proinflammatory factors as interleukins IL-1β and IL-17, interferon-γ, and tumor necrosis factor TNF-α.

2.5.4. Impact of the Absence of Microbiota or Its Disruption

If the normal harmonious interaction between the microbiota and immune system components is disrupted, this poses the risk of the development of various infectious and inflammatory diseases (see the end of subsection 2.2.3 above for their list that, of necessity, is incomplete).

GF animals are characterized by an immature local immune system of the GI tract, a systemic decrease in expression of immune response co-stimulators, insufficiently differentiated dendritic cells, a decreased T and B lymphocyte number, disrupted phagocytosis and T lymphocyte cytotoxic activity, and inadequate interaction between dendritic cells and T lymphocytes, including T_{reg}s ($CD4+CD_{25}^{high}$; El Aidy et al., 2015; Foster et al., 2016). Introduction of the normal human or mouse GI microbiota into the organism of GF mice increases the level of immune response-attenuating

T_{reg}s (Yano et al., 2015). This is attributable to the aforementioned strategy of the microbiota that is aimed at developing host tolerance to it. The polysaccharide A of *Bacteroides fragilis* binds to receptor TLR2 and causes interleukin IL-10 production by $CD4^+$ T lymphocytes, which suppresses inflammation (Ayres, 2016).

The gut microbiota exerts a regulatory influence on the maturation and operation of immune system, starting from the fetal period of prenatal development. A lack of microbiota or its disruption results in attenuated responses of the innate immune system. Gut recolonization by the normal microbiota partially improves the innate immune system, and the effect of the microbiota depends on SCFAs produced by it (Liang et al., 2018).

In the absence of the microbiota or during dysbiosis, the gut-blood barrier and the BBB are disrupted, due to a decrease in expression of tight junction proteins by their cells. Under these conditions, the incidence of such diseases as allergic disorders and IBD is expected to increase. GI microbiota disruption, apart from increasing gut wall and BBB permeability, predisposes the organism to stress-related and neurodegenerative diseases (Liang et al., 2018).

To reiterate, a lack of contact with the symbiotic microbiota or dysbiosis prevent the formation of a sufficient number of T_{reg}s. T lymphocytes only develop into Th1, Th2, and Th17 cells that are actively involved in inflammatory processes. They are stimulated by excessive amounts of spore-forming bacteria that are present in the dysbiotic GI microbiota. The resulting disbalance between pro- and anti-inflammatory immune system factors decreases the tolerance of immunocytes to innocuous (commensal or symbiotic) microorganisms and the host organism's own cells. This poses the risk of chronic inflammatory and autoimmune processes, including, e.g., multiple sclerosis. These processes result in nervous cell apoptosis (programmed death), microglia dysfunction, and neurodegenerative processes that cause memory problems, behavioral disorders, and locomotive symptoms (Liang et al., 2018).

Apart from T lymphocytes, the microbiota exerts an important influence on B lymphocyte differentiation and immunoglobulin production. Immunoglobulins IgA, important protective factors of the immune system,

influence microbiota diversity and composition; in its turn, the symbiotic microbiota regulates IgA synthesis and release (Honda & Littman, 2016). The normal microbiota inhibits IgE production and stimulates IgG formation (McCoy et al., 2017). In the absence of the microbiota, the IgG and IgA contents are decreased and the IgE amount is increased (Gensollen et al., 2016), which may result in allergic problems.

2.5.5. Stress Impact on Microbiota-Immune System Interaction

Detrimental factors cause microecological problems, impairing the immune system and posing the risk of a large number of pathological syndromes and diseases that are characterized by insufficient or, conversely, excessive immune cell activity, as exemplified by diabetes, obesity, asthma, tumors, neural and psychological diseases, etc. (Krishnan et al., 2015).

Chronic social stress in mice impoverishes their microbiota and affects the innate immunity system. It activates dendritic cells, stimulating interleukin-6 production and spleen macrophage responses to microbial antigens and suppressing inflammation-mitigating T_{reg} activity. The intermediary role of the microbiota in the stress factor influence on the immune system is consistent with the fact that no IL-6 production and macrophage activation is detected if GF or antibiotic-treated mice are exposed to stress (Gur et al., 2015; Bharwani et al., 2016). An increase in blood cytokine concentration was found in rats that were repeatedly exposed to electric tail shock. Antibiotic administration prevented accumulation of inflammation-promoting cytokines (Gur et al., 2015).

Immune system alterations are accompanied in stressed animals by behavioral changes that are indicative of the stress impact on the CNS. Stessed mice avoid meeting conspecifics and prefer an empty compartment to that already occupied by another mouse. They display less exploratory behavior in open field tests. Stressed mice spend most time in the dark rather than in the illuminated areas of the experimental chamber, in an analogy to GF mice (see 2.4.1) (Bharwani et al., 2016).

Pathogenic and opportunistic microorganisms and their components and metabolities increase proinflammatory cytokine and chemokine secretion by the cells of the intestinal epithelium, the enteroglia of the ENS, and the innate branch of the immune system (dendritic cells and macrophages) that exert a systemic influence on the organism including the brain. They contribute to the development of depression, anxiety, ASDs, schizophrenia, and bipolar disorder (Kelly & McCusker, 2014; Li & Zhou, 2016).

Constant contact with the Old Friends, i.e., the primary microbial colonizers of the GI tract, presents serious difficulties in modern-day society because of permanent stress, drastic changes in lifestyle and diet, as well as excessive hygiene. This results in disrupting the functions of the immune system that may be weakened (leaving the organism vulnerable to pathogens and tumor cells) or, alternatively, excessively activated, predisposing the organism to allergic and autoimmune problems. Of relevance is the current global spread of inflammatory non-communicable diseases (NCDs). The most dangerous NCDs include cardiovascular diseases, allergic processes, and chronic low-intensity neuroinflammation-dependent neurodegenerative diseases and mental disorders. "The developing immune system is highly dependent on microbial stimulation, and declining biodiversity (or diminished contact with biodiversity via lifestyle changes) is implicated in both the dramatic increase in early-onset inflammatory diseases such as infant allergic diseases, as well as the risk of inflammatory diseases later in life" (Logan et al., 2016).

Infant allergic diseases are directly related to GI dysbiosis-caused abolition of the inhibitory effect of the microbiota on the development of Th2 helpers that are involved in allergic process and are excessively active in allergic children. The data on over 300 children in Canada demonstrated that the GI tract of children with bronchial allergic problems (asthma and obstructive bronchitis) were characterized, during the first three months of life, by significantly decreased numbers of bacteria of the genera *Lachnospira, Veillonella, Faecalibacterium,* and *Rothia.* Adiministration of a combination of these bacteria to GF animals mitigated inflammation in the airways (Logan et al., 2016).

2.5.6. Recognition of Microbial-Associated Molecular Pattens (MAMPs) by Immune Cells

Microbial cell components, especially lipopolysaccharides (LPSs), lipoproteins, flagellin, and CpG repeats-enriched DNA activate macrophages, neutrophils, dendritic cells, and other immune cells that recognize these MAMPs using specific pattern-recognizing receptors (PRRs). They include Toll-like receptors (TLRs), nucleotide oligomerization domain-like receptors (NLRs), C type lectins, and cytosolic multiprotein oligomers of the innate immune system (inflammasomes) responsible for the activation of inflammatory responses (Ivashkin & Ivashkin, 2018; Liang et al., 2018; Shenderov et al., 2018). Similar receptors are present on gut mucosal cells (enterocytes). They recognize microorganisms and initiate an immune response to eliminate pathogens; normally, they are tolerant to the symbiotic microbiota.

Dysbiosis with disruption of the gut wall barrier stimulates the formation of immunoglobulins, e.g., IgA and IgM, in response to potentially dangerous microorganisms translocating from the intestines. It was established that injection of bacterial LPSs into the bloodstream results in a systemic immune response in model animals (Liang et al., 2018).

Nervous cells, including those of the ENS, express receptors that recognize microbial patterns. This enables the nervous system to directly (and not only via the immune system) communicate messages about pathogenic microorganisms, causing a feeling of pain and activating the immune system (Lim et al., 2016). Bacterial LPS-activated TLR4 receptors were detected in the inferior ganglion of the vagal nerve of the rat. The same receptors and other types of TLRS (TLR-3 and TLR-7) are present in the jejunal plexus and the dorsal roots of the spinal cord, in mice and humans. The LPSs are recognized by the TLR4 receptors of the cells of endocrine organs, such as the thyroid, which stimulates the expression of the thyroglobulin gene (Mazzoli & Pessione, 2016).

2.5.7. Effects of Immunocytes-Produced Factors on the Nervous System

Apart from producing their own neuroactive factors, microorganisms induce immunocytes to release low molecular weight compounds that influence the brain and behavior in health and disease. “Immune processes may underpin many of the effects of the microbiome on the brain” (Johnson & Foster, 2018, p.652). “The bacterial genera *Lactobacillus* and *Bifidobacterium*, which are commonly associated with behavioral changes, are also known for their immunomodulatory properties” (ibid.; see also Wells, 2011).

Various intestinal microorganisms produce peptides that are similar to appetite-regulating hormones (ghrelin, leptin, melanocyte-stimulating hormone, etc.); this results in releasing respective immunoglobulins that may cause an autoimmune response, i.e., a response to the organism’s own peptides (van de Wouw et al., 2017). Gut epithelium cells and lymphocytes recognize microbial signals and produce cytokines, serotonin, and corticotropin-releasing factor (CRF) that impact the immune, nervous, and endocrine system (Liang et al., 2018).

Immunocyte-produced interleukins perform a number of different functions. IL-6 causes local and systemic temperature response, IL-1 affects the brain and causes characteristic symptoms of depression and sickness in humans and animals, including loss of appetite, adynamia, social behavior inhibition, and decrease in cognitive capacities. Brain-related symptoms result from disrupting not only the intestinal barrier but also the BBB. Therefore, microbic toxic products and immune system-produced proinflammatory factors translocate into the brain and promote chronic neuroinflammation. Disrupting the two barriers poses the risk of systemic metabolic disorders (Liang et al., 2018), including metabolic syndrome with obesity.

IL-1 of peripheral origin activates the organism’s neuroendocrine response to stress: the parvocellular neurosecretory cells of the hypothalamus produce CRF under the influence of IL-1. Since CRF induces

the release of adrenocorticotrophic hormone (ACTH) by the front lobe of the pituitary, glucocorticoid hormones are produced by the adrenal cortex.

The immunocyte-produced tumor necrosis factor TNF-α brings about a decrease in the number of 5-HT_{2A} serotonin receptors on neurons. The resulting decline in the activity of serotonin-dependent (serotonergic) parts of the nervous system causes sickness and depression (Sampson & Mazmanian, 2015).

Vagal nerve activation produces an anti-inflammatory (nicotin receptor α7-mediated) effect and decreases pathogen-induced release of TNF-α. This results in attenuating the symptoms and increasing the survival chances of animals suffering from sepsis. Septic shock-caused hypotension (blood pressure decrease) does not develop under these conditions (Kelly & McCusker, 2014; Dinan et al., 2015).

Microbially induced interleukin production partly accounts for the somnolescence, fatigue, and psychataxy (lack of concentration) that are characteristic of infectious diseases. Proinflammatory interleukins accumulate, e.g., in the hippocampus, upon pathogen invasion. Along with an increase in IL-1β, IL-6, TNF, and interferon-α levels, a decrease in nerve growth factor (NGF) content results (Kelly & McCusker, 2014).

2.5.8. Microbiota-Nervous System-Immune System Triangle

Trilateral interactivity between the nervous system (especially the brain), the immune system, and the microbiota is essential for the physical and mental well-being of humans (Fig. 9). In particular, the brain sends messages to the immune system that impacts microorganisms. In turn, they influence both the immune and the nervous system. The operation of the whole triangle crucially depends on BASs including neurochemicals produced by all the three systems.

The neurochemical acetylcholine produced by the brain and the vagal nerve (and also immune cells themselves) exerts an influence on macrophages, suppressing TNF, IL-1, IL-6, and IL-18 synthesis by them; acetylcholine also affects the microbiota and is produced by some

microorganisms (Wall et al., 2014 and see 3.4 for details). Adipose cells that form a part of the immune system contain receptors that bind neuropeptides produced, apart from immunocytes themselves, both by nervous cells and by microorganisms (Mazzoli & Pessione, 2016).

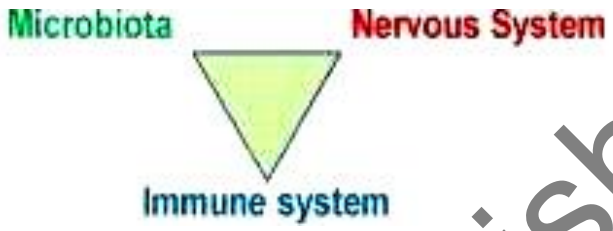

Figure 9. The microbiota-nervous system-immune system triangle.

The neurochemical acetylcholine produced by the brain and the vagal nerve (and also immune cells themselves) exerts an influence on macrophages, suppressing TNF, IL-1, IL-6, and IL-18 synthesis by them; acetylcholine also affects the microbiota and is produced by some microorganisms (Wall et al., 2014 and see 3.4 for details). Adipose cells that form a part of the immune system contain receptors that bind neuropeptides produced, apart from immunocytes themselves, both by nervous cells and by microorganisms (Mazzoli & Pessione, 2016).

Apart from their direct impact on immunocytes, the immunological effects of neurochemicals, including those of microbial origin, may be due to their impact on the nervous system, especially the brain. The impact of neurochemicals on the CNS secondarily modifies their regulatory influence on the operation of the immune system (Idova et al., 2012).

Microorganisms-induced release of proinflammatory cytokines by immunocytes, e.g., during dysbiosis, results in local and systemic inflammatory processes and an increase in the activity of indole-2,3-dioxygenase that catalyzes the conversion of tryptophan to kinurenine. If the organism is under stress, the increase in blood glucocorticoid levels activates tryptophan-2,3-dioxygenase that also can convert tryptophan to kynurenine. As a result, the level of tryptophan, the precursor of serotonin, a major neuromediator, decreases. This brings about brain function disruption and

psychological disorders including depression (O'Mahony et al., 2015). The afferent fibers of the vagal nerve respond to various lymphocyte-secreted neuroactive substances, such as histamine, serotonin, prostaglandins, and cytokines (Mazzoli & Pessione, 2016).

The trilateral interaction between the microbiota, the nervous, and the immune system of the host is highlighted by studies on maternal immune activation (MIA) in which pregnant female mice were treated with immunostimulators. They gave birth to pups that were characterized by a disrupted gut-blood barrier and autism-like behavioral symptoms such as inadequate social behavior, stereotyped movements, and disrupted acoustic communication. Administration of *Bacteroides fragilis* to MIA pups restored their gut-blood barrier and improved their behavior (Rao & Gershon, 2016).

The linkage between the immune and the nervous system within the framework of the triangle accounts for the fact that anxious behavior is mitigated in mice in which T and B lymphocyte differentiation is disrupted. Such mice have a mutation in the DNA recombination-activating *RAG-1* gene that is responsible for the diversity of lymphocyte receptors. Alternatively, the T lymphocyte receptors of these mice lack the β and δ chains or the total T lymphocyte content is decreased (Foster et al., 2016).

The recent *jumping genes* hypothesis is based on the assumption that the evolution of the adaptive immune system was linked with the transfer of mobile genetic elements (transposons) from microbial DNA molecules to animal cells. The transposons contained *RAG* gene homologues that converted into V(*D*)J recombination-enabling genes responsible for a wide variety of T and B lymphocyte receptors (Logan et al., 2016).

Apart from protecting the host organism, the adaptive immune system that evolved under the influence of microbial transposons presumably fulfilled an additional function. It was aimed at optimizing the conditions for the development of the microbiota. The microbial stimulation of the evolution of the adaptive immune system formed a part of a strategy aimed at manipulating the host organism for the benefit of the GI microbiota. The fact that the normal microbiota facilitates T cell differentiation into immunosuppressive T_{reg}s can be explained in terms of this hypothesis

because this secures host immunotolerance to the microbiota and minimizes risks associated with misdirected immune responses (Logan et al., 2016).

The impact of the microbiota on gustatory perceptions and eating habits that was discussed above (in 2.4) involves the immune system. Systemic administration of toxic concentrations of bacterial LPSs to the mouse organism causes tongue inflammation that depends on the effect of the LPSs on Toll-like receptors. This results in decreasing the life time of taste bud cells and changing the taste perception of the mouse. Prolonged oral administration of LPSs to mice causes a decrease in expression of sweet taste receptors. LPS injection into the tongue tissue results in local leukocyte recruitment and inhibition of the sodium-dependent taste perception system (van de Wouw et al., 2017).

Brain-produced signal molecules enter the bloodstream with (i) the interstitial fluid collected by brain capillaries and pumped by the internal carotid artery system or (ii) the cerebrospinal fluid via the subarachnoid space and the cribriform plate. Brain-produced signals reach nasal lymph nodes and thereupon enter the lymph and blood circulation systems. Moving in the opposite direction, cytokines, chemokines, and alarm signals, e.g., ATP, can bypass the BBB and directly translocate from the blood to brain endiothelial cells (Kelly & McCusker, 2014).

2.5.9. Immune System-Mediated Systemic Effects of the Microbiota on the Host Organism

Under the influence of microbial factors, the immune system interacts, apart from the nervous system, with various other tissues and organs of the human organism. Microbiota-stimulated IL-4 and IL-13 production by CD4+ T cells increases cholecystokinine secretion by the enterochromaffin cells of the intestinal epithelium. In addition, enterochromaffin cells release serotonin that upregulates the inflammatory response of the immune system (Mazzoli & Pessione, 2016).

The important role of microorganisms in orchestrating the immune system at the level of the whole organism is highlighted by the relationship between the microbiota and various autoimmune disorders. GF mice display attenuated symptoms of experimental autoimmune encephalomyelitis (EAE) that represents an experimental model of multiple sclerosis (MS) *in vivo*. Such mice are characterized by disrupted formation of the pool of Th17 lymphocytes that is involved in MS progression. Upon colonization of the gut of GF mice with segmented filamentous bacteria, the disease becomes more severe, which is accompanied by an increased Th17 level in the CNS (Lee et al., 2011).

The lack of bacteria in the gut of GF mice is associated with an increased BBB permeability. Apparently, this should result in a more manifest clinical picture of EAE. However, a diametrically opposite effect is observed: autoimmune disorder symptoms are considerably mitigated, and this testifies to the microbiota's stimulatory effect on immunity development. In the absence of the microbiota, the antigen-presenting function of dendritic cells is disrupted. The dendritic cells of GF animals are also characterized by an abnormally decreased capacity to start the inflammatory T cell response (Lee et al., 2011).

Oral administration of antimicrobial drugs including the non-absorbable antibiotics kanamycin, colistin, and vancomycin, results in slowing down the development of EAE in model animals (Yokote et al., 2008). The protective effect of antibiotics during EAE manifests itself in a decrease in the numbers of proinflammatory cells, such as Th1 and Th17 lymphocytes, and an increase in anti-inflammatory activity, including the involvement of T_{reg}s.

The therapeutic potential of antibiotics with respect to multiple sclerosis is emphasized in a recent clinical study conducted in Canada. It was revealed that administration of minocycline, a semisynthetic antibiotic of the tetracycline group, decelerated the transition from isolated multiple sclerosis (MS) syndrome to clinically fully manifested MS. The efficiency of minocycline that decreased the risk of MS progression within 6 months by 18.5%, is comparable with that of MS-modifying drug preparations (Metz & Eliasziw, 2017). The protective mechanism of minocycline is still to be

elucidated, particularly because minocycline exhibits not only antibacterial but also anti-inflammatory activity (Garrido-Mesa et al., 2013).

Interestingly, the immunotropic activity of the microbiota can be enhanced by optimizing the diet. The diet exerts a strong influence on the microbiota. In the absence of complex natural carbohydrates, the microbiota produces little SCFAs (e.g., butyrate) and predominantly carries out proteolytic fermentation that may result in forming potentially toxic proinflammatory compounds, including various amines and ammonia (see 3.8 below). These compounds are implicated in gut dysbiosis and pose the risk of the development of nonspecific ulcerous colitis and colorectal cancer. SCFAs produced by bacteria from food fibers, in contrast, possess anti-inflammatory and anticarcinogenic properties (Carlucci et al., 2016, Chen & Vitetta, 2018).

2.6. PROBIOTICS AND PSYCHOBIOTICS

In order to ameliorate the human microbiota, a wide variety of drugs, biologically active food additives, and functional nutrients are currently used. Much attention is currently given to preparations containing selected strains of lactobacilli, bifidobacteria, and other live microorganisms *(probiotics)*, as well as to soluble food fibers and other organic substances that stimulate their growth *(prebiotics)*. Prebiotics are exemplified by undigestible oligosaccharides degraded by beneficial gut microorganisms that produce SCFAs and other valuable organic acids (Shenderov, 2001; Boddu & Divakar, 2018). Diet optimization including sufficient supply of prebiotics such as fructans contributes to the proliferation of useful bacteria, e.g., *Bifidobacterium*, in the organism (Norris et al., 2013; Shenderov, 2014). Prebiotics also produce anti-inflammatory effects that are attributable to oligosaccharides' capacity to directly interact with the gut epithelium and to significantly decrease proinflammatory cytokine production (Ivashkin & Ivashkin, 2018, p. 15). According to the results of a recent experimental study, a prebiotic (oligofructose-enriched inulin) can reverse middle age-

related immune priming and brain inflammation in mice, giving hope for mitigating midlife brain problems in humans (Boehme et al., 2019).

Figure 10. Actoflor®-S (the picture is Dr. Timur Y. Vakhitov's gift) and Bactistatin®, commercial probiotic preparations.

The effects of probiotics and prebiotics that stimulate the growth of symbiotic bacteria are partly due to low molecular weight exometabolites. For instance, APK preparations produced from the culture liquid of *E. coli* M-17 stimulate the growth of *Lactobacillus delbrueckii* subsp. *bulgaricus* more efficiently than widely used commercial prebiotics based on fructooligosaccharides. Under the influence of APK preparations, the metabolism of lactobacilli is stimulated, which manifests itself in elevated antagonistic activity and an increased rate of milk fermentation that is completed 2-4 hrs earlier if APKs are present (Vakhitov, 2019).

2.6.1. Probiotics

In recent decades, increasing attention has been given to probiotics (this term was coined by the German nutritionist Werner Kollath in the 1950's who contrasted them with risky antibiotics, see: Park, 2009). According to the official definition given by FAO/WHO (2006), probiotics are live

microorganisms that, "when administered in adequate amounts, confer a health benefit on the host". Commercially available probiotics are supplied in the form of drug preparations and biologically active food additives that contain microbial cultures.

There are a variety of other terms used to denote probiotic preparations that are at least partly based on live microrganisms and aimed at improving the microbiota of the human organism (Shenderov, 2001, 2014, 2017; Shenderov et al., 2017):

(1) *symbiotics*, i.e., combinations of several strains of probiotic microorganisms that exhibit complementary activities and produce synergistic effects. For instance, the commercial preparation Bificol contains *Bifidobacterium bifidum* and *E. coli* strains;
(2) *synbiotics*, complex products containing probiotic bacterial strains and growth-stimulating prebiotic substances, e.g., Bioaminolact that is composed of bifidobacteria, *Enterococcus faecium* L-3, and plant extract;
(3) *combiotics*, synbiotics additionally enriched with functional nutrients such as mixtures of vitamins and minerals and phenolic compounds;
(4) *metabiotics*, i.e., biologically active substances that are produced as a result of the metabolic activities of symbiotic (probiotic) microorganisms and exert a positive influence on various kinds of physiological processes: the term was coined 10 years ago by Boris A. Shenderov (see review: Shenderov et al., 2017, p. 27). Some important neuroactive compounds of microbial origin that can be used as metabiotics are given in Table 1.

The meaning of the term "metabiotics" is similar to that of the relatively often used term "postbiotics" that denotes bacterial products whose effects on the signal pathways and functional barriers of the organism are similar to those of live bacterial cells (Patel & Denning, 2013; Aguilar-Toala et al., 2018). They include bacteriocines, organic acids, ethanol, diacetyl, etc. (Shenderov et al., 2017; Kerry et al., 2018).

Table 1. Neuroactive compounds of microbial origin that function as hormones and/or neurochemicals in the host organism and can be used as metabiotics (Shenderov et al., 2017)

Type of chemicals	Examples
Biogenic amines	Serotonin, 3,4-dihydroxyphenylalanine (DOPA), norepinephrine, dopamine, histamine, acetylcholine, tryptamine
Amino acids	Aspartic acid, glutamic acid, glycine, taurine, tryptophan, γ-aminobutyric acid (GABA)
Short-chain fatty acids	Butyric, propionic, acetic, lactic acid
Gaseous substances	NO, CO, H_2S, H_2, CH_4, NH_3

However, the authors prefer using the term *metabiotics* (Shenderov, 2001, 2013b, 2014). This term contains the Greek prefix *meta-* (change, transformation), referring to the metabiotics' ability to initiate a large number of hormonal and neurochemical processes. In contrast, the prefix *post-* (after, posterior to) in the word 'postbiotics" merely emphasizes the "post mortem" nature of these compounds that either work after a microbial cell has been killed and broken down into fragments or represent substances that have been separated from it.

Metabiotics are exemplified by heated dead cells of probiotic bacteria, polysaccharide A formed by *Bacteroides fragilis* that modulates the immune system and protects mice from *Helicobacter hepaticum*-induced colitis, and skeleton P-CWS (fragments of the *Propionibacterium acne* cell wall) that activates the cytotoxic activity of macrophages and, therefore, produces an anticarcinogenic effect. A widely used metabiotic is Hilac-Forte that contains the metabolic products of *E. coli* DSM 4087, *Enterococcus faecalis* DSM 4086, *Lactobacillus acidophilus* DSM 4149, and *Lact. helveticus* DSM 4183; volatile fatty acids and lactate also form a part of this preparation. It helps restore the composition and the functions of the GI microbiota (Shenderov, 2011; Shenderov et al., 2017; Kerry et al., 2018).

Metabiotics as the structural components and/or metabolites of probiotics possess a number of indisputable advantages over probiotics per se: they tend to have a longer shelf life; they are more target-specific and safe in terms of their interaction with the human organism. It is relatively

easy to adjust their dosage in a clinical setting (Shenderov, 2011; Shenderov et al., 2017, 2018).

Probiotics and combined or probiotics-derived preprarations are asigned important roles in terms of the international sustainable development program that emphasizes "Good Health and Well-Being" as one of its main goals. The program was adopted at the *World Summit on Sustainable Development (Rio + 10)* in Johannesburg in 2002 and further developed as a part of the sustainable development package for the period until 2030. For instance, Sustainable Development Goal 3 (SDG 3) envisages helping the population of Earth eradicate diarrhea, IBD, and a large number of infant diseases; special hopes are pinned on prokaryotic, e.g., *Lactobacillus acidophila*, and eukaryotic (*Saccharomyces carlsbergensis* yeast and *Aspergillus niger* mycelial fungus) probiotics. Allergic diseases and many other autoimmune problems are linked to humoral immune system (Th2 branch) disruption. It is known that such probiotics as lactobacilli and bifidobacteria significantly contribute to the normalization of the Th2 branch and the whole immune system and, therefore, can potentially be used for treating such autoimmune disorders (Akinsemolu, 2018). "The total global retail market for all probiotic products was estimated at $ 45.6 billion U.S. for the year 2017, with a predicted compound annual growth rate of 7% (2017–2022)" (MarketsandMarkets, 2017; quoted from Jackson et al., 2019).

The following health-promoting functions of probiotics have been documented in the literature (reviewed, Sanders et al., 2018); these effects are also produced by metabiotics (Shenderov, 2013b; Shenderov et al., 2017):

1. *They help the human organism stabilize the GI microbiota and optimize its qualitative and quantitative composition.* They also suppress harmful microorganisms because they contain antimicrobial factors (SCFAs, bacteriocines and their analogs, hydrogen peroxide, nitric oxide, etc.); live probiotics prevent the invasion of potential pathogens by successfully competing with them for ecological niches in the human organism (Shenderov,

2001, 2014). Both probiotics and metabiotics can potentiate the immune response to pathogens. An eukaryotic probiotic, the yeast *Saccharomyces cerevisiae* strain 905, was established to protect the mouse gut from the pathogenic enterobacteria *Salmonella typhimurium* and *Clostridioides difficile* (Martins et al., 2005). An inhibitory effect on these two pathogens is also produced by probiotic *Lactobacillus, Bifidobacterium, Lactococcus, Streptococcus,* and *Bacillus* strains, because they produce SCFAs including formate, acetate, and lactate (Tejero-Saricena et al., 2013). They also suppress the development of pathogens by increasing their membranes' permeability and oxidizing the sulfhydryl groups of their lipids (Kerry et al., 2018). Feed fermented with probiotic *Lactobacillus* strains increases the resistance of pigs to swine flu (Liang et al., 2018). Using fermented feed improves the meat taste and makes pigs more submissive and less aggressive; therefore, these lactobacilli can be regarded as psychobiotics (see below).

2. *Low molecular-weight compounds contained in probiotics/ metabiotics neutralize toxins and other metabolites that are harmful for the host organism.* These small-size molecules disrupt pathogen-specific communication mechanisms, including quorum-sensing systems. Importantly, while suppressing potentially pathogenic microorganisms, probiotics and metabiotics do not inhibit the functioning of the GI symbiotic microbiota, in contrast to antibiotics (Kerry et al., 2018).
3. *Probiotics and metabiotics supply the host organism with nutrients, antioxidants, growth factors, enzymes, organic acids, polyphenols, vitamins, bile acids, gaseous substances, and other biologically active substances (BASs)* that beneficially influence the salt-water, lipid, amino acid, and energy metabolism, the redox balance at the local (intestinal) and systemic (general) levels, and the development and operation of the peripheral, especially enteric, and central nervous system. The BASs also exercise epigenomic control over the expression of host genes and modulate the systemic responses

of the innate and adaptive immune system (Shenderov, 2001, 2011, 2014; Shenderov et al., 2017).

4. *Probiotics and metabiotics exhibit anticarcinogenic activity* (Patel & Denning, 2013), as exemplified by the strong anticancer effects of the *Lactobacillus acidophilus* 36YL strain on four tested cancer cell lines (AGS, HeLa, MCF-7, and HT-29), in which the strain induces apoptosis (Nami et al., 2014). The strain *Lactobacillus fermentum* NCIMB 5221 suppresses the growth of cancerous cells in the colon and concomitantly stimulates the growth of normal epithelial cells (Kerry et al., 2018).
5. *Probiotics and metabiotics produce anti-allergic, antidiabetic, and anti-inflammatory effects.* The probiotic strain *Lactobacillus plantarum* 06CC2 relieved allergic symptoms in mice treated with the allergen ovalbumin. It decreased the amount of ovalbumin-specific immunoglobulin IgE and the total IgE level. Concomitantly, the concentrations of the anti-allergic factors interleukin-4 and γ-interferon increased (Kerry et al., 2018). The administration of probiotic bifido- and lactobacteria causes an increase in morning melatonin content in the saliva, which is associated with the attenuation of IBS (Mazzoli & Pessione, 2016). Probiotic strains of lactobacilli promote the production of anti-inflammatory interleukin-10 and influence the development of the dendritic cells of the immune system (Mishra et al., 2018).
6. *Probiotics and metabiotics beneficially influence metabolism, and they can be used for treating obesity (metabolic syndrome).* Probiotics and metabiotics also help patients with anorexia and malnutrition. It was revealed that probiotics improve the health state of rodents after a period of starvation (Shenderov, 2008; van de Wouw et al., 2017).
7. *Beneficial microbial agents can potentially be used to improve the symptoms of aging*; this point was already made by Ilya Mechnikov (also known as: Élie Metchnikoff, 1904) in his famous work *Etudes sur la nature humaine: essai de philosophie optimiste.* Administering probiotics to aging subjects results in increasing the

Actinobacteria, *Bacterioidetes*, and *Lactobacillus* concentrations while decreasing the *Clostridioides difficile* concentration in their microbiota. As a result, at least partial amelioration of age-related cognitive problems is achieved (Ivashkin & Ivashkin, 2018, p.17).

8. *These agents promote the growth of blood vessels (angiogenesis)* in the intestinal tissue by producing VEGF (vascular endothelial growth factor; Kerry et al., 2018).
9. *Some probiotics produce a pain-relieving effect*, particularly with respect to abdominal pain. This effect may result in complete analgesia (a lack of pain sensitivity), which is attributable to the capacity of lactobacilli including *Lact. acidophilus* to induce the expression of μ-opioid and canabioid receptors in the intestinal epithelium (Cryan & Dinan, 2012).
10. *Probiotics and metabiotics can relieve stress*. This is characteristic of preparations that are based on bifidobacteria and lactobacilli contained in fermented dairy products. Consumption of dairy products with such metabiotics as the metabolites of bifidobacteria and lactobacilli promotes physical and mental health by ameliorating the patient's microecological system and optimizing the activity level of the brain areas that are responsible for cognitive capacities. Tryptophan metabolism is optimized, which positively influences the production of the essential brain neurochemical serotonin from tryptophan (O'Mahony et al., 2015).
11. *Probiotics and metabiotics regulate the activity of the intestinal part of the immune system, i.e., the gut-associated lymphoid tissue (GALT).* They modulate immune responses, normalize the balance of pro- and anti-inflammatory cytokines, lower the antigen load of GALT, decrease gut wall permeability, increase immunoglobulin IgA secretion, induce the activity of anti-inflammatory T_{reg} cells, and promote the production of anti-inflammatory interleukin-10 (Belkaid & Hand, 2014; Shenderov et al., 2017; Liang et al., 2018).
12. *These agents systemically strengthen the whole immune system and the organism's natural barriers,* including the gut–blood barrier and the BBB by increasing the expression of proteins involved in

> forming tight junctions between cells. In this fashion, they help prevent brain problems and, accordingly, cognitive and behavioral disorders (Liang et al., 2018). Under stress, they improve the gut wall protective function, decrease the concentrations of circulating corticosteroids and proinflammatory cytokines, while increasing those of anti-inflammatory cytokines. The latter contribute to the strengthening of the BBB and the gut–blood barrier and attenuate systemic inflammation (Ivashkin & Ivashkin, 2018, p.13).

It should be emphasized that the useful effects of probiotics and metabiotics are produced not by individual microbial substances, but by a complex ensemble of low molecular-weight compounds (Shenderov, 2011) that are present either in their functional form or as precursors. These microbially produced complexes influence the host organism and its microbiota in combination with other BASs that are ingested or produced by the resident microbiota.

In light of the above, the main requirements to be met by probiotic microorganisms are as follows (Anh, 2015; Shenderov et al., 2017; Boddu & Divakar, 2018; Kerry et al., 2018; Jackson et al., 2019):

- They should be adapted to the local GI tract conditions with characteristic stress factors including a low pH, a high redox potential, and a high osmotic pressure;
- They should efficiently utilize carbohydrates and other GI-typical substrates;
- Probiotic bacterial cells should attach to the gut mucosa;
- They should suppress the development of pathogens; they should not transmit to them genes responsible for resistance to antibiotics and other antimicrobial substances.
- They should promote health by producing low molecular weight compounds, including autoinducers (QS signals), chemokinines, effectors, substrates, cofactors, metabolites, (histo)hormones, and

neurochemicals, which are useful for the host organism and/or its microbiota.

- Probiotics should remain viable during the product storage period; this is a challenge, in view of "environmental factors such as carrier material (including different food matrices), temperature, water activity, and storage time" (Jackson et al., 2019).

The aforementioned requirements can be supplemented by other important properties that are characteristic of the most recently developed probiotics. They include antioxidant, anti-inflammatory, and antimutagenic activity, as well as a positive influence on a human individual's mental state (Shenderov et al., 2017).

Autoprobiotics are a potentially important type of probiotics. They are frozen GI microbiota components of human individuals; their long-term storage enables recolonizing the GI tract of these individuals whenever necessary, in order to treat them for dysbiosis or to rejuvenate their organism (Shenderov, 2001; Ermolenko et al., 2018).

Importantly, probiotic cultures form a part of a large number of dairy products (yogurts and kefir; cottage cheese and other cheese kinds; ayran, kumys, kurunga, and other national dairy items) as well as other food items, including traditional Asian food such as Korean fermented cabbage (*kimchi*) and Vietnamese fermented eggplants (*dua muoi*) and sausages (*nem chua*). For instance, the bacterial strains isolated from the Vietnamese fermented food items meet the above criteria of probiotic microorganisms. These strains release pathogens-suppressing bacteriocines; they include strains that produce an important neurochemical, γ-aminobutyric acid (GABA; Anh, 2015).

Nonetheless, serious questions are raised in the literature with regard to probiotic microorganisms, as exemplified by the following:

- The weight of probiotics administered orally does not exceed several grams; how can they influence the gut microbiota with a total weight of 1-2 kg?

- Can the introduction of bacteria or other microorganisms into the organism elicit an immune or allergic response?
- Low molecular weight products of probiotic microorganisms (including potentially toxic agents such as biogenic amines or oxygen radicals) and microbial cells can rapidly translocate from the GI tract to various host organs and tissues. This poses the risk that probiotics might unexpectedly convert into pathogens, e.g., in a stressed, dysbiotic (antibiotic-treated) or immunocompromised host organism. Clinical cases of probiotic lactic-acid bacteria- or bifidobacteria-caused endocarditis, pneumonia, intestinal abscesses, meningitis, urinary tract infections, and sepsis were reported in the literature (reviewed, Shenderov, 2011, 2017; Shenderov et al., 2017).

Besides, "observations of uneven quality in probiotic products have been reported: examples range from products not meeting claimed active counts and incorrect strain identification… to the tragic infant death linked to a fungus (*Rhizopus oryzae*)-contaminated probiotic product" (Jackson et al., 2019). The work cited rightfully emphasizes the necessity of probiotics' "verification, certification or qualification by an independent third-party organization" (Jackson et al., 2019) as exemplified by a decentralized social network of competent experts (Oleskin, 2014, 2018, and see the discussion concerning networks in human society in the Conclusion section).

2.6.2. Psychobiotics

Probiotics include a subgroup that is denoted as *psychobiotics* (Fig. 11), i.e., live microorganisms that, when administered in adequate amounts, confer a health benefit on patients with psychiatric problems (Cryan & Dinan, 2012).

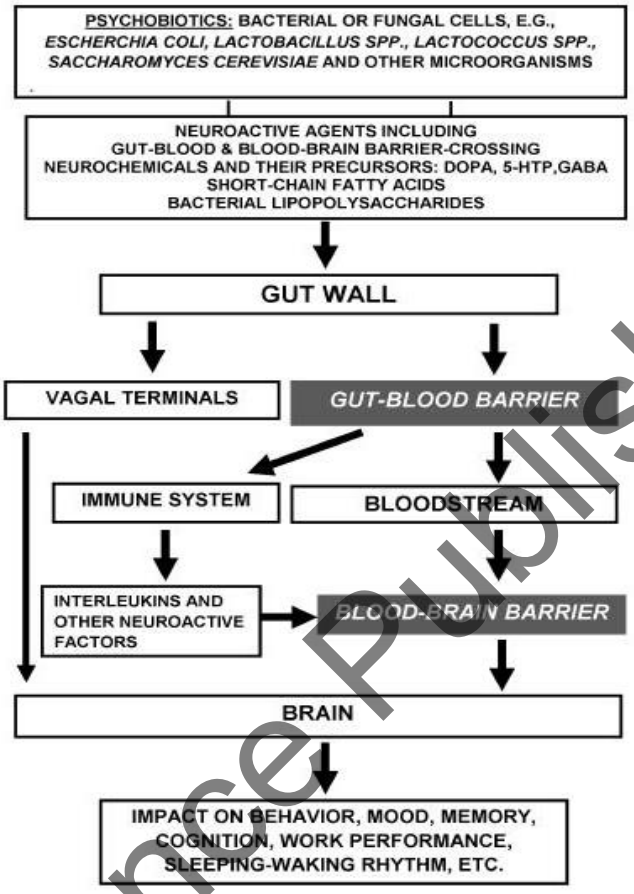

Figure 11. Psychobiotics represent microbial cells, their fragments or products. They produce beneficial effects on the host' nervous system, especially the brain and, therefore, positively influence human behavior, mood, cognition, etc. The Figure demonstrates the three main pathways used by psychobiotics: (i) via the vagus nerve; (ii) via the immune system that produces BBB-crossing neuroactive cytokines and other compounds, and (iii) by crossing the gut-blood barrier and the BBB. Abbreviations: DOPA, *L*-3,4-dihydroxyphenylalanine (the precursor of catecholamines); 5-HTP, 5-hydroxytryptophan (the precursor of serotonin); GABA, γ-aminobutyric acid. Note: In addition to the vagus nerve mentioned in the Figure, the effects of psychobiotics on the brain may be mediated by other neuronal pathways within the peripheral nervous system and its part located in the intestines (the enteric nervous system).

There is a growing body of evidence that probiotics can significantly influence the brain and, therefore, affect behavior, mood, and cognition both in experimental and clinical settings (Parashar & Udayabanu, 2016). Taking account of the data that many low molecular weight compounds produced by probiotics can modify the psychological and behavioral features of humans and animals (Shenderov et al., 2017), we can denote such microbial products as *metapsychobiotics*. They include, e.g., the lipopolysaccharides of *Bifidobacterium breve* 2003 that induce gut epithelial cells to synthesize substances which modulate signal transmission by the afferent axons of nervous cells within the gut–brain axis (Dinan et al., 2015).

Of considerable interest in this context are the recent data that probiotic bacterial strains directly influence the functionally important characteristics of neurons. The amplitude and duration of the electrically induced action potentials of myenteric neurons significantly decrease if the neurons are placed in a medium containing *Bifidobacterium longum* NCC3001 and substrates fermented by this probiotic. The neurons of the spinal dorsal ganglia that innervate the colon do not become overexcited in response to excessive stimulation if incubated in a *Lact. rhamnosus*-containing medium (Ivashkin & Ivashkin, 2018). One of the psychobiotics' mechanism of action is based on mitigating systemic inflammation by suppressing the secretion of proinflammatory cytokines into the bloodstream. Proinflammatory cytokines increase BBB permeability and, therefore, the probability of the migration of potentially pathogenic agents into the brain. Psychobiotics inhibit proinflammatory cytokine production either directly or by increasing the anti-inflammatory cytokine content. Therefore, they decrease the probability of the translocation of pathogenic factors into the CNS and improve the functioning of the BBB (Ivashkin & Ivashkin, 2018).

By modulating the GABA-dependent brain system, the psychobiotic strain *Lact. rhamnosus* JB-1 stimulated exploratory behavior in BALB/c mice in a maze, inhibited their anxiety-like behavior in an open field test and prevented depression-like symptoms in a forced swimming test[18] (Bravo et al., 2011; Bercik et al., 2012; Rohrscheib & Brownlie, 2013; Lyte, 2013b,

[18] A rat is placed in a water-filled cylinder; the lag time after which the rat starts swimming is recorded. The longer the lag, the more depressed is the rat.

2014). After administering *Lact. rhamnosus* JB-1 to the mice, the transcription of the genes encoding the receptors for GABA was increased in the hippocampus and decreased in the prefrontal cortex of the brain (Lyte, 2016; Strandwitz, 2018; Cani et al., 2019).

Severing the vagal nerve (vagotomy) abolished the effect of the psychobiotic. In similar fashion, the anxiolytic (anxiety-relieving) effect of the psychobiotic *Bifidobacterium longum* NC3001 was also removed by vagotomy (Bercik et al., 2012).

A *B. longum* 1714 + *B. breve* 1205 combination ameliorated anxiety-like behavior in mice, and its efficiency was comparable to that of the anxiolytic drug escitalopram (Sampson & Mazmanian, 2015). The practically important conclusion was drawn that such probiotic microorganisms as well as the butyrate-producing *Faecalibacterium* and *Coprocossus* bacteria can be used for treating stress-related mental disorders, including abnormal anxiety and depression (Rohrscheib & Brownlie, 2013).

Administration of *Lact. rhamnosus* and *B. longum* improved the behavior of mice that were infected by the parasite *Trichuris muris* and suffered from colitis caused by dextran sodium sulfate, respectively (Bercik etal., 2011; Foster et al., 2016).

Studies with GF mice demonstrated that colonizing their GI tract with *Lact. plantarum* PS128 increased their motor activity, decreased anxiety in an extended maze test, and increased the concentrations of dopamine and serotonin in the striatum of their brain (We-Hsien et al., 2015; Liu et al., 2016).

Of relevance are also data obtained with rats. Anxiety-like behavior caused by an electric shock is relieved by the psychobiotic strains *Lact. helveticus* R0052 and *B. longum* R0175. Restraint stress in rats (holding a rat in a fixed position) results in depressive behavior which is accompanied by GI dysbiosis. The probiotic strain *Lact. helveticus* NS8 relieves depression and, moreover, restores the normal microbiota composition (Liang et al., 2018). Young rats separated from their mothers display depressive behavior in a forced swimming test, which is relieved by administering the psychobiotic *B. infantis* strain 35624 (Desbonnet et al.,

2010). This psychobiotic increases the blood level of tryptophan, the serotonin precursor, in the rats (Dinan et al., 2015; Cani et al., 2019), which might account for its antidepressant effect, since depression is often correlated with a lowered activity of serotonergic brain areas. Introducing *B. infantis* into the GI tract of maternal separation-stressed rats also increases the brain norepinephrine level, which is lowered by stress (Desbonnet et al., 2010; Strandwitz, 2018). Psychobiotic strains stimulate memorization and learning processes (Sampson & Mazmanian, 2015; Parashar & Udayabanu, 2016; Ermolenko et al., 2018; Cani et al., 2019).

Ameliorating the GI microbiota by administering psychobiotics in studies with animal models reduces inflammation-induced alterations in the gut and improves behavioral symptoms, e.g., in mice with an autism-like disorder. Normalizing the microbiota with psychobiotics was shown to decrease the risk of neurological and psychiatric problems. For instance, administration of *B. infantis* to GF mice at an early age reduces their stress response to the normal level, so that the GF mice become similar to conventional mice in this respect (Clarke et al., 2014).

Alcoholism affects both the physical and mental health state, and it results in significant microbiota changes. Severe alcoholic hepatitis is associated "with a decrease in the abundance of *Bacteroidetes* and an enrichment of *Fusobacteria*, bacteria present mainly in the oral cavity" (Lynch et al., 2019, p.657). Useful bacterial strains such as *Akkermansia muciniphila* "are depleted by alcohol consumption in mice and humans, and supplementation of this bacterium in ethanol-induced experimental liver injury improves intestinal barrier function and relieves liver disease in mice" (ibid.).

The strain *Lact. paracasei* NCC2461 restored the normal composition of the intestinal microbiota and decreased the pain sensitivity of the colon of NIH Swiss mice with disrupted microbiota and antibiotic-enhanced visceral pain sensitivity (antibiotic-induced hyperalgesia). The same psychobiotic mitigated visceral pain in maternal separation-stressed rats whose colon was distended experimentally. *Lact. acidophilus* NCFM induced the expression of pain sensitivity-reducing opioid and cannabioid receptors in the intestinal

epithelium, causing analgesia (a lack of pain sensitivity) in rats (Bercik et al., 2012).

Female mice on a lipid-enriched diet give birth to pups with disrupted social behavior, GI dysbiosis, and a decreased number of oxytocin-producing neurons in the hypothalamus; all these symptoms are improved by treating them with the psychobiotic *Lact. reuteri* MM4-1A (ATCC-PTA-6475; Buffington et al., 2016).

These effects of psychobiotics are apparently due to their positive influence on the hypothalamus–pituitary–adrenal (HPA) axis that is essential for a stress response; the HPA function may be impaired under stress, as well as in GF animals (cf. 2.4.4 above).

In *humans*, anxiety and depression can be efficiently treated with a combination of several probiotics (Foster et al., 2016). Administration of probiotic/psychobiotic strains, e.g., of the species *Lactobacillus casei*, to patients with chronic fatigue syndrome (CFS) and IBS made them less anxious and stressed. The GI microbiota of individuals with CFS became enriched in lactobacilli and bifidobacteria under the influence of the strain *Lact. casei* Shirota (Rao et al., 2009). A psychobiotic strain (strain 35624) of the species *B. infantis* relieved pain in IBS patients and normalized the serum concentrations of proinflammatory cytokines (Bercik et al., 2012). A psychobiotic combination of *Lactobacillus helveticus* and *Bifidobacterium longum* strains improved depressive symptomes after myocardial infarction (Parashar & Udayabanu, 2016).

Apart from relieving depression and anxiety, psychobiotics and dairy products containing them improve mood and cognitive capacities. For instance, the depression-relieving psyhobiotic strain *Lact. rhamnosus* JB-1 promoted information memorization and learning (Lyte, 2013b). The *Lact. acidophilus*, *Lact. fermentum*, and *B. animalis* subsp. *lactis* cocktail ameliorated the cognitive capacities and electroencephalographic data of subjects suffering from diabetes (Parashar & Udayabana, 2016). In healthy volunteers, oral administration of the *Lact. helveticus* B0052 and *B. longum* R0175 combination attenuated stress caused by psychological factors (Kerry et al., 2018).

Recent data suggest that probiotics can be used for treating diabetes-associated brain problems: they can improve cognitive functions and diabetes-induced impairment of synaptic activity (reviewed, Thakur et al., 2019).

Gut-inhabiting bacteria belonging to the genera *Dialister* and *Coprococcus* are regarded as potential psychobiotics. Metagenomic studies revealed that their amounts in the GI tract are decreased in patients diagnosed with depression (Valles-Colomer et al., 2019).

In studies with human subjects, it was also established that a dairy product that contains *Bifidobacterium animalis* subsp. *lactis* (strain number I-2494 in the French National Collection of Cultures of Micro-organisms (CNCM, Paris, France), also referred as DN-173010), *Streptococcus thermophilus* (CNCM strain number I-1630), *Lact. delbrueckii* subsp. *bulgaricus* (CNCM strain numbers I-1632 and I-1519), and *Lactococcus lactis* subsp. *lactis* (CNCM strain number I-1631), lowers the intensity of the brain response to emotional stimuli. According to fMRI data, the brain structures involved in emotion perception become less activated during a test in which subjects recognize the emotions of the faces that are demonstrated to them. Probiotics also relieved sadness and reduced aggressiveness, according to the quiestionnaire filled in by the subjects (Tillisch et al., 2013). Similar results were obtained after 4 weeks of administering of a combination of probiotic strains (*B. bifidum* W23, *B. animalis* subsp. *lactis* W52, *Lact. acidophilus* W37, *Lact. brevis* W63, *Lact. casei* W56, *Lact. salivarius* W24, and *L. lactis* W19 and W58). After this treatment, the subjects exhibited less aggressive-ness, rumination, and other negative behavioral responses to disagreeable stimuli, compared to the control (placebo-receiving) group of subjects (Steenbergen et al., 2015). Using a dairy product with the probiotic *Lact. casei* Shirota improved the mood of patients that had displayed depression symptoms prior to the treatment (Benton et al., 2006).

In contrast, opportunistic and pathogenic bacteria exert a detrimental influence on the human brain and, therefore, behavior. The lipopolysaccharides of staphylococci bring about anxiety and depression and worsen cognitive capacities (Parashar & Udayabanu, 2016). Anxious behavior also

occurs during the infection that is caused by the pathogens *Campylobacter jejuni* and *Citrobacter amalonaticus.* This behavioral effect depends on the vagus nerve and is abolished by severing it in animals (Liang et al., 2018; Strandwitz, 2018).

Antibiotics disrupt the functioning of the GI microbial consortium and worsen cognitive capacities. Specifically, they suppress the operation of working and spatial memory systems. Subsequent administration of psychobiotics improves memory (Liang et al., 2018).

The psychobiotic *B. fragilis* ATCC 9343 normalizes the gut wall permeability and ameliorates autism-like symptoms in mice, including stereotypic behavior, impaired communication, and anxiety-like behavior (Hsiao et al., 2013; Sampson & Mazmanian, 2015). In children with autistic spectrum disorders (ASDs), administration of the psychobiotic strain *Lact. plantarum* WCFS1 improves their performance at school (Kerry et al., 2018).

The *c-fos* transcription regulator genes are activated in the hypothalamus under the influence of psychobiotics, e.g., *Bifidobacterium infantis* and non-pathogenic *E. coli* strains (Parashar & Udayabanu, 2016).

Microorganisms are known to influence the volatile signal substances (pheromones) that are produced by humans and animals. Probiotics/psychobiotics also exert their influence on host pheromones. Interestingly, the fruitfly *Drosophila melanogaster* whose gut is colonized with *Lactobacillus plantarum* prefers mating with fruitflies that also contain the probiotic bacteria in the gut. Hyenas form social groups with different microbiota composition in the odor glands and, therefore, different chemical composition of their pheromones (Sampson & Mazmanyan, 2015). Of behavioral importance is microbially produced trimethylamine. Many human individuals find its “fishy” odor sexually attractive (Shenderov et al., 2017).

Despite the promising data, serious questions should be raised with regard to probiotics and, more specifically, psychobiotics in therapeutic and nootropic terms. One of the issues is whether the microbial agents called psychobiotics in this work really improve cognitive capacities. No compelling evidence has been presented in studies with humans. There are

only data obtained with animal models in which psychobiotics promote memorization and learning (Ivashkin & Ivashkin, 2018). Even a negative influence of probiotics on the brain and psyche of their consumers cannot be ruled out at present. Obviously, further extensive research on probiotics, including psychiobiotics, should address these issues.

Microorganisms inhabit diverse niches in the host organism, especially the gastro-intestinal tract. They release multifarious low molecular weight signal substances and also specifically respond to host signals. This enables their ongoing interactivity with the nervous system, including the brain, and the immune system. This constant dialogue may promote physical and mental health or, alternatively, endanger it. The vitally important interaction along the brain-gut-microbiota axis can be normalized using probiotics including psychobiotics that directly influence human psyche and behavior. Among the evolutionarily conserved signals that are involved in microbiota-host communication, important functions are performed by neuroactive substances (neurochemicals) that provide the subject of the next chapter.

Chapter 3

NEUROCHEMICALS: THEIR INVOLVEMENT IN INTERACTIVITY AMONG MICROBIAL CELLS AND THE MICROBIOTA-HOST DIALOGUE

The brain indisputably plays the central role in terms of human physiology, psychology, and behavior. Its normal operation depends on *neurochemicals*, i.e., predominantly low molecular weight substances that transmit messages between nervous cells (neurons) or from a neuron to a muscular or glandular cell and/or modulate the efficiency of impulse transmission. Neurochemicals are subdivided into the following groups: (1) biogenic amines, including catecholamines (dopamine, norepinephrine, and epinephrine[19]), serotonin, histamine, octopamine, tyramine, and others; (2) amino acids (aspartic, glutamic, and γ-aminobutyric acid, glycine, and others); (3) peptides such as endorphins, enkephalins, dynorphins, substance P, etc.; (4) "gasotransmitters" including nitric oxide, carbon monoxide, and hydrogen sulfide; and (5) purines, e.g., adenosine and ATP. In this work, we

[19] Norepinephrine and epinephrine are also known as noradrenaline and adrenaline, respectively.

do not discuss the differences between *neuromediators (neurotransmitters)* that transmit impulses across the synaptic cleft between nervous cells and *neuromodulators* that modulate neurotransmitter effects and predominantly use the more general term *neurochemicals*, especially as many chemicals, e.g., norepinephrine (Boldyrev et al., 2010), combine these roles.

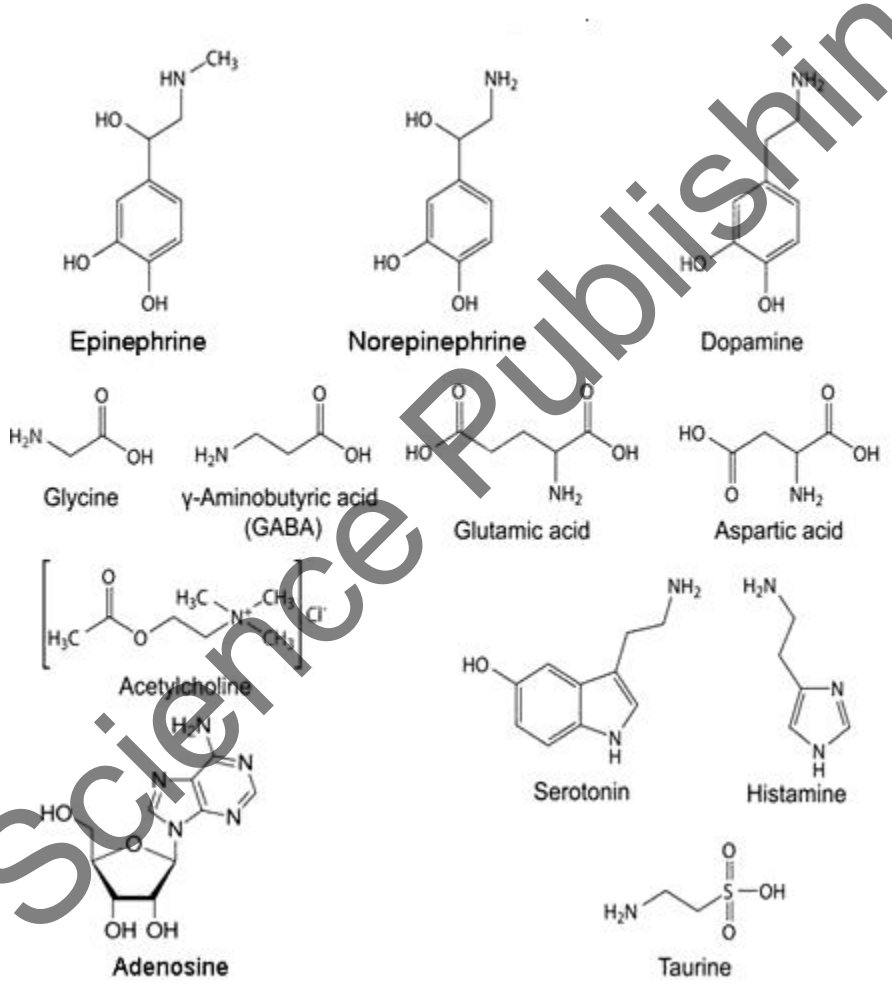

Figure 12. The formulas of some important neurochemicals.

Many neurochemicals are multifunctional agents: they combine the roles of neuromediators, hormones, and local tissue factors (histohormones). Some neurochemicals perform communicative and regulatory functions in diverse taxa of animals (Dubynin et al., 2010), plants (Roshchina, 1991, 2010, 2016), fungi (Buznikov, 1987, 2007), protozoans (Roshchina, 2010, 2016), and bacteria (reviewed, Lyte, 1993, 2010, 2014, 2016; Oleskin et al., 2010, 2016, 2017a,b; Oleskin & Shenderov, 2019), which enables using the more general term *biomediators* (Roshchina, 2010, 2016).

Synthesis of neuroactive compounds by microorganisms forms a part of *microbial endocrinology*, an interdisciplinary area of research that straddles the boundary between microbiology and neurology and focuses on neurochemical agents that are identical, homologous, or functionally analogous in the host organism and the microbiota. "Microbial endocrinology is defined as the study of the ability of microorganisms to both produce and recognize neurochemicals that originate either within the microorganisms themselves or within the host they inhabit" (Lyte, 2014, p.3). Microbial endocrinology actually emphasizes the fact that neuroactive substances formed both by multicellular organisms and microorganisms constitute an "universal language" that enables communication between different kingdoms and empires of life (see Mazzoli & Pensione, 2016).

The evolutionarily primary role of neurochemicals and hormones could be that of microbial communicative signals, according to the hypothesis that eukaryotic communication agents evolved as a result of horizontal gene transfer from prokaryotic microorganisms (Iyer et al., 2004; Lyte, 2014). As pointed out by Lyte (2014), the ubiquitous occurence of neuroendocrine hormones in nonmammal biosystems suggests that their presence in mammal organisms is to be interpreted in terms of their evolutionary prehistory.

Since the present book is mainly concerned with substances identical with or related to those involved in impulse transfer among nervous cells, we suggest an additional term, *microbial neurochemistry*. There are two main aspects of microbial neurochemistry: (i) production of neuroactive substances by microorganisms and (ii) the specific effects of neurochemical agents in microbial systems, including, e.g., the stimulation of microbial

growth and the regulation of the transition from the planktonic to the biofilm lifestyle of microorganisms. To reiterate, the human microbiota uses neurochemicals as signal agents (Lyte, 2014) and, therefore, is comparable to a primitive nervous system (see 1.3.11 above).

The literature data and the authors' own findings presented in this chapter indicate that microbial, dietary, or endogenous SCFAs, biogenic amines (BAs), neuroactive amino acids, neuropeptides, gasotransmitters, and purines interact with various receptors of prokaryotic and eukaryotic cells. This enables modifying the microecological (microbiota-based), immune, and nervous system of animals and humans. For instance, BAs (catecholamines, serotonin, histamine, acetylcholine, and, probably, agmatine) and other low molecular weight compounds perform a large number of endocrine and/or neurochemical functions in the human organism (Oleskin et al., 2016, 2017; Averina & Danilenko, 2017). Recently, much evidence has been presented that the same substances exert a strong influence on the physiological activities and the viability of diverse prokaryotes.

3.1. Interaction of Catecholamines with Microorgansims and the Host Nervous and Immune System

Catecholamines (dopamine, norepinephrine, and epinephrine) are derived from the non-essential[20] amino acid tyrosine whose hydroxylation yields *L*-3,4-dihydroxyphenylalanine (DOPA), the direct precursor of the catecholamine dopamine; its β-hydroxylation yields norepinephrine (noradrenaline). Its subsequent methylation produces epinephrine (adrenaline). In the mammalian organism, catecholamines are predominantly formed by the chromaffin cells of the adrenal medulla and by

[20] Tyrosine is synthesized in the human organism from phenylalanine, an essential amino acid that must be contained in food.

the axons of the sympathetic nervous system that effectuates the organism's response to stress; they are also produced in the brain. Significant catecholamine concentrations are characteristic of the GI tract. For instance, about 50% of the dopamine contained in the human organism is located in the gut (Liang et al., 2018). Neurochemical functions are performed by dopamine and norepinephrine; a direct involvement of epinephrine in the operation of the nervous system is questionable (Boldyrev et al., 2010).

Catecholamines are widely spread in invertebrate animals. They fulfill important functions in insects (Pitman, 1971; Gritsai, 2017), plants (Roshchina, 1991, 2010, 2016; Kulma & Szopa, 2007), and various unicellular organisms (Roshchina, 2010, 2016). In plants, in an analogy to animals (see below), catecholamines are released in response to stress. Tomatoes under stress, e.g., upon exposure to low temperatures, produce significant catecholamine amounts (Lyte, 2014).

3.1.1. Interaction of Catecholamines with Microorganisms

Recently, much evidence has been presented in the literature (Lyte, 1993, 2010, 2011, 2013a, b, 2014, 2016; Freestone et al., 2007) that catecholamines stimulate the growth of various microorganisms (see Table 2 that also contains data on the effects of other neuroactive compounds on microorganisms). Elevating the norepinephrine content in the mouse bloodstream by opening norepinephrine-accumulating sympathetic nerve terminals with the neurotoxin 6-hydroxydopamine resulted in a drastic increase in *E. coli* cell numbers in the mouse cecum (Lyte & Bailey, 1997). *In vitro* treatment of *Salmonella enterica* var. *Typhimurium* cells with norepineprine increased the proliferation rate of this pathogen in various tissues of infected pigs (Verbrugge et al., 2012).

Table 2. Effects of neurochemicals in microbial systems

Neurochemicals	Effects	Subjects and sources
Biogenic amines and their precursors, derivatives, and metabolites		
Catecholamines (dopamine, norepinephrine, epinephrine)	Stimulation of growth and, in pathogens,of virulence, flagellar motility, and adherence to host cells	*Escherichia coli* (commensal and pathogenic strains), *Shigella* and *Salmonella* species, *Pseudomonas aeruginosa* (Lyte & Ernst, 1993; Freestone et al., 1999, 2007; Anuchin et al., 2008); *Bordetella pertussis*, *B. broncioseptica*, (Freestone & Lyte, 2008); *Aeromonas hydrophila* (Kinney et al., 1999); *Helicobacter pylori*, *Haemophilus influenza*, *Klebsiella pneumonia* (reviewed, Shpakov, 2009); *Listeria monocytogenes* (Verbrugge et al., 2012), *Saccharomyces cerevisiae* (Malikina et al., 2010) *Lactobacillus acidophilus* NK-1 (Vodolazov, Zhilenkova, and Oleskin, unpublished)
	Stimulation of growth and medium acidification	
Additional effects of individual catecholamines: Dopamine	Inhibition of cell aggregation Promotion of spore survival and germination	*E. coli* K-12 (Anuchin et al., 2008) *Saccharopolyspora erythraea* (Filippova et al., 2010)
	Stimulation of growth and antibacterial activity	*Lactococcus lactis subsp.lactis* strain 194, F-116, K-205, 729 (Vodolazov et al., 2018)
	Stimulation of luminescence (at low concentrations)	*E. coli* TGI with the *lux* operon (Oleskin et al., 2017c)
Norepinephrine	Stimulation of cell aggregation	*E. coli* K-12 (Oleskin et al., 1998a; Anuchin et al., 2008), *Polyangium* sp. (Oleskin et al., 1998a) *Mycoplasma hyopneumoniae* (Oneal et al., 2008) Increase in the *Clostridium:Bacteroides* ratio (Bailey et al., 2011)
	Growth inhibition	

Neurochemicals	Effects	Subjects and sources
	Balance shift in the human GI tract	*Lactococcus lactis subsp.lactis* strain 194(Vodolazov et al., 2018)
	Stimulation of growth and antibacterial activity	*E. coli* TGI with the *lux* operon (Oleskin et al., 2017c)
	Inhibition of luminescence	
Serotonin	Growth stimulation	Commensal (Oleskin et al.,1998a; Anuchin et al., 2008) and, to a lesser extent, pathogenic (M.Lyte, personal communication) strains of *E. coli*, *Enterococcus faecalis*(Strakhovskaya et al.,1993); *Rhodospirillum rubrum* (Oleskin et al., 1998a); *Polyangium* sp. (Oleskin et al., 1998a); *Candida guillermondii* (Strakhovskaya et al., 1993); *Saccharomyces cerevisiae* (Malikina et al., 2010; Oleskin et al.,2010)
	Stimulation of growth and antibacterial activity	*Lactococcus lactis subsp.lactis* strain 194(Vodolazov et al., 2018)
	Stimulation of growth and medium acidification	*Lactobacillus acidophilus* NK-1 (Vodolazov, Zhilenkova, and Oleskin, unpublished)
	Stimulation of luminescence (at low concentrations)	*E. coli* TGI with the *lux* operon (Oleskin et al., 2017c)
	Stimulation of cell aggregation	*E.coli* K-12(Oleskin et al.,1998a;Anuchin et al., 2008), *Polyangium* sp.(Oleskin et al.,1998a).
	Photo- and radioprotective effects	*S. cerevisiae* (Fraikin et al., 1985)
	Growth inhibition	Chlamydia (Rahman et al., 2005)
	Virulence attenuation	*Candida albicans* (Mayr et al., 2005)
Melatonin	Swarming stimulation	*Enterobacter aerogenes* (Paulose & Cassone, 2016)
Indole	Growth stimulation	*Salmonella enterica* var. *enteritidis* (Vakhitov & Sitkin, 2014)
	Stimulation of biofilm formation	*Pseudomonas aeruginosa*, *Ps. fluorescens*
	Inhibition of biofilm formation	*E. coli*
Histamine	Growth stimulation	*E.coli* K-12(Anuchin et al., 2008).
	Stimulation of cell aggregation	*E. coli* K-12 (Anuchin et al., 2008)
	Stimulation of growth and medium acidification	*Lactobacillus acidophilus* NK-1 (Vodolazov, Zhilenkova, and Oleskin, unpublished)
	Stimulation of luminescence (at low concentrations)	*E. coli* TGI with the *lux* operon (Oleskin et al., 2017c)
Acetylcholine	Regulation of conjugation and growth	Infusorians (reviewed, Roschina, 2010), *Acanthamoeba* sp. (Baig & Ahmad, 2017)
Agmatine	Inhibition of colon colonization	*Cryptosporidium parvum* (Lyte, 2016)
Short-chain fatty acids and their derivatives		
SCFAs in general	Antimicrobial activity	Gram-negative bacteria (Neish, 2009; Shenderov 2013)

Table 2. (Continued)

Neurochemicals	Effects	Subjects and sources	
Acetate	Growth stimulation	*Roseburia* spp., *Faecalibacterium prausnitzi* (Duncan et al., 2004)	
Propionate	Antifungal activity	Various groups of fungi (van de Wouw et al., 2017)	
Phenylbutyrate	Induction of endogenous antimicrobial peptides	Various groups of bacteria (Raqib et al., 2006)	
Neuroactive amino acids			
Aspartate	Regulation of colony macro- and microstructure	*E.coli* (Budrene &Berg, 1991, 2002; Mittal et al., 2003)	
	Growth stimulation	*E. coli* BL	Vakhitov et al., 2000; Vakhitov & Sitkin, 2014; Vakhitov, 2019
	Growth inhibiion	*E. coli* M-17	
Glutamate	Growth stimulation	*E. coli* M-17	
GABA	Increase in resistance to acidification	*Lact. reuteri* (Lyte, 2014)	
	Virulence stimulation	*Ps aeruginosa* (Mazzoli & Pessione, 2016)	
	Virulence and germination stimulation	*C. albicans* (Reyes-Garcia et al., 2012)	
Neuropeptides			
Dynorphin	Stimulation of virulence,pyocyanine production, and antagonistic activity	*Ps. aeruginosa* (Zaborina et al., 2007)	
[Met]^5-Enkephalin	Growth inhibition	*Ps.aeruginosa, Staph.aureus*, *Serratia marcescens* (Zagon & McLaughlin,1992)	
α–MSH	Growth inhibition	*Saph. aureus* (Shireen et al., 2015)	
LL-37 (catelicidin)	Stimulation of virulence and antibiotic resistance	*Ps. aeruginosa* (Strempel et al., 2013)	
Insulin	Regulation of carbon metabolism	*Neurospora crassa* (Lenard, 1992)	
Substance P	Antimicrobial activity	Many gram-positive and gram-negative bacteria and fungi: the data are discordant (Kowalska et al., 2002; Hansen et al., 2006; El Karim et al., 2008)	
Neuropeptide Y			
Gasotransmitter			
Nitric oxide: Low (nanomolar) concentrations	Inhibition of biofilm formation and acceleration of biofilm dispersal	*Ps. aeruginosa* (Barraud et al., 2006), *S. marcescens*, *Vibrio cholerae*, *E. coli* (pathogenic strain BW20767), *Staphylococcus epidermidis, Bacillus licheniformis*, *C. albicans* (Barraud et al., 2009a, b)	
High (micro- and millimolar) concentrations	Stimulation of biofilm formation, cytotoxic and stressor effects	*Ps. aeruginosa* (Barraud et al., 2006); *Azospirillum brasilense*, *Neisseria gonorrhoeae* (reviewed: Medinets et al., 2015); *Mycobacterium tuberculosum* (Robinson et al., 2014)	
Purines			
Adenine nucleotides	Required for host invasion	Uropathogenic *E. coli* strains (UPEC; Andersen-Civil et al., 2018)	

These data apparently account for the fact that cold shock-induced catecholamine release in the bloodstream of mice (Previte et al., 1970) and norepinephrine administration to them (Williams et al., 2006) increase the incidence of *Salmonella* infection. Gangrene and fulminating sepsis with a fatal outcome were reported to develop in patients to whom epinephrine was administered; incompletely sterilized syringes were used that contained surviving *Clostridium perfringens* spores (published in 1929; cited according to: Lyte, 2011, 2014). Interestingly, norepinephrine and epinephrine also increase the sensitivity of plants to bacterial and fungal infections (Lyte, 2014).

The stimulation of microbial growth by catecholamines in an animal organism can be due to both the *direct* and the *indirect effect* of these compounds on microorganisms. Norepinephrine and other catecholamines suppress the synthesis and excretion of immunoglobulin A and phagocyte migration, decreasing the antimicrobial activity of the local immune system (Lyte, 2010, 2011, 2014). By stimulating peristalsis and bile release, catecholamines accelerate the transit of a food bolus through the GI tract and ion transfer through the intestinal epithelium. Therefore, the conditions are created in the GI tract that stimulate the growth of some representatives of the intestinal microbiota, e.g., of bacteria belonging to the genus *Bacteroides* (Verbrugge et al., 2012).

A direct stimulatory effect of catecholamines on microbial growth was revealed in vitro for a wide variety of pathogenic, opportunistic, and saprotrophic bacteria, including *Yersinia enterocolitica*, a number of enterotoxic and enterohemorrhagic strains of *E. coli*, *Shigella* spp., *Salmonella* spp., *Ps. aeruginosa* (Freestone et al., 2007), *Bordetella pertussis*, *Bord. bronchiseptica* (Freestone and Lyte, 2008), *Aeromonas hydrophila* (Kinney et al., 1999), *Helicobacter pylori*, *Haemophilus influenza*, *Klebsiella pneumonia* (Shpakov, 2009), *Listeria monocytogenes* (Verbrugge et al., 2012), some *E. coli* symbiotic strains (Freestone et al., 2007; Anuchin et al., 2008), and the yeast *Saccharomyces cerevisiae* (Malikina et al., 2010; Oleskin et al., 2010). Noreprinephrine enables *Camp. jejunii*, normally requiring low oxygen concentrations (microaero-philic conditions), to grow in the absence of oxygen (Lyte, 2014).

All catecholamines stimulated the growth (estimated from the increase in the culture's optical density and colony-forming unit, CFU, number) and biochemical activity (determined from the pH decrease caused by organic acid formation) of the probiotic strain *Lactobacillus acidophila* NK-1 (Vodolazov, Zhilenkova, and Oleskin, unpublished). Dopamine also promoted the growth and antibacterial activity of all tested strains of the probiotic *Lactococcus lactis subsp. lactis*, whereas the other tested amines, epinephrine and serotonin, exerted a stimulatory effect only on one of the strains, strain 194 (Vodolazov et al., 2018).

Low concentrations of dopamine stimulated, and its high concentrations inhibited, the bioluminescence (light emission) of the *E. coli I* TGI strain containing the luciferase operon (*lux*) of the bacterium *Photorhabdus luminescencens* ZMI. Norepinephrine suppressed bioluminescence at all tested concentrations. The bioluminescence is envisaged as an integral index of the physiological state of *E. coli* cells (Oleskin et al., 2017c).

When applied at high concentrations, catecholamines can produce a cytotoxic effect, which is attributable to their ability to induce oxidative stress. Apart from suppressing the growth of yeast (*S. cerevisiae, Pichia pastoris, Candida albicans,* etc.), high concentrations of dopamine (and 6-hydroxydopamine) kill yeast cells. Addition of antioxidants (ascorbate and glutathione) to the cultivation medium relieves the toxic effect of dopamine (Macreadie et al., 2010).

Catecholamines promote adherence of GI microorganisms to the intestinal mucosa and formation of adherence-enabling type I pili in symbiotic *E. coli* strains, attachment of *Staphylococcus epidermidis* to skin cells, and biofilm formation by these microorganisms (Lyte, 2010, 2011). Apart from cell proliferation, catecholamines stimulated toxin and adhesin formation in an enterotoxic *E. coli* strain (Freestone et al., 2007). Under these conditions, other pathogenic bacteria also increased their virulence and capacity for colonizing the GI tract (Lyte et al., 1996; Clarke et al., 2006; Shpakov, 2009). The adherence of the cells of the enterohemorrhagic *E. coli* strain O157:H7 to the mucosa increases in the presence of norepinephrine because it induces the expression of F5 pili involved in attaching bacterial cells to the intestinal mucosal epithelium (Verbrugge et al., 2012).

In *Salmonella enterica* var. *Typhimurium*, norepinephrine increases the secretion of virulence factors, especially the type III secretion system (the "molecular needle" enabling bacterial protein injection into the host cell cytoplasm) and flagellar motility. Norepinephrine also facilitates the translocation of pathogenic *E. coli* to Peyer glands (the intestinal lymphoid tissue; Lyte, 2011).

In studies with the mouse model, it was established that partial removal of the liver, which results in increasing the norepinephrine concentration in the intestinal lumen, stimulated *Ps. aeruginosa* adherence to the intestinal mucosa (Freestone et al., 2007).

The effects of catecholamines vary depending on their concentrations and the taxonomic position of the tested microorganisms. Norepinephrine, epinephrine, and dopamine stimulate the growth of *Vibrio parahaemolyticus* and *V. mimicus,* but not *V. vulnificus* and *V. cholerae* (Nakano et al., 2007); norepinephrine inhibits the growth of *Mycoplasma hyopneumoniae* by suppressing the expression of the genes required for proliferation (Oneal et al., 2008). Dopamine drastically stimulates proliferation of the yeast *S. cerevisiae;* conversely, norepinephrine produces little effect in this system (Malikina et al., 2010). When added to a solid medium, dopamine and norepinephrine produce different effects on microcolony formation in *E. coli* K-12: norepinephrine stimulates and dopamine inhibits this process (Anuchin et al., 2008). Norepinephrine and epinephrine shift the *Clostridium:Bacteroides* ratio in the gut in favor of *Clostridium* (Bailey et al., 2011).

Dopamine and epinephrine stimulate spore germination in the actinobacterium *Saccharopolyspora erythraea* (strains RIA 1387 and RIA 120) and stabilize the composition of its population. They increase the colony-forming unit (CFU) number of the dominant phenotype, the most efficient producer of the antibiotic erythromycin. Nonetheless, only dopamine and not epinephrine increase the viability of *S. erythraea* spores after 3 months of storage or 10 minutes of freezing and promote their transition to the active vegetative state. Hence, different catecholamines produce different effects in this system (Filippova et al., 2010).

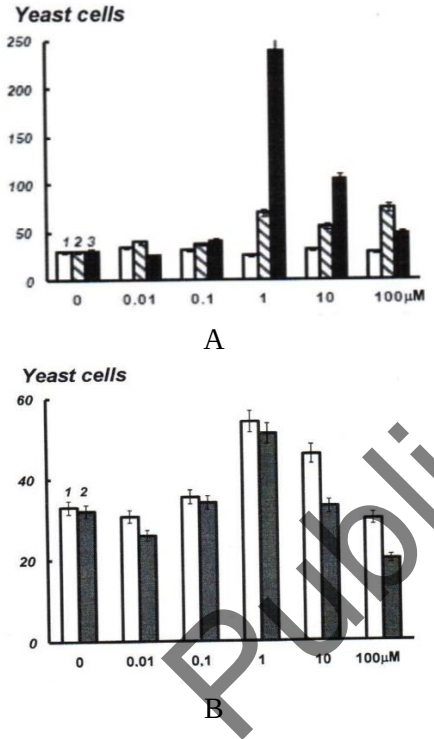

Figure 13. Effects of biogenic amines on *S. cerevisiae* proliferation on Sabouraud agar (15 h culture). A, effects of norepinephrine (1), apomorphine (2), and dopamine (3); B, effects of serotonin (1) and histamine (2). Vertical axis, cell number per field of view. According to: Malikina et al., 2010, modified.

Norepinephrine and dopamine alter the gene expression profile in a number of prokaryotes, such as *Mycoplasma hyopneumoniae, Salmonella enterica* serovar *Typhimurium,* and *Vibrio parahaemolyticus* (Lyte, 2014).

Two different hypotheses have been suggested to account for the catecholamine effects. According to the first of them, these compounds can chelate ferric iron, removing it from the lactoferrin and transferrin of the blood serum and other biological liquids. Catecholamine-bound iron becomes available to microorganisms that use specific carriers – siderophores such as enterobactin (Lyte et al., 1996) – to transfer it into the cell. As a result, the growth of iron-dependent strains of *E. coli, Salm. enterica* var. *enteritidis, Camp. jejuni, Bord. bronchiseptica, Ps. aeruginosa, Listeria monocytogenes,* and coagulase-negative staphylococci is stimulated

(Verbrugge et al., 2012). This mechanism accounts for the fact that adding norepinephrine to enterobacteria in a nutrient-low, iron-limited medium (that to a certain extent resembles the environment in the host organism) increases their cell numbers by several orders of magnitude (Lyte, 2011). The lower the inoculum cell number, the more significant is the catecholamine effect. It is the presence of catecholamines in the host organism that makes it possible for a very small number of pathogen cells, e.g., those of *E. coli* O157:H7, to cause food-born infections (Lyte, 2011).

In accordance with the alternative hypothesis, the catecholamine effects on bacteria should be interpreted in terms of quorum-sensing communication (Clarke et al., 2006; Bansal et al., 2007). Catecholamines operate as signal AI-3 analogs (see 1.3.3 above). Like AI-3, they bind to histidine kinases QseC and QseE in *E. coli*. Therefore, these receptors can be regarded as functional analogs of the receptors of eukaryotic cells even though they differ from eukaryotic receptors (known as G proteins) in structural terms (Clarke et al., 2006; Hughes et al., 2009). Genes related to the histidine kinase gene (*qseC*) were revealed, apart from *Haemophila influenza* and representatives of the genera *Salmonella* and *Shigella*, in a large number of bacteria including *Erwinia carotovora, Thiobacillus denitrificans, Psychrobacter* sp., and in the fungus *Aspergillus nidulans* (Shpakov, 2009). This suggests that such receptors may be involved in controlling the development of multispecies communities.

Bacterial receptors QseC are functionally similar to the α-adrenergic receptors of eukaryotes. In the pathogenic *E. coli* O157:H7 strain, *Salm. enterica,* and *Yersinia enterocolitica,* their interaction with norepinephrine, epinephrine, and the AI-3 signal is blocked by α-adrenoreceptor-blocking agents such as phentolamine, phenoxybenzamine, and prazosin, but not by the β-adrenoreceptor-blocking agents propranol and labetalol (Clarke et al., 2006; Freestone et al., 2007). It was established that the stimulatory action of norepinephrine on *E. coli* O147:H7 adhesion to the cecal epithelium and internalization in Peyer glands is prevented by pretreating the intestinal tissue with phentolamine (Freestone et al., 2007; Lyte, 2011).

In light of the available data, it seems likely that, by interacting with autoinducer AI-3 and catecholamines, microbial receptor systems

participate in the "talk" among microbial cells and the chemical "dialogue" between the microbiota and the host organism. Stress agents and catecholamine synthesis-promoting factors may influence the abundance, composition, and operation of the microbiota of the GI tract and, presumably, of other mucous membranes and of the skin. During the course of long-term co-evolution of the host organism and the microbiota, neuroactive substances of prokaryotic and/or eukaryotic origins became an integral part of the "alerting system" used by both the host cells and pathogenic or opportunistic bacteria (Trueba & Ritz, 2013). The antagonists of adrenergic and dopaminergic receptors are of potential medical interest. For instance, andenergic receptor antagonists can inhibit the AI-3-, epinephrine-, or norepinephrine-dependent quorum-sensing cascade in the pathogenic strain *E. coli* EHEC, preventing the expression of its virulence genes. These antagonists can become a new class of antimicrobial preparations (Clarke et al., 2006).

The catecholamine-mediated dialogue in the microbiota–host system is bidirectional. Apart from responding to host-produced catecholamines, microorganisms are also capable of producing them (Table 3; it also contains data on other microbially produced neuroactive compounds).

High-performance liquid chromatography (HPLC) with amperometric detection was used to identify and quantitatively determine catecholamines in the cultures of a large number of prokaryotic and eukaryotic microorganisms (Tsavkelova et al., 2000).

Norepinephrine was present at concentrations of 0.2-2 μM in the biomass of *Bacillus mycoides, B. subtilis, Proteus vulgaris,* and *Serratia marcescens;* dopamine at concentrations of 0.5-2 μM was found in the biomass of the majority of the tested prokaryotes. These catecholamine concentrations considerably exceed those in human blood, which contains 0.1-0.5 nM free (unbound) dopamine and 1-2 nM norepinephrine (Eldrup, 2004).

Table 3. Production of neurochemicals by microorganisms

Neurochemicals	Subjects	Sources
Biogenic amines and their precursors		
Dopamine	*Bacillus cereus, B. mycoides, B. subtilis, Staphylococcus aureus, E. coli, Ps. aeruginosa, Serratia marcescens, Proteus vulgaris, Saccharomyces cerevisiae*	Tsavkelova et al., 2000; Shishov et al., 2009; Malikina et al., 2010; ; Oleskin et al., 2010
	Morganella morganii, Klebsiella pneumonia, Hafnia alvei	Özogul, 2004
	Lactobacillus helveticus NK-1, *Lact. delbrueckii* subsp. *bulgaricus*	Oleskin et al., 2014a, b
	Lactococcus lactis subsp. *lactis*, strains K-205 and F-116	Vodolazov et al., 2018
Norepinephrine	*B. mycoides, B. cereus, B.subtilis, P. vulgaris, S. marcescens, E. coli, S. cerevisiae, Pennicilum chrysogenum*	Tsavkelova et al., 2000; Shishov et al., 2009; Malikina et al., 2010; Oleskin et al., 2010
	Lact. helveticus 100ash, *Lact. helveticus* NK-1, *Lact.casei* K3III24, *Lact. delbrueckii* subsp. *Bulgaricus*	Oleskin et al., 2014 a, b
DOPA	*E. coli* K-12, *S. cerevisiae, B. cereus*	Shishov et al., 2009; Malikina et al., 2010; Oleskin et al., 2010
	Lact. helveticus 100ash, *Lact. helveticus* NK-1, *Lact.casei* K3III24, *Lact. delbrueckii* subsp. *bulgaricus*	Oleskin et al., 2014a, b
	Lactococcus lactis subsp. *lactis*, strains K-205 and F-116	Vodolazov et al., 2018
	Toxoplasma gondii	Rohrscheib & Brownlie, 2013
Serotonin	*Staph. aureus*	Hsu et al., 1986
	Enterococcus faecalis	Strakhovskaya et al., 1993
	Rhodospirillum rubrum, B. subtilis, Staph. aureus, E. coli K-12, *S. cerevisiae*	Oleskin et al., 1998a; Tsavkelova et al., 2000; Shishov et al., 2009; Malikina et al., 2010
	Morganella morganii, Klebsiella pneumonia, Hafnia alvei	Özogul, 2004
	Lactococcus lactis subspecies *cremoris* MG 1363, *L. lactis* subspecies *lactis* IL 1403, *Lact. plantarum* NCFB2392.	Özogul et al., 2012
	Lact. helveticus 100ash	Oleskin et al., 2014a, b
Histamine	*Morganella mogranii, Proteus vulgaris, Pr. mirabilis, Klebsiella* spp., *Enterobacter aerogenes, Enterococcus. faecalis, Citrobacter freundii, Raoultella orhithinolytica, Pantooea agglomerans, Vibrio alginolyticus, V. fischeri, V. harveyi, Acinetobacter lowfli, Pseudomonas fluorescens,*	Devalia et al., 1989; Halász et al., 1994; Shenderov, 1998; Roig-Sagués et al., 2002; Özogul, Özogul, 2005, 2007; Roshchina, 2010; Gardini et al., 2012; Helinck et al., 2013; Lin et al., 2014; Doeun et al., 2017; van de Wouw et al., 2017

Table 3. (Continued)

Neurochemicals	Subjects	Sources
	Ps. putida, Ps. aruginosa, Aeromonas spp., *Clostridium* spp., *Photobacterium* spp., *Branhamella catarrhalis,Haemophilus parainfluenza, Streptococcus thermophilus, Bacillus licheniformis, B. coagulans, Lactobacillus buchneri, Lact. reuteri, Lact. casei, Lactococus lactis*; the yeast *Debaryomyces hansenii* and *Yarrowia lypolytica*	
Tyramine	*Lactobacillus brevis, Lact. plantarum, Lact. delbrueckii, Lact. casei, Lactococcus lactis, Leuconostoc mesenteroides*, the yeast *D. hansenii* and *Y.lypolytica*	Roig-Sagués et al., 2002; Doeun et al., 2017
Indole	*E. coli, Bacteroides ovatis, Clostridium bifermentes, Ps. aeruginosa, Ps. fluorescens*	Smith & Macfarlane, 1996; Lee et. al., 2007b; Vega et al., 2012
Acetylcholine	*Bacillus* spp., *Lactobacillus* spp.	Wall et al., 2014; Johnson & Foster, 2018
Neuroactive amino acids		
Agmatine	*Lactobacillus* spp.	Reviewed, Oleskin et al., 2017a
Glutamate	*E. coli, Corynebacterium glutamicum, Brevibacterium lactofermentum, B. flavum, Lactobacillus helveticus* 100ash, *Lact. helveticus* NK-1, *Lact. casei* K3III24, *Lact. delbrueckii* subsp. *bulgaricus*	Vakhitov & Sitkin, 2014; Oleskin et al., 2014a b; Mazzoli & Pessione, 2016
Aspartate	*E. coli*	Vakhitov & Sitkin, 2014
GABA	*Lactobacillus brevis, Lact. rhamnosus, Lactococcus lactis, Lact. helveticus* 100ash, *L. helveticus* NK-1, *Lact.casei* K3III24, *Lact. delbrueckii* subsp. b*ulgaricus, Bifidobacterium adolescentis*, and other lactobacilli and bifidobacteria,	Lee et al., 2010; Barrett et al., 2012; Ko et al., 2013 ; Liao et al., 2013; Oleskin et al., 2014a, b; Mazzoli & Pessione, 2016; Yunes, 2017
Glycine	*Lact. helveticus* 100ash, *Lact. helveticus* NK-1, *Lact.casei* K3III24, *Lact. delbrueckii* subsp. *bulgaricus*	Oleskin et al., 2014a, b
Taurine		
Short-chain fatty acids		
SCFAs in general	Various representatives of the GI micrbiota	Reviewed, Oleskin & Shenderov, 2016; Oleskin et al., 2017a
Propionate	*Propionibacterium* spp.	MacFabe, 2012

Neurochemicals	Subjects	Sources
Neuropeptides		
β-Endorphin	*Tetrahymena pyriformis, Amoeba proteus*	Lenard, 1992
[Met]^5-Enkephalin	*Staph. aureus*	Zagon & McLaughlin, 1992
Insulin	*E. coli,Neurospora crassa*	Lenard, 1992
Corticotropin	*Tetrahymena pyriformis*	
Somatostatin	*B. subtilis, Plasmodium falciparum*	
α-Factor, a homologue of gonadotropin-liberating factor	*S. cerevisiae*	
Gasotransmitters		
Nitric oxide	Manymicroorganisms including *Firmicutes, Actinobacteria, Proteobacterium,* and archaeans (e.g., *Euryarchaeota*)	Zumft, 1993; Barraud et al., 2006; Ramírez-Mata et al., 2014; Medinets et al., 2015
Carbon (mono)oxide	Many hemoxidase-containing microorganisms	King & Weber, 2007; Tinajero-Trejo et al., 2013;
Hydrogen sulfide	*E. coli* and many other GI bacteria	Carbonero et al., 2012; Olas, 2015
Ammonia	Many urease-containing microorganisms, including peptostreptococci, ruminococci, bifidobacteria, lactobacilli, clostridia, bacteroids, streptococci, and the yeast *Candida albicans*	Shenderov, 1998; Burrus, 2012; Richardson et al., 2013; Oleskin & Shenderov, 2016

In the matrix-rich bacterium *B. subtilis* (the M variant), neurotransmitters (norepinephrine and dopamine) are mainly contained in the matrix fraction. This fact supports the idea that these amines function as cell-cell communication signals, because the hydrophilic biopolymer components of the matrix promote the diffusion of low molecular weight signal molecules within the colony (biofilm). A majority of the tested microorganisms also contain the products of oxidative deamination of biogenic amines such as dihydrophenylacetic acid (DHPAA) produced from dopamine and 5-hydroxyindoleacetic acid (5-HIAA) that results from degrading serotonin (Tsavkelova et al., 2000).

Micromolar concentrations of dopamine were also detected in *Morganella morganii* (2.46 mg/L, ~16 μM), *Klebsiella pneumonia* (1.06 mg/L, ~7 μM), and *Hafnia alvei* (0.73 mg/L, ~5 μM) that were isolated from fish products (Özogul, 2004). Some researchers are convinced that dopamine is ubiquitous in the world of pro- and eukaryotic microorganisms:

"in bacteria, fungi, protozoans… dopamine seems present wherever it is sought" (Vidal-Gadea & Pierce-Shimomura, 2012, p.440). *S. cerevisiae* and *Penicillium chrysogenum* contain norepinephrine (0.21 and 21.1 μM, respectively; Tsavkelova et al., 2000).

Table 4. Concentrations of biogenic amines and their metabolites in microbial cells

Subject	NE	DA	DHPAA	5-HT	5-HIAA
Bacillus cereus	-	2.13	0.69	0.85	0.95
B. mycoides	0.32	0.25	0.81	-	0.33
*B.subtilis:*Total fraction	0.25	0.36	-	-	0.42
Cells	-	-	-	-	
Matrix	0.26	0.34	-	-	0.52
Staph. aureus	-	1.35	1.54	2.2	-
E. coli	-	1.61	2.64	-	0.81
Proteus vulgaris	0.6	0.73	0.46	-	0.4
Ps. aeruginosa, var. R	-	-	1.62	-	2.7
Ps.aeruginosa, var. S	-	-	3.74	-	2.1
Serratia marcescens	1.87	0.6	1.47	-	0.51
Zoogloea ramigera	-	-	14.2	-	0.34
Saccharomyces cerevisiae	0.21	-	-	-	0.26
Penicillum chrysogenum	21.1	-	88.9	-	10.8

The cells were ultrasonically disintegrated, BA contents were measured by HPLC with an amperometric detector (data from the authors' work: Tsavkelova et al., 2000). All concentrations are expressed in micromoles/kg of biomass. Designations: NE, norepinephrine; DA, dopamine; 5-HT, serotonin; DHPAA, dihydrophenylacetic acid; 5-HIAA, 5-hydroxyindolylacetic acid.

Using the *E. coli* model, it was established (Shishov et al., 2009) that maximum (micromolar) catecolamine concentrations accumulate during the lag phase of culture growth. In light of these data, it should be suggested that neuromediator amines behave as triggers that activate growth processes and cell division during the initial phase of the ontogeny of the microbial culture. This is comparable with the effects of other known autoregulatory compounds. The biomass of *E. coli*, *S. cerevisiae*, *B. cereus*, and lactobacilli also contained DOPA, the catecholamine precursor in animal cells, and the products of oxidative deamination of catecholamines (DHPAA and homovanillic acid).

Analysis of the data available in the literature gives grounds for the suggestion that the metabolic pathways of neuromediator amines are universal for prokaryotic and eukaryotic organisms and follow the pattern shown below:

Tyrosine → DOPA → *Dopamine* → *Norepinephrine* (products of norepinephrine oxidation not shown) ↓

DHPAA→ Homovanillic acid

These pathways involve the enzymes that catalyze catecholamine synthesis (hydroxylases and decarboxylases of aromatic amino acids) and degradation (monoamine oxidases, MAOs). There is evidence that such MAOs are actually present in microorganisms. For instance, *Sarcina lutea* contains MAO that catalyzes the oxidation of dopamine but not of histamine and diamines (Yagodina et al., 2000).

The fact that microorganisms carry out all the animal-specific stages of the catecholamine synthesis and degradation pathway is consistent with the hypothesis that cell-cell signalling in vertebrates, including impulse transmission across the synaptic cleft between neurons resulted from horizontal gene transfer from the microbiota (Lyte, 2011).

The culture liquid of *E. coli* grown in the M-9 medium, a synthetic mineral medium with glucose, contained nanomolar concentrations of extracellular serotonin, dopamine, and norepinephrine at the later stages of bacterial growth (Shishov et al., 2009, see Figure 14). These concentrations are sufficiently high to enable the neuromediators to bind to the specific receptors of the GI tract.

Of special interest is the fact that an *E. coli* culture grown on the M-9 medium contained the catecholamine precursor DOPA that was present at micromolar concentrations both intracellularly and in the culture liquid. Presumably, DOPA functions as a long action range regulator; its conversion into dopamine that stimulates *E. coli* growth (Anuchin et al., 2008) can proceed within cells that take up DOPA. It has been known for over one hundred years that the lag phase is shortened and cell proliferation in a bacterial culture is stimulated under the influence of the cell-free supernatant

of an exponential-phase culture (Rahn, 1906; Penfold, 1914). This phenomenon may partly be accounted for by the effects of extracellular DOPA, along with those of other autostimulators.

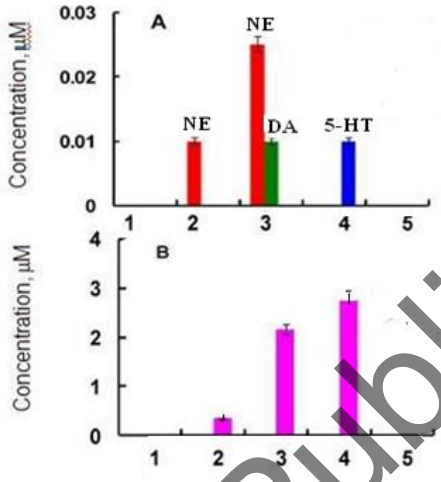

Figure 14. Concentrations of biogenic amines (A) and DOPA (B) in the cultural liquid of *E. coli* K-12 on M-9 medium. Horizontal axis: 1, lag phase; 2, early exponential phase; 3, late exponential phase; 4, stationary growth phase; 5, supernatant of the medium with the inoculum. Designations: DA, dopamine; NE, norepinephrine; 5-HT, serotonin. According to Vladimir Shishov's Cand Sci. (Ph. D.) dissertation (2010).

Unlike *E. coli*, the yeast *S. cerevisiae* only intracellularly accumulates neurochemicals (dopamine, norepinephrine, and serotonin), the products of their metabolism (homovanillic acid and dihydroxyphenylacetic acid), and the precursor DOPA. They were present at micromolar concentrations in yeast biomass if the yeast was grown in a synthetic medium that contained no neurochemicals. They are not released into the medium. If the neurochemicals-containing Sabouraud medium was used, the concentrations of all tested compounds decreased during the cultivation of the yeast, which was indicative of their active uptake from the medium by *S. cerevisiae* cells (Malikina et al., 2010; Shishov, 2010; Oleskin et al., 2010). Apparently, neuromediator amines do not function as intercellular communicative factors in *S. cerevisiae* populations. Nevertheless, since yeast responds to exogenous neuromediators (see the preceding section), the amines may be

involved in regulating the development of yeast cultures by other ecosystem components.

Catecholamines and serotonin are chemically similar to the aromatic alcohols phenylethanol and tryptophol that function as autoregulators in *S. cerevisiae.* These alcohols control cell differentiation during the transition from solitary cells to branched filaments (pseudomycelium) in a nitrogen-limited medium (Chen & Fink, 2006). Presumably, yeast cells respond to the neuromediators because they represent functional analogs of the yeast autoregulators. Several yeast species contain the autoregulator tyrosol that is structurally related to tyrosine, the DOPA precursor (Chen et al., 2004). Tyrosol belongs to alkylhydroxybenzenes that control the formation of dormant forms in a large number of prokaryotes and yeasts (El'-Registan et al., 2006).

Of paramount importance is the presence of catecholamines in dairy products that are fermented by probiotic bacteria. For instance, norepinephrine and dopamine were present at concentrations of 0.1-2 μM and 1-10 μM, respectively, in various yogurt samples, whereas the growth substrate per se (unskimmed milk) maximally contained 0.09 μM norepinephrine and lacked dopamine. DOPA was present in the yogurts at concentrations of 80-250 μM, while its content in milk did not exceed 57 μM (Zhilenkova et al., 2013).

Starter strains of lactobacilli (*Lactobacillus helveticus* 100ash, *Lact. helveticus* NK-1, *Lact. casei* K3III24 and *Lact. delbrueckii* subsp. *bulgaricus*) differed in catecholamine production activity. On media with milk (1%) or pancreatic digest of caseine, dopamine was only synthesized by *Lact. helveticus* NK-1 and *Lact. delbrueckii* subsp. *bulgaricus*; all the tested strains, except *Lact. casei* K3III24, enriched both kinds of media in norepinephrine. All strains formed DOPA, and its maximum concentration (over 5 μM) was attained with strain *Lact. helveticus* NK-1 (Oleskin et al., 2014a, b, Table 5). Since DOPA passes the gut–blood barrier and the BBB and, therefore, is used to treat Parkinson disease (Dubynin et al., 2010), these results hold much promise with respect to the employment of DOPA in the form of dairy products fermented by efficient producers of this catecholamine precursor.

Table 5. Maximum concentrations of neuroactive compounds in the tested cultures of lactobacilli

Compound	Strain	Medium	Concentration, μM	
			Cuture liquid	Medium (control)
DOPA	NK-1	PDC	5,37±1,00	0,70±0,10
Dopamine	NK-1	Milk	0,07 ±0,01	0,04±0,01
Norepinephrine	*Lb*	PDC	3,47±0,55	0,69±0,05
DHPAA	100ash;K_3III_{24}	Milk	0,15±0,03	0,03±0,01
Homovanilic acid	100ash	PDC	0,08±0,02	0
Serotonin	100ash	PDC	0,40±0,15	0
5-HIAA	*Lb*	PDC	0,08±0,02	0
Glutamate	K_3III_{24}*	Milk	0,62±0,10	0,20±0,03
Glycine	100ash*	Milk	0,31±0,10	0,13±0,02
Taurine	K_3III_{24}	Milk	0,47±0,05	0,13±0,02
GABA	*Lb*	Milk	0,90±0,04	0,02±0,01

Designations: PDC, medium with pancreatic digest of caseine; 100ash, strain *Lactobacillus helveticus* 100ash; NK-1, strain *Lact. helveticus* NK-1; K3III24, strain *Lact. casei* K3III24; *Lb, Lact. delbrueckii* subsp. *bulgaricus*. Data from the authors' work: Oleskin et al., 2014b: see also Oleskin et al., 2014a.

DOPA production was also documented in the parasitic protozoan *Toxoplasma gondii*. In the brain tissue of its intermediate hosts (mice or rats), toxoplasma cells convert tyrosine to DOPA that is thereupon transformed into dopamine. Therefore, the dopamine concentration in the hippocampus and the amygdala increases by approximately 14%. As a result, the behavior of the rodent becomes more active; it stops avoiding cats. Moreover, it finds the odor of cat urine attractive. This increases the probability of the ingestion of a toxoplasma by the definitive host (Rohrscheib & Brownlie, 2013).

Upon invading the human brain, *T. gondii* causes mental symptoms including delirium and hallucinations. Apart from increasing dopamine production, it alters the expression of the genes that are involved in the functioning of other neurochemical systems that depend on serotonin, glutamate, and γ-aminobutyric acid. The production of toxoplasma antibodies is increased in human individuals with serious mental problems including schizophrenia. It was hypothesized that *T. gondii* is implicated in

the development of schizophrenia, which is often associated with an increase in dopamine concentrations in functional brain areas (Yolken &Torrey, 2015).

The content of dopamine and norepinephrine in the cecal lumen of GF mice increased after intragastric treatment with the *Clostridium* cocktail, i.e., a mixture of 46 *Clostridium* species (belonging to the *coccoides* and *leptum* groups). In the intestine of the control mice, 90% of the dopamine and 40-50% of the norepinephrine pool were in the bound form, whereas 90% of the catecholamine pool of the *Clostridium*-treated mice was in the unbound form. From these results, the conclusion was drawn (Asano et al., 2012) that the intestinal microbiota is essential for conversion of catecholamines into biologically active forms in the gut lumen.

3.1.2. Interaction of Catecholamines with the Nervous System

The catecholamines dopamine and norepinephrine are major neurotransmitters. They also function as (neuro)hormones: norepinephrine is an adrenal hormone, and dopamine the hypothalamic neurohormone that suppresses lactation in females. Epinephrine is an adrenal hormone. Catecholamine levels in the organism are known to increase under stress. The functional role of catecholamines in the organism is related to the activation of the sympathetic nervous system that is responsible for "fight or flight" behavior. Catecholamines are involved in cognition, information memorization, and emotions, as well as in regulating the endocrine system (Oleskin et al., 2017; Averina & Danilenko, 2017). Catecholamines also perform more specific functions (see Table 6).

Dopamine causes vasodilation (at moderate concentrations)[21], stimulates sodium excretion and urine production by the kidneys, and suppresses intestinal movement and insulin secretion by the pancreas (Bronwen & Knights, 2009; de Backer et al., 2010). Importantly, "the stomach… is essentially a dopaminergic organ producing the highest amount of dopamine in the body" (reviewed, Lyte, 2016).

[21] High dopamine concentrations, conversely, cause vasoconstriction.

Table 6. Functions of neurochemicals in the nervous system

Neurochemicals	Neurophysiological and psychological effects	
Dopamine	Activation of the sympathetic nervous system; involvement in cognition, information memorization, and emotions	Maintenance of the wakeful state and stimulation of hedonic behavior; involvement in effectuating voluntary movements
Norepinephrine		Stimulation of locomotor activity aggressiveness and mitigation of anxiety
Serotonin	Regulation of the emotional state, memorization and learning processes, and dominant behavior. Appetite suppression. "Putting the brain asleep" at high concentrations.	
Histamine	Involvement in regulating appetite, pain sensitivity, the cognitive activity of the brain, and the sleep-wake rhythm	
Acetylcholine	Regulation of brain processes related to motivation, attention, memory, learning, plasticity, and the general activity level of the brain	
Agmatine	Hypothetic neurochemical function, consistent with the data on synthesis of agmatine in the brain, its accumulation in synaptic vesicles, and release upon membrane depolarization	
Glutamate	Main excitatory neurochemical in the CNS that exerts a stress-relieving effect and is involved in learning and information memorization	
GABA	Main inhibitory neurochemical in the CNS. Involvement in regulating the sleep-wake cycle, locomotor activity, conditioned reflex formation, and information memorization and recognition	
Glycine	Inhibitory neurochemical with a stress-relieving and relaxing effect	
Aspartate	Auxiliary excitatory neurochemical. Mood improvement, mitigation of the state of fatigue	
SCFAs	Mitigation of depression and anxiety, pain relief. Antidepressant effect (especially butyrate). Propionate at high concentrations causes locomotive behavior disruption and accelerates autism progression. Appetite suppression (acetate).	
Nitic oxide	Involvement in pain perception; mood improvement during grooming behavior.	
Hydrogen sulfide	Involvement in neuronal activity regulation, cognitive activities, and memory. Neuroprotective effect.	
Opioids (endorphins, enkephalins, and dynorphins)	Inhibition of impulse transmission, pain-relieving effect. Mood improvement, which may result in euphoria. Soporific effect at high concentrations	
Substance P	Involvement in pain perception, anxiety stimulation	
Neuropeptide Y	Pain relief, stress and anxiety mitigation, food intake stimulation	
Cholecystokinin	Involvement in foraging behavior and pain perception. A fragment of the cholecystokinin molecule causes anxiety and fear.	
Adenine nucleotides and other purines	Inhibitory action on excitatory brain synapses. Sedation (tranquilization); regulation of initial sleep stages.	

(Boldyrev et al., 2010; Dubynin et al., 2010; Sitdikova & Zefirov, 2010; Duan et al., 2015; Oleskin & Shenderov, 2016; Oleskin et al., 2017a, b; Averina & Danilenko, 2017.)

The biological activity of dopamine is due to its binding to D receptors. They are subdivided into five types ($D_{1\text{-}5}$). All D receptors are coupled with G proteins. The receptors activate (the D_1 and D_5 receptors) or, conversely, inhibit (the $D_{2\text{-}4}$ receptors) the adenylate cyclase enzyme, thereby increasing or decreasing the level of intracellular cyclic adenosinomonophosphate (cAMP). The recently discovered trace amine-associated receptor 1 (TAAR1) also influences intracellular adenylate cyclase activity (Grandy et al., 2016); apart from dopamine, TAAR1 binds octopamine, tyramine, and β-phenylethylamine normally present in the nervous system at trace concentrations. The predominant D_1 receptors account for about ¾ of all dopamine receptors in the human organism (Dubynin et al., 2010).

As a CNS neurotransmitter, dopamine is produced by the neurons of several parts of the brain, including the substantia nigra, the tegmentum, and some hypothalamic nuclei (Dubynin et al., 2010). Release of dopamine by the ventral tegmentum results in its spreading along the axons toward the nucleus accumbens of the hypothalamus and the prefrontal cortex. The dopaminergic system of the brain induces active wakefulness, promotes hedonic, i.e., pleasure-seeking, behavior (Berridge & Robinson, 1998), and enhances the positive emotions that are caused by enjoying, e.g., tasty food or a videotape (Arias-Carrión & Pöppel, 2007). Anticipating a reward results in increasing the dopamine concentration in the brain, and many addictive drugs stimulate dopamine release or block dopamine reuptake by dopamine-producing neurons. The *substantia nigra*, a part of the dopamine-dependent (dopaminergic) brain system, is of paramount importance for the motivation and emotional regulation of maternal behavior (Markov, 2011).

To reiterate, the dopamine precursor DOPA passes the gut-blood and the blood-brain barrier. Therefore, DOPA-producing microorganisms, including both probiotics, e.g., lactobacilli (Oleskin et al., 2014a, b), and potential pathogens such as *Bacillus cereus* (Shishov, 2010; Oleskin et al., 2010), can cause euphoria, due to the conversion of micriobial DOPA to dopamine in the brain. Such euphoria should be particularly impressive and bizarre when induced by pathogens and developing in spite of a severe bacterial infection and a worsening health state.

Dopamine is also produced by the neurons of the *substantia nigra* in the brainstem and reaches the striatum via their axons. Dopaminergic neurons are involved in maintaining locomotive activity and enabling humans or animals to make voluntary movements while suppressing the involuntary ones. Increasing the activity of the dopaminergic systems of the brain results in lowering the activation thresholds for various forms of locomotion. The body seems to become lighter and more supple and resilient; the feeling of fatigue is relieved if dopaminergic synapses are in operation. This dopamine-dependent sensation is also caused by dancing and doing complex physical exercises. Dopamine is necessary for switching from one stage of cognitive work to another. Dopamine is implicated in controlling hormone release; for instance, it decelerates prolactin liberation and, therefore, inhibits lactation (milk production by mammary glands).

A decrease in the activity of dopaminergic brain systems (olfactory and visual cortex zones, frontal cortex lobe, amygdala, thalamus, hypothalamus, etc.) results in apathy and loss of initiative. Decelerated cognitive processes, shaking extremities (tremor), and compulsive movements are typical of Parkinson's disease that is characterized by the destruction of dopamine-producing *substantia nigra* neurons. Substances that increase brain dopamine levels stimulate physical and mental activity. Some of them represent addictive drugs exemplified by amphetamine that promotes dopamine excretion into the synaptic cleft.

Norepinephrine activates the brain and stimulates locomotive behavior (Dubynin et al., 2010). Norepinephrine increases cerebral blood supply and is involved in emotions associated with risk-taking and learning. Norepinephrine is dubbed the rage hormone because its release into the bloodsream results in aggressive behavior and a significant increase in muscular strength. Norepinephrine promotes vigilant behavior, stimulates information memorization and retrieval, and is implicated in "fight or flight" behaviors.

The neurochemical and hormonal activities of norepinephrine are due to its binding to α- and β-adrenergic receptors. α-Receptors are subdivided into the α_1-subtype that increases the intracellular inositol-1,4,5-triphosphate and Ca^{2+} concentrations by activating phospholipase C and the α_2-subtype that

inhibits adenylate cyclase and, therefore, decreases the intracellular cAMP concentration. In contrast, β-type receptors (β1, β2, and β3) represent G proteins; they activate adenylate cyclase upon binding norepinephrine.

In the brain, norepinephrine is predominantly produced by the neurons of the locus coereleus, the lateral reticular formation, the medulla oblongata, and the nuclei of the solitary tract (Boldyrev et al., 2010); their axons form dense synapses in various parts of the CNS, including the cerebellum and the brain cortex (Dubynin et al., 2010). Norepinephrine accelerates heart beat, elevates blood pressure, stimulates glucose transfer from depots to the bloodstream, increases the blood supply of skeletal muscles while decreasing that of the GI tract, and suppresses bladder empting and intestinal peristalsis. This inhibitory effect on the persitalsis facilitates the attachment of microbial biofilms to epithelial cells. Norepinephrine dilates the pupil and increases tear production.

The effects of norepinpephrine and its methylated derivative epinephrine on the heart are due to their stimulatory influence on myocardial β-adrenoreceptors, which results in increasing the cardiac output and accelerating cardiac contraction.

In the brain, norepinephrine is predominantly produced by the neurons of the locus coereleus and some other parts of the brainstem. The hormonal effect of the norepinephrine is supplemented by its neurotransmitter activity that is aimed at mobilizing the brain under stress. Minimum norepinephrine levels, especially in the locus coeruleus, are characteristic of the sleep state, particularly during REM sleep associated with dreaming; they increase in the wakeful state (Berridge et al., 2012). Norepinephrine and other catecholamines are released under the influence of various stressors, including trauma, hemorrhage, and emotional states associated with fear and anxiety. Under severe stress, the pain-relieving (analgetic) effect of norepinephrine comes to the forefront (Dubynin et al., 2010).

3.1.3. Interaction of Catecholamines with the Immune System

Immunocytes respond to biogenic amines; they synthesize and release them (Table 7), including catecholamines. Among catecholamine receptors, β_2-adrenoreceptors (β_2-ARs) are predominantly expressed by immune cells.

Stimulation of β_2-ARs chiefly results in an anti-inflammatory response of immunocytes including macrophages and monocytes. Antigen-presenting dendritic cell express both α- and β-adrenoreceptors. The binding of catecholamines to α-ARs mainly causes the stimulation of the immune response, whereas their interaction with β-ARs is more likely to inhibit the immune system and to mitigate inflammation.

Incubation of dendritic cells with norepinephrine after stimulating their Toll-like receptors with agonists results in decreasing the secretion of IL-12, IL-6, TNF-α, and IL-23 while increasing the production of IL-10. This can cause immunosuppression and Th1 priming disruption.

Norepinephrine can aggravate Th2-associated diseases including various allergic problems. Th1-dependent diseases, such as multiple sclerosis and type 1 diabetes, in contrast, are treatable with catecholamines and β_2-AR agonists (Cosentino & Marino, 2012). Dopamine, norepinephrine, and epinephrine are synthesized by various immune cells. The pathways of their synthesis and metabolism are similar to those in the cells of the nervous and the endocrine system. Both immune and neuroendocrine cells express tyrosine hydroxylase and catecholamine-degrading enzymes such as the MAO and the catechol-O-methyltransferase enzymes (Jiang et al., 2006; Cosentino et al., 2013).

Presumably, norepinephrine secretion by immunocytes is acetylcholine- and calcium-dependent, in an analogy to adrenal chromaffin cells (Jiang et al., 2006). Dopamine receptors are expressed on the surface of all kinds of immune cells including T and B lymphocytes, dendritic cells, macrophages, neutrophiles, NK cells, and T-regulatory cells (Cosentino et al., 2013).

Table 7. Functions of neurochemicals in the immune system

Neurochemicals	Effects in the immune system
Dopamine	Complex and partly contradictory effects involving multiple receptors. Overwhelmingly, catecholamines exhibit anti-inflammatory and immunosuppressive activity. Norepinephrine can promote the development of Th2-asociated diseases, such as allergic processes (Orlova et al., 2012; Cosentino & Marino, 2012; Cosentino et al., 2013; Levite, 2016).
Norepine-phrine	
Serotonin	Both compounds are implicated in effectuating and potentiating immune responses at the initial inflammation stages. However, at the final inflammation stages, they may be involved in mitigating inflammation. Their immunotropic effects can be both stimulatory and inhibitory, depending on the microenvironment. Serotonin activates phagocytosis at low IFN-γ levels and inhibits this process at high IFN-γ levels. As for Th1-dependent pathological processes, e.g., rheumatoid arthritis, serotonin can attenuate inflammation. Histamine's capacity to stimulate T lymphocyte differentiation can be used for treating autoimmune diseases such as multiple sclerosis. Histamine decreases the risk of an immunocyte attack on the myeline sheath of neurons (Zampeli & Tiligada, 2009; Ley et al., 2010; Arreola et al., 2015; Gao et al., 2015; O'Mahoni et al., 2015; Shajib & Khan, 2015).
Histamine	
Acetylcholine	Immunosuppressive and anti-inflammatory activity. Of paramount importance is the interaction of acetylcholine with the nAchRα_7 receptor that results in suppressing proinflammatory cytokine production; stimulation of the efferent activity of the vagal nerve inhibits the systemic inflammatory response (Ley et al., 2010)
Agmatine	Inhibition of the inducible NO synthase, an anti-inflammatory and neuroprotective effect (Satriano, 2004; Uranchimeg et al., 2010; Ahn et al., 2012; Chai et al., 2016)
GABA	Predominantly, an anti-inflammatory effect that is due to suppressing T lymphocyte activity and downregulating proinflammatory cytokine production. Protection from experimental autoimmune encephalomyelitis, type 1 diabetes, contact dermatitis, and other autoimmune problems (Auteri et al., 2015; Prud'homme et al., 2015; Bhandage et al., 2018).
Glycine	Predominantly, an anti-inflammatory effect; inhibition of the secretion of proinflammatory cytokines and stimulation of the synthesis of anti-inflammatory mediators (van den Eynden et al., 2009)
Glutamate and aspartate	Complex immunotropic effects; predominantly immunosuppressive activity at high concentrations (chacteristic of glutamate; Ganor & Levite, 2014).
SCFAs	Inhibition of neutrophil adherence and chemotaxis and suppression of immunocyte migration from the bloodstream to the inflammation area Acetate and butyrate suppress T cell proliferation and activation, decrease the antibody content in the bloodstream, and induce apoptosis in immunocytes. Butyrate and propionate increase the production and stimulate the activity of extrathymic (intestinal) T_{reg} cells (Shenderov, 2013a, b; Verbeke et al., 2015; Correâ-Oliveira et al., 2016)
Nitric oxide	Complex and partly contradictory effects on all parts of the immune system. A cytotoxic effect at high concentrations (used by T killers)
ATP and other purines	"Danger signals" stimulating inflammation; adenosine chemotactically attracts neutrophils (Barletta et al., 2012). Both stimulatory and inhibitory effects on immune responses (*Codis EU Research Results*, 2013)

Autoimmune, leukemic and lymphoma T cells also express dopamine receptors. Dopamine at low concentrations (10^{-8}M) behaves as an important autocrine and paracrine signal (Levite, 2016). Dopamine's anti-inflammatory role is associated with the suppression of macrophage functions. In the model of murine peritoneal macrophages, it was established that dopamine inhibits the synthesis of the inflammation activator IL12p40 in response to bacterial LPSs and enhances the production of anti-inflammatory factor IL-10 (Orlova et al., 2012); these effects are mediated by β-ARs (Ley et al., 2010). Dopamine can suppress the response of preactivated T cells (Cosentino et al., 2013).

Evidence has been also presented that dopamine can stimulate the operation of the immune system under certain conditions. For instance, it activates dormant naïve T cells. Dopamine stimulates their adherence to fibronectin via binding to D_2/D_3 receptors. Dopamine stimulation of D_1 receptors on human T_{reg}s ($CD4+CD_{25}^{high}$) causes inhibition of their immunosuppressive activity and a decrease in IL-10 and TGF-β (transforming growth factor β) production (Orlova et al., 2012; Cosentino et al., 2013). By "suppressing immunosuppressors", dopamine is expected to activate immune responses. In support of this suggestion, there is evidence that dopamine inhibits the second major component of the immunosuppression system, i.e., MDSCs (myeloid derived suppressor cells).

Recent research has revealed that dopamine acts on the D_1 receptors of MDSCs and considerably reduces their inhibitory activity with respect to T_{reg} proliferation and secretion; in this fashion, it boosts antitumor immunity. Thus, the overall dopamine's mode of action is complex and to an extent contradictory (reviewed, Oleskin et al., 2017a).

3.2. INTERACTION OF SEROTONIN WITH MICROORGANSIMS AND THE HOST NERVOUS AND IMMUNE SYSTEM

Serotonin (5-hydroxytryptamine), a derivative of the amino acid tryptophan, combines the functions of a neurotransmitter and a locally acting hormone (histohormone) that is involved in upregulating the smooth muscle tone. Much serotonin is contained in blood thrombocytes that are responsible for blood clotting upon blood vessel injury (Dubynin et al., 2010). Over 90% of the total serotonin pool of the human organism are synthesized in the GI tract via metabolizing tryptophan that enters the organism with food. There are 14 different types (including subtypes) of serotonin receptors that are located on various kinds of cells exemplified by enterocytes, neurons including those of the GI tract, and immune cells. In the GI tract, serotonin is synthesized by specialized enterochromaffin cells in the intestinal mucosa and by mesenteric nervous cells (Yano et al., 2015).

3.2.1. Interaction of Serotonin with Microorganisms

Serotonin slightly stimulated the growth of *Aeromonas hydrophila* at very high concentrations (Kinney et al., 1999) and caused a statistically significant increase in the growth of *Enterococcus faecalis* (Strakhovskaya et al., 1993), *E. coli*, *Rhodospirillum rubrum* (Oleskin et al., 1998), and the yeast *Candida guillermondii* (Strakhovskaya et al., 1993) and *Saccharomyces cerevisiae* (Malikina et al., 2010; Oleskin et al., 2010).

The capacity of serotonin to stimulate plant growth (radish seed germination; Roshchina, 1991) is attributable to its chemical similarity to auxin, or indole-3-acetic acid, a plant hormone. In studies with *S. cerevisiae,* a photo- and radioprotective effect of serotonin was established (Fraikin et al., 1985).

Serotonin at a concentration of ~1 μM stimulated cell aggregation and microcolony formation in *E. coli* K-12 (Oleskin et al., 1998a; Anuchin et al., 2008), *Rhodospirillum rubrum*, and the myxobacterium *Polyangium* sp. (Oleskin et al., 1998a). At concentrations of 25-100 μM and above, in contrast, serotonin caused deaggregation of *E. coli* and *Polyangium* sp. cells, suppressed intercellular matrix formation, and inhibited the growth of these bacteria (Oleskin et al., 1998a).

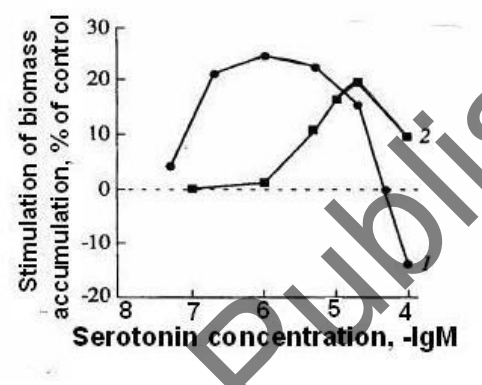

Figure 15. Stimulation of biomass accumulation by serotonin in *E. coli* K-12 (1) and *Rhodospirillum rubrum* (2). According to: Oleskin et al., 1998a. Designation: -lgM is -lg [Serotonin concentration in moles per liter].

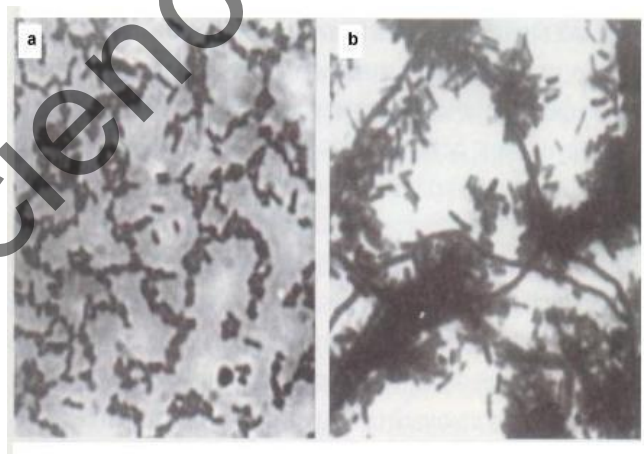

Figure 16. Effect of serotonin on microcolony formation in *E. coli* K-12 on LB agar: a, control; b, with 1 μM serotonin. According to: Oleskin, 2001. Magnification, 1500.

Serotonin stimulated the growth (estimated from the increase in the culture's optical densityand colony-forming unit, CFU, number) and biochemical activity (determined from the pH decrease caused by organic acid formation) of the probiotic *Lactobacillus acidophila* NK-1 (Vodolazov, Zhilenkova, and Oleskin, unpublished). Low serotonin concentrations stimulated, and high concentrations inhibited, the bioluminescence (light emission) of the *E. coli* TGI strain containing the luciferase operon (*lux*) of the bacterium *Photorhabdus luminescencens* ZMI (Oleskin et al., 2017c).

The stimulatory effect of serotonin on pro- and eukaryotic cells can be hypothetically attributed to the presence of receptors to serotonin and, presumably, to related compounds, e.g., indole. The inhibitory effect of high concentrations may be nonspecific: serotonin can behave as a membrane uncoupler, similar to bacterial d_2 factors (El'-Registan et al., 2006; Bukharin et al., 2005).

Serotonin suppressed the development of intracellular chlamydia (Rahman et al., 2005) and attenuated the virulence of *Candida albicans* (Mayr et al., 2005).

Of relevance in this context are data on the inhibitory effect of compounds that suppress the reuptake of serotonin by serotonin-releasing cells and thereby increase its extracellular concentration. These selective serotonin reuptake inhibitors (SSRIs) suppress the growth of some bacteria that predominantly belong to gram-positive species (O'Mahony et al., 2015). SSRIs inhibit the growth of clinically isolated pathogenic yeast (*Candida parapsilosis*) and mycelium fungi of the genus *Aspergillus* (*A. fumigatus, A. flavus,* and *A. terreus*). Sertraline and fluoxetine (Prozac) that are used as antidepressants exhibit the strongest inhibitory activity. Serotonin attenuates the virulence of the pathogen *C. albicans* (Mayr et al., 2005).

Apart from responding to serotonin, microorganisms produce physiologically significant concentrations of this neurotransmitter. Serotonin production is sufficiently widely spread in the microbial world, including the symbiotic and parasitic microbiota of the human organism (Shenderov, 1998; Rook et al., 2013; Liang et al., 2018). A low concentration of a serotonin-like substance was detected in *Rhodospirillum rubrum* cells (Oleskin et al., 1998a). Serotonin was present in *Bacillus*

subtilis and *Staphylococcus aureus* cells at concentrations of ~1 μM (Tsavkelova et al., 2000), which are comparable to its concentrations in the bloodstream. The blood normally contains 0.5–1.5 μM serotonin (McPherson & Pincus, 2011). The product of enzymatic oxidation of serotonin, 5-HIAA, was present at micromolar or submicromolar concentrations in the aforementioned microorganisms, as well as in other tested species, even though they lacked serotonin per se. The maximum concentrations of 5-HIAA (~10 μM) were detected in the biomass of the eukaryote *Penicillium chrysogenum* (Tsavkelova et al., 2000).

High serotonin concentrations were detected in the cultures of the bacteria *Morganella morganii* (4.96 mg/L, i.e., ~28 μM serotonin), *Klebsiella pneumonia* (3.23 mg/L, ~18 μM), and *Hafnia alvei* (2.69 mg/L, ~15 μM) (Özogul, 2004). To reiterate, serotonin is chemically related to the plant growth hormone auxin (indole-3-acetic acid) that, apart from plants, is synthesized by a number of bacterial species (Yano et al., 2015).

Studies on the dynamics of serotonin synthesis during the growth of *E. coli* and *S. cerevisiae* (Shishov et al., 2009; Malikina et al., 2010; Oleskin et al., 2010) demonstrated that *intracellular* serotonin concentrations, similar to catecholamine concentrations, tend to decrease with the aging of the culture. Low *extracellular* serotonin concentrations (~10 nM) were, in contrast, only detectable in the culture liquid of *E. coli* at the late growth stages.

Yeast released neither serotonin nor catecholamines, even though it accumulated these substances inside its cells.

The biomass of *E. coli* and *S. cerevisiae* contained 5-hydroxytryptophan, the serotonin precursor in the animal organism, and 5-HIAA, the product of oxidative deamination of serotonin. In *E. coli,* 5-HIAA was also present in the culture liquid. Taken together, these facts suggest that serotonin synthesis and degradation pathways in microrganisms are likely to include the following animal organism-specific enzyme steps:

Tryptophan → 5- Hydroxytryptophan → *Serotonin*→ 5-HIAA.

This pathway should involve enzymes that are homologous or functionally analogous to the animal enzymes tryptophan hydroxylase (catalyzing the tryptophan → 5-hydroxytryptophan conversion), aromatic amino acid decarboxylase (catalyzing the 5-hydroxytryptophan → serotonin conversion), and MAOs (responsible for oxidative deamination). Such enzymes, including serotonin oxidation-catalyzing MAOs, were detected in a number of microbial species (Yagodina et al., 2000).

There is evidence that serotonin is synthesized by *Lactococcus lactis* subsp. *cremoris* MG 1363, *L. lactis* subsp. *lactis* IL 1403, and *Lactobacillus plantarum* NCFB2392 (Özogul et al., 2012). It was also established that serotonin is present in fermented food items including Chinese rice wine (particularly its semi-sweet variety) that also contains other neuromediator amines (histamine and tyramine) (Ye et al., 2012). Serotonin was also detected in the culture liquid of *Lactobacillus helveticus*100ash at a concentration of 0.4 μM, but not in that of *Lact. helveticus* NK-1, *Lact. casei* K3III24, and *Lact. delbrueckii* subsp. *bulgaricus*. The serotonin metabolite 5-HIAA was synthesized by *Lact. helveticus* 100ash and NK-1 and *Lact. delbrueckii* subsp. *bulgaricus* (Oleskin et al., 2014a, b).

In *Pseudomonas aeruginosa*, serotonin functions as the signal in the quorum-sensing system *lasI-lasR*. An increase in its concentration results in increasing *Ps. aeruginosa* virulence and biofilm formation both *in vitro* and in the organism of an infected mouse (Knecht et al., 2016). Serotonin released by the pathogenic amoeba *Entoamoeba histolytica* causes diarrhea, a characteristic symptom of the amoebic infection (McGowan et al., 1983).

Apart from producing serotonin, a large number of microorganisms including spore-forming bacteria can stimulate its synthesis by the enterochromaffin cells of the mucosa of the colon. There is evidence that this effect is mediated by microbial metabolites such as SCFAs (El Aidy et al., 2015; Li & Zhou, 2016; Ivashkin & Ivashkin, 2018) including propionate and butyrate, as well as deoxycholate, α-tocopherol, tyramine, and aminobenzoate (Yano et al., 2015). GF mice have blood tryptophan concentrations that are considerably (40%) higher than those of colonized mice. However, the plasma serotonin level of colonized mice is 2.8 times higher than that of GF mice.These data testify to an involvement of gut

bacteria in the conversion of tryptophan to serotonin in the enterochromaffin cells of the intestinal epithelium, which is one of the main sources of serotonin in the organism.

Presumably, the gut microbiota can also influence the serotonin level in the organism including the brain by suppressing tryptophan conversion to kynurenine, a process that competes with tryptophan conversion to serotonin (Kennedy et al., 2017).

The involvement of the microbiota in the formation of the serotonin pool in wild-type animals is consistent with the fact that the serum of GF mice contains very low amounts of serotonin. Introduction of clostridial microbiota into the GI tract of these mice results in normalizing the level of serum serotonin (Krishnan et al., 2015).

Luminal tryptophan in the gut is microbially converted to tryptamine, the hydroxy group-lacking "relative" of serotonin that is also regarded as a neuroactive agent in the literature (van de Wouw et al., 2017). Tryptamine-forming microorganisms in the human intestine include *Clostridium sporogenes*, *Ruminococcus gnavus*, and *Lact. bulgaricus.* On average, they are detectable in 10% of the human population.

Under the influence of the enzymes N-acetyltransferase and methyltransferase, serotonin converts to *melatonin*, the main pineal gland-produced hormone; melatonin mediates the organism's responses to the evironmental light-dark rhythm and, therefore, is directly involved in regulating the circadian rhythms of the organism. Melatonin is likely to be implicated in host-microbiota communication because it exerts an influence on the GI microbiota. It accelerates cell swarming in *Enterobacter aerogenes*, but produces no effect on *E. coli* or *Klebsiella pneumoiae* (Paulose & Cassone, 2016).

A large number of bacterial species, including those inhabiting the animal/human intestines (*E. coli*, *Bacteroides ovatis*, and *Clostridium bifermentus;* Smith & Macfarlane, 1996), synthesize high concentrations of indole (up to 600 μM and above), the bicyclic backbone of serotonin (Domka et al., 2006). Indole inhibits biofilm formation in *E. coli* (Bansal et al., 2007; Lee et al., 2007a) and, conversely, stimulates biofilm formation in *Ps. aeruginosa* and *Ps. fluorescens* (Lee et al., 2007b). Indole accelerates

the growth of *Salmonella enterica* var. *enteridis* (Vakhitov & Sitkin, 2014) and stimulates the formation of antibiotic-tolerant persister cells (Vega et al., 2012). In intestinal epithelial cells, indole was reported to induce the expression of the genes that are responsible for the barrier function, mucin formation, and the synthesis of anti-inflammatory cytokine IL-10, while concomitantly suppressing the synthesis of cytokine IL-8 (Bansal et al., 2010). Some derivatives of indole, such as 7-hydroxyindole and 5-hydroxyindole, inhibited biofilm formation in saprotrophic *E. coli* strains, whereas another derivative, isatin (indole-2,3-dion), stimulated biofilm formation in enterohemorrhagic *E. coli* strain EHEC (O157:H7; Lee et al., 2007a). The effect of indole on microbial biofilms seems to be due to its capacity to function as an analog of autoinducers AI-1 (N-AHLs) in bacterial QS systems (Ryan & Dow, 2008; Karatan & Watnick, 2009).

3.2.2. Interaction of Serotonin with the Nervous System

Serotonin is a major neurochemical implicated in a large number of human physiological and behavioral processes. Serotonin is a neurochemical factor related to the feelings of well-being and happiness. It influences cognitive capacities, hedonic (reward-seeking) behavior, learning, and memorization (Young, 2007).

Serotonin as a major neurotransmitter is produced by the nervous cells of nine raphe nuclei of the brainstem that are denoted as nuclei B1–B9. Serotonin from the lower nuclei spreads along the axons toward the cerebellum and the spinal cord. From the upper nuclei, it is transferred to most parts of the brain. The effects of serotonin are due to its binding to seven main receptor types that are called 5-$HT_{1–7}$; receptors 5-HT_1 include subtypes 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C}, and 5-HT_{1D}; receptors 5-HT_2 subtypes 5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C} (Hannon & Hoyer, 2008). Only receptors of the 5-HT_3 type are associated with ion channels, while all other serotonin receptors are coupled with G proteins and act via secondary intracellular messengers. Receptors 5-HT_2 activate phosholipase C; other types of receptors modulate adenylate cyclase activity and thereby change cAMP

concentrations (receptors 5-$HT_{1,\ 4–7}$)[22]. Serotonin receptor gene expression is under the influence of the GI microbiota. In GF mice, the 5-HT_{1A} expression level in the hypothalamus and amygdale is decreased (Rohrscheib & Brownlie, 2013).

In addition, serotonin exerts a non-receptor effect called "serotonylation" on protein molecules by directly attaching to them. For instance, serotonin "serotonylates" small-size GTPase enzymes; this process influences the transmission of cell signals in thrombocytes, smooth muscle cells, and the insulin-secreting cells of pancreas islets (Walther et al., 2003; Paulmann et al., 2009).

Serotonin is involved in downregulating the wakefulness level, in contrast to wakefulness-promoting catecholamines; it is also implicated in the functioning of sensory systems and in controlling the learning process, emotions, and the motivation level. Serotonin decelerates the spreading of nerve impulses from a local focus, thereby limiting the brain areas that are directly involved in perceiving a stimulus. The inhibitory effect of serotonin on pain perception underlies its pain-relieving influence. LSD, an antagonist of serotonin receptors 5-HT_2, disrupts the image perception process, which results in illusions and hallucinations (Dubynin et al., 2010). A typical serotonin-dependent locomotive response, "wet dog shaking", involves 5-HT_2 receptors (Boldyrev et al., 2010).

Serotonin is implicated in regulating the emotional state and memorization and learning processes. It suppresses appetite by binding to receptor 5-HT_{2C} and influences social and sexual behavior (reviewed, Oleskin & Shenderov, 2013, 2019; Oleskin et al., 2016, 2017). A high serotonin level in the blood or hemolymph corresponds to a high rank in the hierarchies of various animals ranging from crustaceans to primates[23] (McGuire, 1982; Raleigh & McGuire, 1994; Masters, 1994). Injecting serotonin into a lobster's hemolymph induces dominant behavior. In

[22] 5-HT_{5A} receptors inhibit, and several other types of serotonin receptors activate the intracellular adenylate cyclase enzyme.

[23] However, dominant males in monkey species other than vervets (cynomolgus and talapoin monkeys) are distinguished by lower levels of serotonin-dependent activity than subordinate males (Kaplan et al., 2002); dopamine seems to be involved in establishing dominance-submission relationships in some primates. Dominant monkeys reveal a higher activity level of D_2 receptors to dopamine than their subordinate conspecifics (Morgan et al., 2002).

contrast, octopamine, a substance chemically related to catecholamines, elicits submissive behavior (Kravitz, 1988). The dominant individual in a group of green African vervets has more serotonin and its oxidation product, 5-HIAA, in the blood serum than the submissive individuals (Raleigh & McGuire, 1994; Masters, 1994).

Serotonin at relatively high concentrations "puts the brain asleep": serotonin-releasing raphe nuclei contain the sleep center in the brain (Dubynin et al., 2010).

Serotonin is necessary for the formation of the nervous system during ontogeny, the development of the brain is disrupted in its absence (O'Mahony et al., 2015). Serotonin is essential for the interaction between the nervous, immune, and digestive system (including its microbiota). Serotonin regulates intraorganismic systems that are responsible for maintaining a constant body temperature and effectuating sensory and locomotor activities. There is a negative correlation between the brain serotonin level and such psychological states as depression and anxiety; these states are mitigated by serotonin binding to receptors 5-HT_{2A}. Aggressiveness in humans and animals decreases if serotonin binds to receptors 5-HT_{1B}.

Apart from depression and anxiety, human behavior becomes impulsive if the activity of serotonin-dependent (serotonergic) brain structures is low. This is fraught with violence and criminal behavior. Drugs that increase effective serotonin concentrations by blocking serotonin reuptake (Prozac, Zoloft, etc.) or inhibiting serotonin degradation (monoamine oxidase inhibitors) serve as antidepressants. Brain serotonin-dependent (serotonergic) system disruption may contribute to the development of Parkinson's disease, schizophrenia, hepatic encephalopathy, autism, and drug addiction. Serotonin receptors are involved in regulating circadian rhythms and body temperature. Some of the receptors are also implicated in panic and obsessive behavior (Boldyrev et al., 2010).

A lack of serotonin in serotonin-dependent brain structures (the serotonergic system) is responsible for conditions such as seasonal affective disorder (SAD) and premenstrual syndrome (PMS). The symptoms of both disorders include depression, anxiety, and often impulsive behavior. SAD is

associated with an increase in the synthesis of melatonin in the brain, which inhibits the activity of the serotonergic system. When the day becomes short, this leads to an increase in density of brain serotonin transporters that take up neuronally released serotonin from the synaptic cleft. The resulting lack of serotonin in the synapses may cause a loss of energy, a longer sleep time, and other SAD symptoms (Praschak-Riederetal., 2008).

Beyond the CNS, serotonin performs multifarious functional roles: it promotes vasoconstriction and platelet aggregation and regulates the blood pressure level, the bladder function, and the synthesis of prolactin, corticosterone, oxytocine, and other hormones. Serotonin is present in insect venoms and plant stings, and it increases pain intensity (Chen & Lariviere, 2010). Serotonin controls the synthesis of prolactin, corticosterone, oxytocin, and other hormones. It also represents an important enteric nervous system-regulating agent (Yano et al., 2015). It regulates peristalsis and is involved in the development of feeling of nausea and indigestion (Mazzoli & Pessione, 2016). If food contains GI tract-irritating substances, gut enterochromaffin cells produce elevated serotonin concentrations and promote intestinal motility. This may result in diarrhea; if serotonin accumulates in the blood and is not taken up by blood platelets, 5-HT_3 receptor activation may cause a vomiting response (Rang, 2003).

Excessive serotonin concentrations resulting from administering a combination of serotonin level-increasing drugs, may cause the serotonin syndrome. It manifests itself in high body temperature, agitation, muscle tremor, pupil dilation, nausea, and diarrhea; this may be followed by more serious, life-endangering, symptoms (Volpi-Abadie et al., 2013). Since serotonin is produced by GI microorganisms, including *E. coli* and some lactobacilli, serotonin overproduction by them may also result in the development of the serotonin syndrome.

The microbiota-produced serotonin "backbone", *indole,* influences enteroendocrine cells (EECs), inducing the synthesis of glucagon-likeprotein 1 (GLP-1). Due to its similarity both to serotonin and melatonin, indole produces a soporific effect, in an analogy to both neuroactive substances. Somnolescence and falling asleep was observed under the

influence of bacteria-produced muramylpeptide that is also structurally similar to serotonin (Mazzoli &Pessione, 2016).

3.2.3. Interaction of Serotonin with the Immune System

The serotonin that is produced by GI cells helps adjust the activity of local inflammatory responses. This is mandatory for maintaining the GI barrier function and developing sufficient food tolerance. The immunocytes of other organs require sufficient serotonin amounts for normal functioning (Arreola et al., 2015). The importance of serotonin from the immunological viewpoint is highlighted by the fact that lymphocytes, monocytes, macrophages, and dendritic cells have multiple serotonin receptors (O'Mahony et al., 2015). Apart from possessing a wide variety of serotonin receptors, immune cells produce their own serotonin. Adipose cells and blood platelets release micromolar serotonin concentrations in an inflamed area (Ley et al., 2010).

Although some immune cells, e.g., dendritic cells, do not express tryptophan hydroxylase (TPH1), the key enzyme for serotonin synthesis, they store and excrete serotonin. Such cells use systems that enable serotonin uptake from the environment (serotonin reverse transporter, SERT; Arreola et al., 2015). Other kinds of immunocytes including activated T cells express TPH1 and are potentially capable of synthesizing their own serotonin (O'Connell et al., 2006).

Immune cells may possess several types of serotonin receptors. Receptor 5-HT_3 is particularly widespread in immunocytes; it has been detected on the surface of T lymphocytes, monocytes, and dendritic and adipose cells. The expression of serotonin receptors varies depending on the immunocytes' physiological state, e.g., on whether they are naive or activated (Arreola et al., 2015). Naïve T cells express 5-HT_7 receptors. Upon activation, they are characterized by active 5-HT_{1B} and 5-HT_{2A} receptors (Leon-Ponte et al., 2007). Monocytes can express the mRNA that is necessary for the synthesis of 5-HT_{1E}, 5-HT_{2A}, 5-HT_3, 5-HT_4, and 5-HT_7 serotonin receptors (Arreola et al., 2015).

Serotonin can influence the proliferation, differentiation, and activity of immune cells. The effects of serotonin are complex. Serotonin can both stimulate and block the operation of immunocytes, depending on the types of the serotonin receptors involved, their degree of activation, and the impact of the supplementary factors of the micro-environment (Arreola et al., 2015). For instance, serotonin activates phagocytosis at low levels of interferon-γ (IFN-γ) in the medium but inhibits this process at high levels of IFN-γ (Ley et al., 2010). Serotonin is a chemotactic factor for immune cells including neutrophils and eosinophils (Ley et al., 2010).

Serotonin modulates the operation of monocytes in a complex fashion. Under its influence, TNF-α secretion by monocytes is decreased, LPS-induced IL-12p40 secretion by activated monocytes is increased, and the migration of activated antigen-presenting cells is stimulated. Serotonin also increases LPS-dependent IL-6, IL-1β, and IL-8/CXCL8 secretion. In mouse peritoneal macrophages, serotonin stimulates phagocytosis by activating 5-HT_{1A} receptors in a dose-dependent manner. Tryptophanhydroxylase 1-deficient mice that do not synthesize serotonin are distinguished from wild-type mice by a decreased level of intestinal inflammation in studies in which experimental colitis is caused. This appears to be due to the decreased macrophagal infiltration of the intestine and the limited amount of proinflammatory cytokines in serotonin-deficient mice (Ley et al., 2010). Bronchial asthma is characterized by increased serotonin production by adipose cells. Treatment with tianeptine that stimulates serotonin reuptake by serotonin-releasing cells results in lowering the blood serotonin level, mitigation of asthma symptoms, and improvement of the operation of the respiratory system. Methysergide, an antagonist of 5-HT_2 serotonin receptors, inhibits airway inflammation in the lungs if asthma is induced in model animals; it also limits eosinophil migration to the inflammation area and cytokine formation by Th2 cells.

Th2 cell activation-related pathological processes such as colitis and atopic dermatitis are accompanied by an increase in serotonin level in the organism (Ley et al., 2010). Nonetheless, human alveolar macrophages that are activated by serotonin and LPSs secrete decreased amounts of TNF-α

and IL-12 but increased amounts of the immunosuppressive factors IL-10, NO, and prostaglandin E2.

As far as Th1 cells-related disorders such as rheumatoid arthritis are concerned, serotonin predominantly exerts an inflammation-limiting effect that can be due to the synthesis of prostaglandin E2 (Ley et al., 2010). The presence of 5-HT_2 receptors on immune cells limits the activation of *in vitro*-stimulated macrophages (Arreola et al., 2015). Activation of 5-HT_{1A} receptors is mandatory for the proliferation of T lymphocytes and synthesis of inflammation mediators (IL-2 and IFN-γ) by them. It was established that the stimulation of 5-HT_7 receptors on naïve T cells is necessary for the early stage of T lymphocyte activation.

Inhibiting serotonin synthesis with para-chlorophenylalanine results in disrupting T cell activation and proliferation (Leon-Ponte et al., 2007; Arreola et al., 2015). Administration of para-chlorophenylalanine diminishes the intensity of the symptoms of dextrane sulfate-induced colitis and experimental pneumonia (Shajib & Khan, 2015).

Intracellular serotonin can bind to the components of signal transduction pathways. Intense reuptake of serotonin was detected in immunocytes that contain the GTPases (RhoA and Rab4) which covalently bind serotonin. This nonreceptor mechanism of interaction between serotonin and immune cells causes the activation level of G receptor-mediated signal pathways to increase (Shajib & Khan, 2015).

Serotonin and histamine (see below) represent efficient inflammation mediators. Nonetheless, their immunotropic effects can be both inflammation-stimulating and inflammation–inhibiting, depending on the micro-environment. Presumably, these compounds are implicated in inducing and potentiating the inflammatory response at its initial stages, but they may promote inflammation attenuation at the final stages of this process. This pattern could account for the seemingly contradictory data on the immunotropic effects of serotonin and histamine that have been presented in the literature.

3.3. INTERACTION OF HISTAMINE WITH MICROORGANSIMS AND THE HOST NERVOUS AND IMMUNE SYSTEM

Histamine, a derivative of the amino acid histidine, is a multifunctional agent that combines the functions of a neuromediator and a histohormone (a local inflammatory factor). Histamine is involved in regulating intestinal functions (Marieb, 2001). It is synthesized by adipose cells, leukocytes including basophils and eosinophils, and the enterochromaffin-like cells of the intestinal epithelium. Under normal conditions, histamine predominantly exists in an inactive bound form. Under pathological conditions (anaphylactic shock, burns, freeze burns, hey fever, urticaria, and allergic diseases) and under the influence of some chemical factors, free histamine concentrations may drastically increase.

3.3.1. Interaction of Histamine with Microorgansims[24]

Histamine is released by both prokaryotic and eukaryotic cells. Histamine produced by microbial decarboxylation of histidine is present in food items that are stored for a long time, e.g., in fish (particularly in bonito, scad, saury, mackerel, tuna, herring, sprat, and salmon), cheese, meat, wine, beer, sauerkraut, and pickled food (Halász et al., 1994; Ladero et al., 2008; Hwang et al., 2010; Roshchina, 2010, 2016; Lin et al., 2014). The capacity for histamine synthesis and its release into the cultivation medium was established in a wide variety of bacteria, including *Morganella mogranii, Proteus vulgaris, Pr. mirabilis, Klebsiella pneumoniae, Kl. oxytoca, Kl. planticola, Enterococcus faecalis, Enterobacter aerogenes, Citrobacter freundii, Raoultella orhithinolytica, Pantooea agglomerans,Psychrobacter* sp., *Vibrio fischeri, V. harveyi*, (Devalia et al., 1989; Shenderov, 1998; Özogul, Özogul, 2005, 2007; Roshchina, 2010, 2016; Helinck et al., 2013; Lin et al., 2014; Doeun et al., 2017), *Streptococcus thermophilus* (Gardini et

[24]Another microbially produced amine, tyramine, is also mentioned in this subsection.

al., 2012), *Vibrio alginolyticus*, *Acinetobacter lwofii*, *Ps. aeruginosa*, *Ps. putida*, *Aeromonas* spp., *Clostridium* spp., *Photobacterium* spp. (Özogul, Özogul, 2005, 2007; Lin et al., 2014), *Lactobacillus buchneri*, *Lact. reuteri*, and other lactobacilli (Halász et al., 1994; Roig-Sagués et al., 2002; Gardini et al., 2012; Doeun et al., 2017; van de Wouw et al., 2017), *Bacillus licheniformis* AJ and *B. coagulans* (Roig-Sagués et al., 2002), and the yeast *Debaryomyces hansenii* and*Yarrowia lypolytica* (Doeun et al., 2017). Histamine concentrations in food are routinely measured as an important criterion of food quality (Doeun et al., 2017).

Apart from functioning as a neurotransmitter, histamine is involved in inflammatory and allergic responses in the human organism. This largely accounts for the symptoms of poisoning caused by its toxic doses (75 mg/kg of food product and above; Ladero et al., 2008; Roshchina, 2010). Such concentrations occur in thawed fish (Doeun et al., 2017). Toxic concentrations (up to 180 mg/kg) of microbial histamine may be present in a large number of food items; they cause poisoning, inflammation, and allergic responses that manifest themselves in skin itching, nausea, diarrhea, rash, headache (histamine migraine), fever sensation, and hypotension (Gardini et al., 2012; Ladero et al., 2008; Roshchina, 2010). Studies with 1549 strains of pharyngeal origin that were isolated from children with an acute asthma attack revealed that 21.7% of them produce histamine. Its concentrations were 3,65 to 9.47 μg/mL with *Pr. spp.*, *Acinetobacter spp.*, *E. coli*, *Enterobacter spp.*, *Kl. pneumonia*, *Ps. aeruginosa*, and *Lact. acidophilus* and 0.3 to 2.76 μg/mL with *Streptococcus spp.*, *Staph. aureus*, *H. influenzae*, *B. spp.*, *Candida albicans*, and *Corynebacterium spp.* (Voropaeva, 2002). Histamine produced by gram-negative pathogens such as *Branhamella catarrhalis*, *Haemophilus parainfluenzae*, and *Ps. aeruginosa* (Devalia et al., 1989) can exacerbate the relapses of chronic bronchitis or pneumonia, particularly in patients suffering from heritable cystic fibrosis (Roshchina, 2010). Histamine enhances the virulence of the aforementioned gram-negative pathogens but not that of the gram-positive species *Staph. aureus* and *Streptococcus pneumonia* (Devalia et al., 1989).

Tyramine, a biogenic amine performing a neuromediator function in insects (Gritsai, 2017), is mainly produced by gram-positive bacteria by

decarboxylating tyrosine. In mammals, tyramine promotes the release of catecholamines and neuropeptide Y from intestinal mucosa cells and stimulates rapid cecal contractions (Yano et al., 2015). Unlike histamine, tyramine raises the blood pressure; it is present at dangerous concentrations in some food items, e.g., cheese (up to 1500 mg/kg in Cheddar cheese; Halász et al., 1994). Among lactic-acid bacteria, *Lactobacillus brevis, Lact. plantarum, Lact. delbrueckii,* and *Leuconostoc mesenteroides* form tyramine but not histamine; *Lact. casei* and *Lactococcus lactis* form both amines (Roig-Sagués et al., 2002). Of the 127 bacterial strains that were isolated from pheasant carcasses, at least 107 synthesized biogenic amines including histamine and tyramine (Buňková et al., 2016). Tyramine is also produced by the yeast *Debaryomyces hansenii* and *Yarrowia lypolytica* (Doeun et al., 2017).

Some bacteria synthesize β-phenylethylamine or tryptamine that belong, like tyramine, to *trace amines* present in the nervous system at very low concentrations. Tryptamine formation was established in *Lact. delbrueckii* subsp. *bulgaricus,* and β-phenylethylamine formation was detected in species of the genera *Leuconostoc* and *Enterococcus* (Mazzoli & Pessione, 2016).

Apart from synthesizing histamine and trace amines, microorganisms are capable of enzymatically oxidizing these biogenic amines with monoamine oxidases, in an analogy to animals. The MAO of the fungus *Aspergillus niger* catalyzes the oxidation of histamine along with other amines (benzylamine, phenylethylamine, and some aliphatic amines). The MAO of the bacterium *Sarcina lutea* is only specialized in oxiding histamine, i.e., represents a specific tyramine oxidase (Yagodina et al., 2000).

Histamine significantly stimulates biomass accumulation, cell aggregation, and colony formation in *E. coli* K-12 (an approximately twofold stimulation is attained with ~0.1 μM histamine; Anuchin et al., 2008), as well as cell proliferation in *S. cerevisiae* (Malikina et al., 2010). In *S. cerevisiae*, the amplitude of the effect of histamine approximately equaled that of serotonin (~70% stimulation with 1 μM of either of the neurochemicals) and was less significant than the dopamine effect (Malikina

et al., 2010; Oleskin et al., 2010). Histamine stimulated the growth and biochemical activity (estimated from acid formation) of the probiotic strain *Lact. acidophilus* NK-1 (Vodolazov, Zhilenkova, and Oleskin, unpublished). Histamine stimulated the bioluminescence of *E. coli* TGI at low concentrations and inhibited this process at high concentrations (Oleskin et al., 2017c).

The data on the stimulation of the growth and biochemical activities of various representatives of the human microbiota raise the issue whether the currently widespread antihistamine drugs that inactivate histamine receptors should suppress the development of symbiotic microorganisms and what should be the consequences of this inhibitory effect for the whole microbiota-gut-brain axis and, therefore, for human physical and mental well-being? This important issue still awaits further research.

3.3.2. Interaction of Histamine with the Nervous System

In the brain, histamine is produced by the tuberomammilar cells of the hypothalamus that spread their axons to various areas of the brain including the cortex. Histamine is involved in regulating appetite, pain sensitivity, cognitive activities, including the learning process, and the sleep-wake rhythm (histamine promotes awakening and maintains the active brain state). Accordingly, BBB-crossing antihistamine drugs cause somnolence. Histamine facilitates locomotive activity, stimulates thirst, suppresses food-seeking behavior, and inhibits pain sensitivity (Boldyrev et al., 2010; Dubynin et al., 2010).

There is evidence that histamine exerts a neuroprotective effect, which opens up new potentialities for the use of histamine-producing microbiota. Histamine can be used to treat convulsions, ischemia, and stress (Yanai & Tashiro, 2007).

Histamine binds to G protein-coupled H receptors. They are classified into four types that are denoted as H_1, H_2, H_3, and H_4. Presumably, there is an additional receptor type that is based on a chloride channel (Panula et al., 2015; Wouters et al., 2015). In the CNS, histamine mainly binds to H_1 and

H_3 receptors. The chloride channel-based receptor might also contribute to histamine binding. High concentrations of histamine receptors are characteristic of the thalamic striatum, the hippocampus, the substantia nigra, and the amygdala (Westfall et al., 2017).

Beyond the nervous system, histamine stimulates gastric juice secretion, blood vessel dilation, and capillary permeability for immunocytes and protein factors. Histamine influences body temperature, lowers blood pressure, regulates allergic responses and wound healing, and, due to its combined effects inside and outside the CNS, promotes penile erection and libido (White & Rumbold, 1988; Bioldyrev et al., 2010; Westfall et al., 2017). Histamine induces adrenal medulla excitation, resulting in catecholamine release, arteriole constriction, and heart contraction acceleration. Apart from classical synapses between nervous cells, histamine is released by open nerve terminals and spreads via intercellular fluids including the cerebrospinal fluid (Boldyrev et al., 2010).

Despite their low CNS concentrations, trace amines including β-ethylamine can operate as co-transmitters and signal transmission modulators in the nervous system. Presumably, they are implicated in regulating an individual's mood swings and the feelings of hunger and satiety; they are also involved in the development of such mental disorders as attention deficit hyperactivity syndrome (ADHD), bipolar disorder, and Parkinson's disease (Mazzoli & Pessione, 2016).

3.3.3. Interaction of Histamine with the Immune System

As one of the main inflammation mediators, histamine mediates the proinflammatory influence of immunocytes on the non-immune cells of the organism. Apart from adipose cells, histamine is synthesized and deposited in macrophages, lymphocytes, thrombocytes, and other immunologically relevant cells (Zampeli & Tiligada, 2009). Immunocytes mainly express H_1, H_2, and H_3 receptors for histamine. H_1 receptors chiefly exert a proinflammatory influence that is linked to the formation of interleukins IL-1α, IL-1β, IL-6, and others, whereas H_2 receptors are mainly responsible for

immunosuppression that partly results from enhancing the secretion of anti-inflammatory interleukin IL-10 (Gao et al., 2015). The more recently discovered H_4 receptors are chemotactic factors and stimulators of cytokine secretion by various immunocytes and also perform an anti-inflammatory function (Zampeli & Tiligada, 2009, Gao et al., 2015).

Presumably, it is due to these receptors that the probiotic bacterium *Lactobacillus reuteri* exerts an anti-inflammatory effect. *Lact. reuteri* converts food histidine to histamine, suppresses the operation of Toll-like receptors (TLRs), and inhibits the expression of proinflammatory factor TNF-α (Westfall et al., 2017).

Histamine is of paramount importance in terms of Th1/Th2 immune system differentiation. Histamine can shift the balance in favor of the Th1 immune response via H_1 receptors or nonspecifically inhibit both Th1 and Th2 responses via H_2 receptors. In addition to cytokine production, histamine regulates dendritic cell and T_{reg} activity in the inflammation area (Westfall et al., 2017). With the involvement of H_4 receptors, indirect activation of transcription factor STAT6 is attained; presumably, histamine can also activate Th2 receptors (Zampeli & Tiligada, 2009).

Like serotonin, histamine is involved in the development of various inflammatory and autoimmune diseases. Increased histamine levels are detected in patients with Alzheimer's disease, which corroborates the hypothesis that this disease is linked to chronic low intensity inflammation in the nervous system (Westfall et al., 2017). Nonetheless, despite the negative health effects of histamine exemplified by the hypersensitivity response, it can also produce a protective effect.

The capacity of histamine to stimulate T lymphocyte differentiation can promote the treatment of autoimmune problems including multiple sclerosis (MS). It enables decreasing the risk of animmunocyte attacks on the myelin sheath of neurons. Such attacks result in a loss of signal transmission capacity and MS-characteristic neurodegeneration (Jadidi-Niaragh & Mirshafiey, 2010). Hence, histamine-producing microorganisms can potentially be used for treating MS.

Histamine can modulate the functioning of the immune system in many different ways. The extent and direction of histamine effects vary depending

on the micro-environment of immune cells and the type(s) of their histamine receptors.

3.4. Interaction of Acetylcholine with Microorgansims and the Host Nervous and Immune System

Acetylcholine, one of the major neurotransmitters of both the CNS and the peripheral (both sympathetic and parasympathetic) nervous system, performs a wide variety of functions in diverse forms of life (Baig et al., 2018). It was hypothesized that its direct precursor choline was incorporated by unicellular organisms, over a billion years ago, in membrane phospholipids including phosphatidylcholine (Dean, 2009). Subsequently, choline was used in other metabolic pathways and one of them lead to acetylcholine synthesis.

3.4.1. Interaction of Acetylcholine with Microorganisms

Acetylcholine is synthesized by diverse microorganisms including bacilli and lactobacilli (Wall et al., 2014; Johnson & Foster, 2018). As for unicellular eukaryotes, acetylcholine is produced by the protozoan *Acanthamoeba* sp. (Baig et al., 2018). The presence of acetylcholine receptors in unicellular eukaryotes and its regulatory influence on conjugation in infusorians and the growth and proliferation of *Acanthamoeba* sp. (that possesses a homologue of the neuronal muscarine receptor for acetylcholine) also suggest that acetylcholine is a highly evolutionarily conserved signal (Roschina, 2010, 2016; Baig & Ahmad, 2017).

3.4.2. Interaction of Acetylcholine with the Nervous System

Historically, acetylcholine was the first neuromediator to be discovered in 1915 (by Henry Dale); its role in nervous impulse transmission was established by Otto Loewi. The Nobel Prize was awarded to both of them in 1936. In the brain, acetylcholine is responsible for motivation, attention, locomotive behavior, new movement initiation, locomotive stereotype formation, memory, learning, behavioral plasticity, and wakefulness regulation which involves the brainstem reticular formation and basal ganglia.

Memory problems and dementia associated with Alzheimer's disease as well as abrupt uncontrollable movements typical of Huntington's disease result from disrupting the brain acetylcholine system and specifically affecting hippocampal pyramidal cells that are connected to acetylcholine-dependent neurons belonging to other CNS parts. Acetylcholine stimulates the perception of sensory stimuli upon awakening and is involved in rapid eye movement (REM) sleep associated with dreaming (Boldyrev et al., 2010; Riedel et al., 2011).

Acetylcholine is produced in the brain by the neurons of the mesencephalic tegumentum, the septum nuclei, the basal ganglia, and the corpus striatum.

The effects of acetylcholine are due to its binding to two types of receptors (Dubynin et al., 2010): (i) nicotine receptors that are responsible for tobacco addiction and (ii) muscarine receptors that bind muscarine contained in the fungus *Amanita muscari*; each type includes several subtypes. Nicotine receptors (nAchRs) that are coupled with ion channels for sodium, potassium, and calcium are subdivided into muscular receptors that are blocked by the poison curare and neuronal, hexamethonium-blocked, receptors. Muscarine receptors (mAchRs) are coupled with G proteins and include five subtypes ($mAchR_1$-$mAchR_5$). The $mAchR_1$, $mAchR_3$ and $mAchR_5$ receptors activate phospholipase C upon binding acetylcholine, which results in increased intracellular inositol-1,4,5-triphosphate and Ca^{2+} concentrations. The $mAchR_2$ and $mAchR_4$ receptors decrease the cAMP level by inhibiting the adenylate cyclase enzyme. The

subtypes of muscarine receptors differ in terms of their location in brain structures and beyond the brain; their effects are also different. In the CNS, the functions of these receptors are related to emotional behavior and image recognition (Boldyrev et al., 2010).

Acetylcholine is released in neuromuscular junctions; it elicits skeletal (striated) muscle contraction. Disrupting this function by inhibiting the acetylcholine-degrading enzyme, choline esterase, has serious consequences ranging from convulsions to paralysis. Outside the CNS, important acetylcholine effects include deceleration of cardiac contraction, stimulation of GI peristalsis, induction of smooth muscle contraction, and regulation of the bronchial, perspiratory, lacrimal, and salivary glands (Boldyrev et al., 2010). Acetylcholine binds to the muscarine receptors of vascular endothelium, resulting in nitric oxide production, vasodilation, and blood pressure decrease (Kellogg et al., 2005).

3.4.3. Interaction of Acetylcholine with the Immune System

In various tissues, macrophages and other immune cells express nicotine ($nAchR_{\alpha 2}$, $nAchR_{\alpha 4}$, $nAchR_{\alpha 7}$, $nAchR_{\beta 2}$, and $nAchR_{\beta 4}$) and muscarine ($mAchR_1$, $mAchR_4$, and $mAchR_5$) acetylcholine receptors (Ley et al., 2010). Of paramount importance is the interaction of acetylcholine with the $nAchR_{\alpha 7}$ receptor that results in inhibiting the transfer of transcription factor NF-κB into the cell nucleus and, accordingly, in suppressing the production of proinflammatory cytokines TNF, IL-1β, IL-6, and HMGB1 (Ley et al., 2010). This mechanism appears to account for the anti-inflammatory effect of acetylcholine that is exerted via (i) afferent impulses to the CNS and (ii) efferent impulses in the branches of *nervus vagus*. Interestingly, acetylcholine does not suppress the secretion of anti-inflammatory cytokine IL-1 (Ley et al., 2010). Acetylcholine and the inhibitors of choline esterase manifest anti-inflammatory activity both *in vivo* and *in vitro* (Silva-Herdade & Saldanha, 2013). Apart from possessing acetylcholine receptors, lymphocytes and macrophages contain the complete cholinergic system.

They can synthesize acetylcholine. Choline acetyltransferase mRNA was detected in peripheral T lymphocytes (Anderson & Tracey, 2012).

3.5. Interaction of Agmatine with Microorgansims and the Host Nervous and Immune System

Agmatine, (4-aminobutyl) guanidine, like other cadaveric decomposition-produced amines *(ptomaines)*, such as cadaverine and putrescine, is formed via enzymatic decarboxylation of amino acids. Specifically, agmatine results from arginine decarboxylation, although it can also be produced by putrescine deamination (Doeun et al., 2017).

3.5.1. Interaction of Agmatine[25] with Microorganisms

Agmatine is released into the medium by diverse microorganisms including some representatives of *Lactobacillus.* Putrescine and cadaverine are also produced by microorganisms, e.g., by many bacterial strains inhabiting pheasant carcasses (Buňková et al., 2016). Puterscine is present in many kinds of wine (Doeun et al., 2017).

Ptomaine producers can be controlled by their antagonists in the microbial world. By displaying aggressive behavior against ptomaine producers, the antagonists prevent the accumulation of these toxic compounds, e.g., in stored ground meat where a combination of *Lactobacillus sake* and an unidentified strain, G-106 was used. While decreasing putrescine and cadaverine production, the *Lact. sake*–G-106 mixed culture did not inhibit the release into the medium of other biogenic amines, such as histamine and tyramine (Roig-Sagués & Eerola, 1997). Some microorganisms exemplified by the fungus *Aspergillus niger* form

[25]Putrescine and cadaverine are also briefly discussed, even though no data on their operation in the capacity of neuromediators are available.

enzymes (MAOs) that oxidize ptomaines, including agmatine (Yagodina et al., 2000).

Agmatine exerts specific effects on microorganisms. For instance, it suppresses gut colonization by the parasitic protozoan *Cryptosporidium parvum* andprevents the infection (Lyte, 2016).

As for the other ptomains, putrescine stimulates the bioluminescence of the GM strain *E. coli* TGI containing the *lux* operon from *Photorhabdus luminescencens* ZMI (Sorokina et al., 2019), while suppressing cell proliferation (CFU formation) in this strain (Vodolazov, Sorokina, & Oleskin, unpublished).

3.5.2. Interaction of Agmatine with the Nervous System

Following the discovery of endogenous agmatine synthesis in mammals in 1994, it was revealed that agmatine influences numerous molecular targets in the organism, such as ion channels, membrane transporters, and nitric oxide-synthesizing systems; it also affects polyamine metabolism, protein ADP-ribozylation, matrix metalloproteases, NADPH oxidase, etc. (Lyte, 2016). Therefore, it has been suggested that agmatine functions as a neuromediator (Piletz et al., 2013). Although no specific agmatine receptors have been detected up to now, agmatine has been revealed to bind to the receptors of other neuromediators. It binds to α_2-adrenergic and imidazoline receptors and blocks NMDA receptors, i.e., behaves as a meuromodulator and co-transmitter that affects the operation of other neurotransmitter systems.

Agmatine was established to lower blood pressure and decelerate the heart rhythm, decrease the glucose concentration in the blood, and stimulate the filtration function of the kidneys (Raasch et al., 2001; Satriano, 2004; Piletz et al., 2013).

Putrescine and cadaverine, as well as the polyamines spermine and spermidine are involved in regulating the CNS response to stress (Bienenstock & Collins, 2010).

3.5.3. Interaction of Agmatine with the Immune System

The impact of agmatine and other ptomaines on the immune system and the whole microbiota-nervous system-immune system complex still awaits further research. Hypothetically, agmatine influences the infectious process by affecting the pathways of conversion of arginine to (i) nitric oxide that kills infectious agents and (ii) ornithine, the polyamine precursor that promotes cell proliferation in the inflammation area (Satriano, 2004; Uranchimeg et al., 2010; Ahn et al., 2012; Chai et al., 2016).

Agmatine inhibits the inducible NO synthase and exerts an anti-inflammatory effect. If administered after ischemic brain damage, admatine decreases the $CD11b^+$ macrophage number in the spleen. It also reduces the T_{reg} cell number. Administration of agmatine limits neural inflammation after experimental disruption of cerebral blood circulation in rats, decreases the necrosis area, and produces a vasoprotective effect (Uranchimeg et al., 2010). In RAW 264.7 macrophages, agmatine induces the activation of nuclear trascription factor Nrf2 and stimulates the production of antioxidant enzymes, which may contribute to its neuroprotective activity (Ahn et al., 2012; Chai et al., 2016).

3.6. Interaction of Neuroactive Amino Acids with Microorgansims and the Host Nervous and Immune System

Neuroactive amino acids including glutamic and aspartic acid, glycine, taurine, and γ-aminobutyric acid (GABA) are present in the mammalian organism in the free and the bound form. They are formed via metabolic transformation of nutrients by intestinal and microbial enzymes. These amino acids are utilized by pro- and eukaryotic cells as nutrient substrates. For instance, glutamate is one of the main nutrient substrates for intestinal cells (enterocytes). Nevertheless, amino acids, often at low concentrations,

serve as signal molecules that operate within the whole microbiota-nervous system-immune system triangle.

3.6.1. Interaction of Amino Acids with Microorganisms

Although microorganisms utilize amino acids as nutrient substrates, they also recognize them as signals. The specific regulatory influence of neuroactive amino acids is exemplified by the data that glutamate[26] (along with lysine, methionine, and succinate) stimulates and aspartate (along with lactate and formate) inhibits the growth of the probiotic strain *E. coli* M-17. Under the same conditions, aspartate, in contrast, produces a stimulatory effect on the strain *E. coli* BL (Vakhitov et al., 2000; Vakhitov & Sitkin, 2014). Overall, the effects of these autoregulators vary depending on their dose, the tested strain, the culture growth phase, and medium composition.

The neurotransmitter *γ-aminobutyric acid (GABA)* increases the resistance of *Lact. reuteri* to medium acidification (Lyte, 2014). GABA stimulates the expression of the pathogenic factors of *Candida albicans*, which manifests itself in the intensification of the synthesis of phospholipase B1-encoding mRNA, germ tube formation, and subsequent hypha development. In combination, these events contribute to the development of infection (Reyes-Garcia et al., 2012). It was also revealed that GABA stimulates the virulence of *Ps. aeruginosa* by regulating the expression of six pathogenicity-related protein factors (Mazzoli & Pessione, 2016).

The macro- and microstructure of *E. coli* colonies and, presumably, biofilms is formed under the influence of *aspartate* (Budrene & Berg, 1991, 2002) as an attractant. Complex structural patterns including concentric circles and hexagonal lattices result from the superposition of the two concentration gradients of aspartate that are formed in the colony center and, in addition, produced by the cells on the colony periphery. Bacteria form

[26]Since organic acids are predominantly present in the form of ions in biological systems, it is common to write *glutamate* and *aspartate* instead of *glutamic* and *aspartic acid*, respectively. This rule only does not apply to *γ-aminobutyric acid* that is routinely abbreviated as *GABA.*

concertedly moving clusters that generate complex patterns on the agar surface in the presence of aspartate (Mittal et al., 2003).

Additional evidence of specific interaction between neuroactive amino acids and microorganisms is provided by the data that they possess amino acid-binding receptors. *Pseudomonas fluorescens* contains a periplasmic protein with a high affinity for GABA; the protein is related to one of the subunits of the ionotropic $GABA_A$ receptor of mammals. GABA-binding receptors were also detected in *Ps. aer*uginosa (PctC) and *Ps. putida* (McpG). A potassium-dependent glutamate channel (GluR0) was revealed in the cyanobacterium *Synechocystis* PCC6803 (Mazzoli & Pessione, 2016).

Microorganisms including GI microbiota representatives produce large amounts of neuroactive amino acids. Coryneform bacteria, such as *Corynebacterium glutamicum*, *Brevibacterium lactofermentum*, and *Brevibacterium flavum*, are widely used as industrial glutamate producers (Mazzoli & Pessione, 2016). The glutamate-synthesizing capacity is characteristic of lactic-acid bacteria, e.g., of the tested strains of the species *Lactobacillus plantarum*, *Lact. paracasei*, and *Lactococcus lactis* subsp. *lactis* (Zhilenkova et al., 2013; Oleskin et al., 2014a, b; Mazzoli & Pessione, 2016; Vodolazov et al., 2018).

As for GABA, both prokaryotes and eukaryotes synthesize it via glutamate decarboxylation. Glutamate decarboxylase was detected in gram-positive and gram-negative bacteria, in which it is linked to systems responsible for pH maintenance and proton-motive force generation. Analysis of metagenomic data during the recent Human Microbiome Project revealed that glutamate decarboxylase-encoding genes are present in a considerable part of the tested representatives of the human intestinal microbiota (reviewed, Mazzoli & Pessione, 2016).

Among microbial producers of amino acid neurochemicals, an important role is played by lacto- and bifidobacteria that represent valuable probiotics and are widely spread within the microbiota of the gastrointestinal and urogenital tracts of mammals, including the human species. Amino acids accumulate in fermented dairy items and fermented products prepared from vegetables, meat, and fish. From the human GI tract, four GABA-producing *Lactobacillus* strains and one *Bifidobacterium* GABA producer were

isolated. *Lact.brevis* DPC 6108 proved to be the most efficient GABA producer on a glutamate-containing medium (90% of the glutamate were converted to GABA, Barrett et al., 2012).

In human blood plasma and spinal fluid, GABA is present at concentrations of ~0.6 and ~0.3 μM, respectively (Abbott et al., 1982), which are close to those produced by lactobacilli. A strain of *Lact. delbrueckii* subsp. *bulgaricus* synthesized 0.3 μM GABA on a milk-containing medium (Oleskin et al., 2014a, b).

A GABA-producing strain, *Lactobacillus brevis* FPA 3709, was used to enrich soybean milk with GABA. Such soybean milk relieved depression in rats: it took them less time to start swimming in a forced swimming test, and GABA was as efficient as the antidepressant fluoxetine (Ko et al., 2013).

In the human organism, GABA is a prerequisite for normal pain sensitivity of the intestine and for the operation of the immune system. GABA mitigates inflammation processes and allergic responses by suppressing the activity of T lymphocytes (Auteri et al., 2015). An imbalance in the GI microbiota frequently results in decreased microbial production of GABA, which increases the risk of irritated bowel syndrome and other inflammatory intestinal diseases (Babin et al., 1994).

GABA is also present in plants, in which it behaves as a signal involved in plant growth regulation (Ramesh et al., 2016).

3.6.2. Interaction of Amino Acids with the Nervous System

Neuroactive amino acids are involved in regulating the impulse transmission rate in the nervous system. They differ in their effects on the nervous system. Amino acids such as glutamate and aspartate activate specific structures in the nervous system. Other amino acids including GABA and glycine exert an inhibitory influence on the nervous system. Besides, amino acids produce multiple effects on the whole organism.

Of note are the roles of glutamate and GABA. Both amino acids along with glutamine form a part of a cycle that is necessary for the homeostatic operation of the CNS. Disruption of the GABA-glutamate-glutamine

interconversion is associated with mental problems including anxiety, depression, bipolar disorder, and schizophrenia.

GABA and glutamate receptors are present in pre- and postsynaptic neurons and in glial cells such as astrocytes.

The major excitatory neurotransmitter glutamate is contained in the neocortex, olfactory bulbs, hippocampus, substantia nigra, cerebellum, and eye retina (Boldyrev et al., 2010). Glutamate is the predominant neurochemical in the nervous system of verterbrates (Meldrum, 2000); it is present in over 90% of all synapses in the human brain. Glutamate stimulates impulse transmission in the CNS and the energy metabolism of brain cells. It is involved in ammonia detoxification, helps improve behavioral symptoms in mentally retarded children, and mitigates stress. Glutamate is implicated in cognitive activities including learning and information memorization. Normally, almost no glutamate can cross the BBB[27]; it is synthesized in the brain from α-ketoglutarate in a transaminase-catalyzed process.

Gutamate accumulates in intracellular vesicles and is thereupon liberated into the synaptic cleft and bound by glutamate receptors (GluRs) that are subdivided into:

1. membranotropic receptors (mGluRs) with seven domains in their membrane-bound structure; there are at least 8 types of mGluRs, they include group I receptors that activate phospholipase C and increase the inositol-3-phosphate and diacylglycerol concentrations and group II and group III receptors that inhibit adenylate cyclase and lower the cAMP level;
2. ionotropic receptors (iGluRs) can be divided into at least three types that bind N-methylaspartate (NMDA receptors), α-amino-3-hydroxy-5-methyloxazole-4-proprionate (AMPA receptors), and kainate (kainate-sensitive receptors; Boldyrev et al., 2010; Mazzoli &Pessione, 2016), respectively.

[27]Glutamate penetrates into the CNS if the BBB is disrupted, e.g., under stress.Translocation of large glutamate amounts into the brain may have serious consequences (see 4.2 below).

NMDA receptors are present in the CNS from the spinal cord to the neocortex, they are especially abundant in the hippocampus (Dubynin et al., 2010).

In addition to the CNS, metabotropic glutamate receptors ($mGluR_4$ receptors) are present in the mucosal cells of the stomach and the duodenum. Similar $mGluR_4$ receptors and $mGluR_7$ receptors are located in the colon epithelium, which is essential for its direct interaction with the glutamate-producing microbiota. Enteroendocrine cells that form a part of the gut wall contain TAS1R3, a subunit of the receptor responsible for the umami taste of glutamate (Mazzoli & Pessione, 2016).

γ-Aminobutyric acid (GABA) performs the function of the main inhibitory neurochemical in the brain. The maximum GABA concentrations are characteristic of the substantia nigra, hypothalamus, cerebellum, and globus pallidum (Boldyrev et el., 2010). There are three main classes of GABA receptors, denoted as $GABA_A$, $GABA_B$, and $GABA_C$. The five-subunit $GABA_A$ and $GABA_C$ receptors form a part of ligand-gated ion channel complexes. Their activation results in increasing their permeability for chloride and bicarbonate, respectively. Metabotropic $GABA_B$ receptors, more widely spread in the peripheral nervous system, are G protein-coupled receptors that open or close ion channels.

GABA actively influences numerous impulse transmission processes. It is involved in brain cell metabolism. GABA plays a major role in regulating the sleep-wake cycle, locomotor activity, vascular tone, and information memorization and recognition. Despite the overall inhibitory effect, GABA behaves as a mild psychostimulant because it activates bioenergetic processes in the brain tissue and improves the utilization of glucose and other energy sources. GABA exhibits moderate antihypoxic and antiseizure activity, produces a sedative effect, promotes concentration, improves sleep, and can be used as as a non-addictive tranquilizer. GABA improves memory, promotes the restoration of locomotor activity and speech in patients with cerebral vascular disorders, ameliorates glucose utilization by brain cells, and facilitates the disposal of toxic metabolic products (Hevia et al., 2015)

GABA receptor ligands are considered potential drugs for treating CNS diseases and mental problems, including Parkinson's and Alzheimer's disease, sleep disorders (insomnia and narcolepsy), and epilepsy. Huntington's disease is also characterized by a decreased GABA concentration in the brain. In infancy, GABA predominantly exerts an excitatory, not inhibitory, influence on developing neurons. The influence of GABA on the GI tract limits its pain sensitivity (Auteri et al., 2015; Yunes et al., 2016; Averina & Danilenko, 2017),

An imbalance in the GI microbiota frequently results in decreasing microbial GABA synthesis. This poses the threat of the development of IBS and other intestinal problems including Crohn's disease and even colon cancer (Babin et al., 1994). Transmembrane GABAergic receptors are located on the neurons of the central and peripheral nervous system and on the cells of the GI tract, the kidneys, the lungs, the liver, and other organs (Averina & Danilenko, 2017). GABA ionotropic and metabotropic receptors are widely spread in the enteric nervous system (Auteri et al., 2015). The metabotropic receptors of the GI tract ($GABA_B$) perform a number of important functions, including intestinal motility regulation and signal transmission from the gut to the brain (Mazzoli & Pessione, 2016). The airway epithelium contains a GABAergic system that is activated by allergens. It seems to be implicated in asthma development (Xiang et al., 2007).

GABA is produced by the insulin-secreting β cells of pancreatic islets and released into the bloodstream by them. GABA binds to the receptors of pancreatic α cells, suppressing the production of the insulin antagonist glucagon by them (Rorsman et al., 1989). GABA promotes the survival and proliferation of β cells (Purwana et al., 2014) and the conversion of α cells to β cells, thereby improving diabetic symptoms (Ben-Othman et al., 2017). An additional GABA source in the peripheral blood is the brain. Low molecular weight factors can migrate from it, entering the bloodstream via the recently investigated *glymphatic* system (Plog & Nedergaard, 2018).

Some GABA molecules cross the gut-blood barrier and the BBB (Boonstra et al., 2015). It seems likely, therefore, that many GABA effects, including its relaxing and tranquilizing influence, the stimulation of

memory, creative work, and attention, sleep normalization, the antioxidant effect, and others, are due to the combined impact of endogenous, microbial, and dietary GABA. Recently, it has been revealed that the GABA-producing bacteria *Bifidobacterium dentium* decrease the abdominal pain sensitivity of rats at the level of the dorsal roots of the spinal cord (Mazzoli & Pessione, 2016).

Glycine functions as a neuromediator in the brainstem and the spinal cord; it inhibits neuronal activity by suppressing the release of glutamate, an excitatory amino acid (see above), from neurons. Glycine promotes GABA formation and helps glutamate and aspartate perform their signal functions. Glycine potentiates the responses of glutamate NMDA receptors by binding to a special glutamate binding site on them. In the spinal cord, glycine inhibits motoneuron activity. Glycine also stimulates the functioning of the pituitary, improves metabolic processes in CNS cells, exerts an antistress effect, improves intellectual working capacity, produces a sedative effect, improves sleep, and enhances the organism's adaptive potential. The structure of the 5-subunit glycine receptor is generally similar to that of the $GABA_A$ receptor.

Aspartate, an excitatory amino acid, improves mood and prevents the state of fatigue. Aspartate promotes ammonia removal from the organism. Aspartate binds to the same receptors as glutamate but it is less widely spread in the CNS. The maximum aspartate content is characteristic of the midbrain. Much aspartate is contained in the inferior olivary nucleus of the medulla oblongata. It is also present in the dorsal and ventral grey matter of the spinal cord (Boldyrev et al., 2010; Dubynin et al., 2010).

The expression of the genes of amino acid receptors is under the influence of the symbiotic microbiota of the GI tract. In GF mice, the expression level of the gene encoding subunit NR2B of the glutamate NMDA receptor in the hypothalamus and the amygdala of the brain is abnormally low (Rohrscheib & Brownlie, 2013).

3.6.3. Interaction of Amino Acids with the Immune System

Like other neurochemicals, amino acids are synthesized by immune cells including T cells, macrophages, and dendritic cells (Bhat et al., 2010; Fuks et al., 2012). $GABA_A$ and $GABA_B$ receptors are present on the surface of many immunocytes. Immunocytes possess α_1, α_2, β_1, β_3, δ, and plausibly other subunits of $GABA_A$ receptors (Jin et al. 2013). The immunotropic effects of GABA are complex; they vary depending on the GABA receptor types involved.

GABA's anti-inflammatory effect is due to suppressing T lymphocyte activity (Auteri et al., 2015). This is consistent with the fact that activation of GABA receptors on T cells and macrophages results in inhibition of the production of proinflammatory cytokines including interleukins IL-1β, IL-2, IL-6, IL-12, interferon-γ, and TNF-α (Bhandage et al., 2018). The work cited presents evidence that GABA influences (predominantly inhibits) the secretion of a wide spectrum of cytokines by immunocytes, e.g., CD4+ T cells.

The cytokines are involved in the operation of the Th1 and Th2 branches of the immune system. Type I diabetes results in increasing the levels of 26 cytokines in the blood; GABA administration inhibits the release of 16 of these cytokines (Bhandage et al., 2018). In preclinical tests, GABA behaves as a protector with respect to experimental autoimmune encephalomyelitis, type 1 diabetes, contact dermatitis, and other immune disorders. GABA can be administered orally and is commercially available (Prud'homme et al. 2015).

Despite the evidence for the anti-inflammatory activity of GABA, there are literature data on the proinflammatory effects of this compound *in vivo* under certain conditions. Microbially produced GABA can to an extent promote inflammatory processes in the GI tract, including those that are caused by *Cl. difficile* (Dann et al. 2014).

Glycine can also exert immunotropic effects. Various cells of the immune system, including T lymphocytes and neutrophils, possess surface receptors for glycine. There is evidence that glycine exhibits anti-inflammatory activity *in vivo* and *in vitro*. In its presence, the secretion of

proinflammatory mediators such as IL-1 and TNFα is suppressed, while the synthesis of the anti-inflammatory mediator IL-10 is stimulated. *In vivo*, glycine mitigates the symptoms and prevents the consequences of experimental endotoxic shock. These effects are caused by very high glycine doses, corresponding to a 5% level of glycine in nutrients consumed per day (van den Eynden et al. 2009). The data concerning the immunological impact of glycine are partly controversial: there is evidence that it produces proinflammatory effects in patients with periodontitis (Rausch-Fan et al. 2005).

Excitatory amino acids also possess immmunoregulatory properties. Immunocytes can secrete glutamate and express various glutamate receptors on their surface. Human T cells express NMDA receptors for glutamate that are involved in T cell activation. T cells' activities including adherence, migration, and proliferation are stimulated in the presence of glutamate. High glutamate concentrations predominantly produce immunosuppressive effects. Glutamate plays a major role in the development of some autoimmune disorders. MS is associated with a considerable increase in the number of $GluR_3$ receptors on T cells. In addition, the proliferation of autoreactive T cells is stimulated (Ganor & Levite, 2014). Conversely, the activation of $GluR_4$ receptors by glutamate presumably exerts a protective effect with respect to inflammatory neurodegenerative diseases. $GluR_4$ facilitates the induction of T regulatory cells in patients with MS (Hansen & Caspi, 2010).

3.7. Interaction of Short-Chain Fatty Acids (SCFAs) with Microorgansims and the Host Nervous and Immune System

Short-chain fatty acids are saturated unbranched fatty acids with short carbon chains. Of paramount importance in biological terms are SCFAs with two to four carbon atoms in the chain, i.e., acetic, propionic, and butyric acid. Since they are mostly present in biological systems as anions, they will

be referred to acetate, propionate, and butyrate, respectively. All the SCFAs represent volatile liquids under normal conditions, due to their low molecular weight.

SCFAs are among the major intermediate and final products of fermentation of complex dietary, bacterial, and endogenous biopolymers, including mucins, glycoproteins, and the proteins of shedded epithelial cells. Their concentrations may be as high as 70-140 mM in the upper and 20-70 mM in the lower part of the colon; acetate is the predominant SCFA. Apart from the gut, significant SCFA concentrations are present in the oral cavity that contains 6-38 mM acetate, 1-13 mM propionate, and up to 5 mM butyrate. In the female reproductive system, the acetate concentrations is up to 120 mM (in its lower part). In this system, SCFA concentrations vary depending on the presence of infection or local inflammation (Correâ-Oliveira et al., 2016). GF mice are distinguished by lowered SCFA levels (vandeWouwetal., 2017).

In the GI tract, SCFAs are involved in regulating (Verbeke et al., 2015; El Aidy et al., 2016; Shenderov, 2008, 2013a, b, 2016; Oleskin & Shenderov, 2016):

- colonial resistance;
- cell differentiation, proliferation, and apoptosis;
- mucin and glycopeptide synthesis;
- maintenance of the electrolyte and energy balance;
- production of antimicrobial peptides, neurotransmitters, and hormones;
- intestinal motility;
- immune system operation.

Many effects of these acids are due to their functions in energy metabolism, epigenomic processes, GI microbiota regulation, and the operation of nervous and immune cells within the GI tract and beyond it (Shenderov, 2013a, b, 2016; Oleskin & Shenderov, 2016; El Aidy et al. 2016; van de Wouw et al., 2017). SCFAs are important regulators of carbohydrate and lipid metabolism in the GI tract, the liver, and other organs

(Shenderov, 2013a, b). They serve as a major energy substrate for many representatives of the symbiotic microbiota of the GI tract (Erofeev et al., 2012).

Upon entering colonocytes and other host cells, SCFAs are rapidly metabolized. Acetate in the form of acetyl-CoA is directly used in the citric acid cycle and serves as an energy source for mitochondria. 59-80% of the per diem energy for colon cells (colonocytes) are supplied in the form of butyrate; propionate is utilized by liver cells (hepatocytes; Shenderov, 2013a, b; Hamer et al., 2008; Neish, 2009; van de Wouw et al., 2017). As far as SCFAs are concerned, it is only acetate that is directly transferred to the bloodstream at significant concentrations (van de Wouw et al., 2017). In various tissues, glucogenesis and lipogenesis partly depend on the available amounts of acetic and propionic acid. They modify the levels of glucagon, acetyl-CoA, insulin, and other compounds (Shenderov, 2008; 2013; MacFabe, 2012). Low butyrate concentrations reversibly decrease the permeability of the GI mucosa by activating cell AMP proteinase and strengthening the junctions between intestinal epitheliocytes (Canani et al., 2011). Butyrate also stimulates mucin synthesis and secretion and promotes mucous membrane repair by accelerating cell migration (Canani et al., 2011). Propionate facilitates intestinal smooth muscle contractions, dilates intestinal arteries, stimulates serotonin release from gut enterochromaffin cells, and inhibits stomach motility (MacFabe, 2012).

3.7.1. Interaction of SCFAs with Microorganisms

SCFAs are used as an important energy source by many representatives of the GI microbiota (Erofeev et al., 2012). Acetate markedly stimulates the growth of intestinal bacteria such as *Roseburia spp.* and *Faecalibacterium prausnitzi* that synthesize another SCFA, butyrate (Duncan et al., 2004). SCFAs at high concentrations exhibit antimicrobial activity, particularly with regard to gram-negative bacteria (Shenderov, 2013a; Neish, 2009). Butyrate and propionate suppress the growth of *Salmonella* but stimulate the proliferation of lactobacilli. The antimicrobial activity of physiological

SCFA concentrations has been detected in the GI tract and the female genital system (Shenderov, 2013a). Propionate is widely used in Europe as a food additive (E280–E282) because it exhibits antifungal activity (van de Wouw et al., 2017).

Phenylbutyrate induces the production of endogenous antimicrobial peptides, LL-37 and its homologue, catalycidine CAP-18, in intestinal epitheliocytes and phagocytes. A combination of these antimicrobial peptides and butyric acid produces a strong synergistic bactericidal effect on *Shigella in vitro* (Raqib et al., 2006).

SCFA-dependent microbiota-host interaction is bidirectional or, rather, multidirectional (see 2.3 above). Microorganisms specifically respond to host-produced SCFAs and also synthesize them, predominantly by degrading host-indigestible dietary fibers.

Literature data indicate that SCFAs, and especially acetate and butyrate, are involved in maintaining the GI barrier and preventing bacterial translocation from the intestinal lumen to the bloodstream. The contribution of SCFAs to gut colonial resistance partly depends on their utilization as energy sources by various kinds of cells, suppression of the growth and proliferation of food-born microorganisms, stimulation of the growth of symbiotic microbiota, and the maintenance of the integrity of gut mucosa and crypts (Shenderov, 2013a, b; Correâ-Oliveira et al., 2016).

Overweight and obese individuals are characterized by markedly increased levels of SCFAs, especially propionate, which is correlated with a shift in the *Firmicutes:Bacteroidetes* ratio in favor of *Bacteroidetes*. Risperidone that is used for treating mental disorders in children and adolescents and causes weight gain (as a side effect), increases SCFA production by their GI microbiota. These data seem to be not quite consistent with the fact that SCFA administration to mice brings about a weight loss and normalizes the composition of the microbiota (van de Wouw et al., 2017).

Human individuals with a loss of appetite (anorexia) and a decreased weight are characterized by lowered levels of acetate and propionate; their levels remain low even if their weight becomes normal (van de Wouw et al., 2017).

SCFAs are not only synthesized by the GI microbiota. Propionate is formed by the representatives of *Propionibacteria* that inhabit the skin and cause acne. Propionate is also produced by the oral microbiota that is implicated in gum inflammation (gingivitis; MacFabe, 2012).

3.7.2. Interaction of SCFAs with the Nervous System

Approximately 60% of GI tract diseases are accompanied by neuropsychological disorders. They may result from changes in the energy level of nervous cells that depend on the availability of SCFAs. Alternatively, individual SCFAs can produce their specific effects on the nervous cells in the CNS and the peripheral nervous system. SCFAs can affect calcium influx into cells, intracellular pH maintenance, lipid metabolism, the gap junction-dependent cell barrier function, gene expression, and immune system activity (MacFabe, 2012).

SCFAs, including those of microbial origin, can cross the gut-blood barrier and the BBB (Westfall et al., 2017) and, therefore, directly influence brain biochemistry.

SCFAs promote the maturation and active operation of microglial cells that represent CNS immunocytes (macrophages). SCFAs also strengthen the BBB; they prevent microbial LPSs from disrupting the BBB by systemically activating the immune system of the organism (Osadchiy et al., 2019).

Apart from regulating CNS activity via energy metabolism, some of the SCFAs can modify the synthesis, in nervous and endocrine cells, of the following neuroactive substances: histamine, serotonin, catecholamines, tyramine, 5-aminovaleric acid, GABA, leptin, peptide YY, hydroxybutyrate, β-alanine and other amino acids (Vinolo et al., 2011; Kimura et al., 2011; Clarke et al., 2014; El Aidy et al., 2016), glucagon-like peptide (GLP-I;Tolhurst et al., 2012), catecholamines, and other hormones and neuromediators (MacFabe, 2012; Oleskin & Shenderov, 2013, 2016; Clarke et al., 2014; El Aidy et al., 2016;). SCFAs also influence amino acid synthesis by inhibiting GABA-dependent and stimulating glutamate-

dependent processes. SCFAs affect neuropeptide formation; for instance, they increase encephalin levels.

Many of the systemic and CNS-specific effects of SCFAs apparently depend on their binding to molecular targets. SCFAs modify gene activity by stimulating the histone acetyltransferase and inhibiting the histone deacetylase enzyme. Among SCFAs, butyrate is the most efficient inhibitor of histone deacetylases that belong to class I and IIa. The inhibition results in promoting chromatine acetylation and unwinding. This epigenetic effect facilitates the access of repair enzymes to the DNA (Westfall et al., 2017). This promotes the improvement of the health state of patients with an excessive activity of these enzymes that is characteristic of Parkinson's disease, depression, and schizophrenia.

The molecular targets of SCFAs include G-protein-coupled receptors (GPCRs), such as FFAR2, or GPR43, and FFAR3, or GPR41. The weight decrease ("slimming down") under the influence of exogenous SCFAs is due to their binding to these receptors. Mice with abnormally high FFAR2 densities do not gain weight even on a fat-enriched diet, while FFAR2-deficient mice are obese even despite a normal diet. FFAR3 is characteristic of ENS cells, afferent vagal fibers, and other parts of the peripheral nervous system (van de Wouw et al., 2017).

SCFAs such as propionate, butyrate, and their derivatives interact with ENS ganglion receptors, which enables them to influence the energy homeostasis of nervous cells. By upregulating or downregulating their energy budget, SCFAs can modify their activity (Clarke et al., 2014; El Aidy et al., 2016). Data obtained with rat pheochromocytome cells indicate that propionate but not butyrate can regulate the expression of the gene coding for tryptophan hydroxylase, the key enzyme of the serotonin biosynthesis pathway, and, therefore, produce an effect on brain neurochemistry (Clarke et al., 2014). SCFA-binding receptors FFAR3 (GPR41) are also characteristic of several types of enteroendocrine cells within the whole GI tract from the stomach to the colon (Mazzoli & Pessione, 2016). Propionate promotes the release of glucagon-like protein 1 (GLP-1) and peptide YY by activating FFAR2 (Ivashkin & Ivashkin, 2018).

Apart from a local direct influence on the intestine, butyrate produces extra-intestinal, systemic effects that impact the host CNS and behavior (Bienenstock & Collins, 2010; Shenderov, 2013a, b; Oleskin & Shenderov, 2013, 2016; Clarke et al., 2014; El Aidy et al., 2016). One-time or repeated (for 28 days) injection of butyric acid mitigates depression and anxiety. This seems to be due to an increased synthesis of the *brain-derived neurotropic factor (BDNF)* in the cells of the hippocampus and the frontal cortex (Schroeder et al., 2007). The brain serotonin concentration increases under the influence of acetate (Ivashkin & Ivashkin, 2018). Butyrate activates the synthesis of the *glial cell line-derived neurotrophic factor* (*GDNF*; Westfall et al., 2017).

An important molecular target of butyrate as a histone deacetylase inhibitor is the *Forkhead box (FOXO)* gene locus. It represents a longevity factor and includes genes that code for antioxidant agents and are involved in stress responses. Under pathological conditions, e.g., during Alzheimer's disease, the FOXO locus may activate apoptosis factors, resulting in nervous cell death (Westfall et al., 2017).

The effects of butyrate are not only confined to the nervous system. In the presence of butyrate, the number of cholinergic (acetylcholine-dependent) neurons in the enteric nervous system is increased, which is due to epigenetic mechanisms such as regulation of histone acetylation (van de Wouw et al., 2017). SCFAs facilitate the maturation of microglial cells in GF mice (van de Wouw et al., 2017).

A rise in luminal butyrate concentration brings about an increase in the number of receptors (GPR41; GPR43; and 5-HT_4) on the membranes of colon mucosal endocrinocytes that interact with butyrate. The local and systemic influence of SCFAs that is due to the regulation of intestinal motility, appears to be linked to their effects on the neurohumoral mucosal receptors of the terminal part of the ileum and other areas of the colon. These effects of SCFAs on intestinal motility are dose-dependent (Tazoe et al., 2008; Soret et al., 2010; Canani et al., 2011; MacFabe, 2012; Oleskin & Shenderov, 2016). Butyrate directly stimulates the activity of vagal afferent fibers and inhibits amyloid accumulation in the brain of patients with

Alzheimer's disease, which improves their memory and cognitive capacities (Westfall et al., 2017).

Butyrate administered to volunteers by enema decreased visceral pain sensitivity. It also relieved the sense of discomfort in the colon area by elevating the sensitivity threshold of visceral mechanoreceptors and increasing the production of peptide YY that suppresses phasic contractions of the circular smooth muscles of the colon (Canani et al., 2011; Erofeev et al., 2012).

The butyrate derivative *γ-hydroxybutyrate, or γ-hydroxybutyric acid (GHBA)* bears much similarity to GABA. GHBA inhibits histone deacetylase, exerts an antihypoxic, sedative, and myorelaxant influence, and significantly increases the systemic dopamine level while producing little effect on the concentrations of other catecholamines. Aqueous solution of GHBA sodium salt has been used for a long time as an anesthetic. In studies with mouse nervous cells, it was established that, like butyrate, GHBA behaves as a neuroprotector: it suppresses neuronal apoptosis (Westfall et al., 2017).

Recently, GHBA has received much attention because it and its derivatives are widely used by drug addicts to modify the time course of the effect of ecstasy-type drugs. GHBA is metabolized via the Krebs cycle pathway, which gives grounds for the suggestion that numerous gut microbiota representatives may produce and metabolize GABA and GHBA.

Propionate produces a number of useful effects such as increasing insulin efficiency, decreasing the blood cholesterol level, and limiting appetite. However, high propionate concentrations cause health problems and affect behavior; they may cause mental retardation. Propionate also brings about seizures, affects locomotive behavior, and causes metabolic acidosis (acidification of an organism's internal medium) and GI symptoms. The parents of autistic children reported that their behavioral symptoms and GI problems increased after consuming processed food items that were rich in carbohydrates (used by bacteria to synthesize SCFAs including propionate) or contained propionate as a preservative (MacFabe, 2012). Intraventricular administration of propionic acid to rodents results in autism-like behavioral disorders (Shultz et al., 2009; MacFabe, 2012).

Among SCFAs, acetate is maximally active in crossing the gut-blood and the blood-brain barrier; its effect at the CNS level manifests itself in a decrease in appetite (Frost et al., 2014).

Many CNS diseases, including Parkinson's disease, are associated with siginificant microbiota changes (see 2.5); importantly, they are often characterized by a decreased SCFA level in the intestinal content, which affects the enteric nervous system and downregulates GI motility.

By stimulating BDNF synthesis (see above), butyrate prevents the destruction of dopaminergic brain neurons. A decrease in microbial butyrate production, therefore, promotes neurodegenerative processes in the brain that are characteristic of Parkinson's disease (Westfall et al., 2017).

3.7.3. Interaction of SCFAs with the Immune System

As mentioned above, SCFAs, predominantly acetate and butyrate, are actively involved in maintaining the colonial resistance of the GI tract. This function of SCFAs is based on (i) their utilization as an energy source by host cells and beneficial bacteria: (ii) suppression of the growth of extraneous microorganisms; (iii) stimulation of the growth of the symbiotic microbiota; and (iv) maintenance of the integrity of the structure of the intestinal mucosa and crypts. Microbial SCFAs also possess the capacity to modulate host immune responses. This is achieved via (a) activation of chemoattractant membrane receptors including free fatty acid receptors (GPR41 and GPR43), the niacine/butyrate receptor (GPR109a), and the olfactory receptor Olfr-78 that are located on immune and intestinal epithelial cells and (b) inhibition of histone deacetylases (Shenderov, 2013b; Correâ-Oliveira et al., 2016). Microbial SCFAs promote the functional differentiation of B lymphocytes that produce IgA in the blood plasma (Rees et al., 2018).

Research on animal models revealed that modifying the microbiota of pregnant females with a diet enriched in fibers results in forming an increased amount of SCFAs and preventing the development of allergic diseases in their offspring. Allergic responses can be suppressed by directly

introducing SCFAs, e.g., acetate, into the maternal organism during the pregnancy period. Upon entering the fetal bloodstream. SCFAs stimulate T_{reg} cell formation. Airway eosinophil cell numbers and serum IgE concentrations are decreased, and Th2-dependent immune responses with IL-5 and IL-13 production are inhibited. It is of relevance that females with low SCFA levels give birth to children with an increased risk of developing allergic problems, such as recurrent bronchial obstruction during the first life year (Logan et al., 2016).

The anti-inflammatory effect of microbial SCFAs seems to at least partially account for the health-promoting influence of the SCFAs-enriched Mediterranean diet that decreases the risk of allergic processes, depression, and cardiovascular diseases (Logan et al., 2016).

SCFAs can inhibit neutrophil chemotaxis and suppress immunocyte migration from the bloodstream to the inflammation area. SCFAs are also implicated in regulating the production of cytokines (TNF-α, IL-2, IL-6, and IL-10), eicosanoids, and chemokinines (MCP-1 and CINC-2). Acetate and butyrate affect inflammatory neutrophil and macrophage responses by inducing, in epithelial and killer cells, the production of cytokines that regulate leukocyte chemotaxis and suppress the formation of adhesion molecules. The same acids inhibit sirtuin and NF-κB activity, attenuate the LPS-induced response (Canani et al., 2011; Shenderov, 2013a, b; Correâ-Oliveira et al., 2016), suppress T cell proliferation and activation (Correâ-Oliveira et al., 2016), decrease the antibody concentration in the peripheral bloodstream (Erofeev et al., 2012; Faith et al., 2014), and induce apoptosis in lymphocytes, macrophages, and neutrophils (Shenderov, 2013a, b; Correâ-Oliveira et al., 2016). Presumably, SCFAs such as butyrate and propionate but not acetate, as well as other metabolic products of symbiotic bacteria, can modify the balance between the proinflammatory and anti-inflammatory mechanisms. They promote the release and increase the activity of intestinal T_{reg} cells via activation of the dendritic cells that are involved in their formation. These effects of SCFAs on T_{reg}s are apparently due to their impact on epigenetic gene regulation in immune cells (Faith et al., 2014; Correâ-Oliveira et al., 2016).

Apart from SCFAs, of significant importance are unsaturated long-chain fatty acids (LCFAs) such as arachidonic and docosatetraenoic acid. LCFAs perform important functions in the brain; they influence impulse transmission in the CNS. Omega-3 (ω-3) unsaturated fatty acids are involved in essential neurochemical and immunological processes; their effects include (Sarris et al., 2015):

- Regulation of neurotransmitter (norepinephrine, dopamine, and serotonin) synthesis, degradation, and reuptake by neurotransmitter-producing nervous cells as well as neurotransmitter-receptor interaction;
- Anti-inflammatory and anti-apoptotic activities;
- Membrane fluidity increase;
- Neurogenesis (formation of new nervous cells);
- Activation of BDNF expression.

LCFAs-mediated communication is bidirectional: LCFAs influence the GI microbiota, while the microbiota, including lactobacilli and bifidobacteria, synthesize LCFAs exemplified by BBB-crossing linoleic acid (Wall et al., 2014).

3.8. Microbiota-Host Interaction: The Role of Gasotransmitters

Gases formed in the animal/human organism, including those produced via microbial fermentation in the GI tract, are involved in the operation of the microbiota-immune system-nervous system triangle. NO, CO, and H_2S are among the most ancient molecules that can perform neurochemical functions. Presumably, some other gases (H_2, CH_4, NH_3, CO_2, and others) are also neuroactive. The host tissue-dependent and microbial synthesis of gases with neurochemical functions is catalyzed by specific enzymes.

For example, the production of NO from arginine is catalyzed by NO synthases (NOSs), while CO formation by heme oxygenases (HOs) is associated with the heme degradation pathway. H_2S is predominantly synthesized from L-cysteine, and this reaction is catalyzed by at least three different enzymes (Althaus & Clauss, 2013; Gadalla & Snyder, 2010; Wang, 2012; Oleskin & Shenderov, 2016). The composition and amount of gases synthesized in the human organism varies depending on the GI area involved (Hezel & Weitzberg, 2015). Gasotransmitters produce their effects on the cells that synthesize them, on adjacent cells, and on remote tissues/organs (Sitdikova & Zefirov, 2010; Tinajero-Trejo et al., 2013). Gaseous signal molecules do not bind to specific receptors on cell membranes and do not accumulate in synaptic vesicles; upon their synthesis, they are usually released from the synthesizing cells.

Gases can easily penetrate into the cells of the nervous, vascular, and immune system, as well as into those of other systems. They interact with intracellular enzymes and ion channels. Many host- or microbiota-produced gases are capable of post-translational modification of various proteins (Oleskin & Shenderov, 2016). This modification may result in oxidative stress, mitochondrial function disruption, and other problems that cause damage to biological macromolecules and even cell death.

Nevertheless, there is gaseous signal-protein interaction that does not pose the risk of cell death (Triantafyllou et al., 2014). Such interaction is exemplified by redox signaling (Kim-Shapiro & Gladwin, 2014), i.e., "transduction of signals coding for cellular processes in which the integrative elements are electron transfer reactions involving free radicals or related species, redox-active metals (e.g., iron, copper, etc.) or reductive equivalents" (Laurindo, 2018).

The GI tract of adult humans contains about 20 mL of various gaseous products. The volume of intestinal gases that is produced per day varies between 400 and 1,200 mL. Nitrogen, oxygen, hydrogen, methane, carbon dioxide, and H_2S account for 20−90%, 3.9−10%, 20.9−50%, 7.2−10%, 9−30%, and 0.00028% of the total volume, respectively. In addition, other

gases such as ammonia, carbon monoxide, nitrous oxide, acetaldehyde,[28] and sulfur dioxide also accumulate in the GI tract.

These gaseous substances enter the GI tract with air and food; in addition, they are formed by various eukaryotic and prokaryotic cells via enzymatic or non-enzymatic processes. All the above molecules belong to the smallest biologically active molecules. Most gas molecules are removed from the intestines: they can be absorbed and delivered to the bloodstream and, subsequently, released via the respiratory system. H_2 and CH_4 are only formed by microbial fermentation in the GI tract; after entering the bloodstream, they reach the lungs and are removed with the exhaled air. Their quantity varies to a large extent, depending on a human individual's diet.

The synthesis of gaseous neurotransmitters by eukaryotic and prokaryotic cells involves well characterized enzymes; the molecular targets of most of the gaseous agents have been identified, and their physiological or pathological effects on tissues and organs have been elucidated. Unfortunately, the determination of the endogenous concentrations of these gases still presents serious difficulties because currently used measurement techniques have their limitations; gas molecules are highly reactive and short-lived. Ion channels and transporters that are the molecular targets of many gas molecules are exemplified by cation channel-containing TRP receptors. They are activated by a wide spectrum of extra- and intracellular stimuli. Mammals have six families of such receptors (TRPC, TRPV, TRPM, TRPA, TRPP, and TRPML). Gases covalently bind to the prosthetic metal complexes of receptor proteins or noncovalently attach to their regulatory subunits. Once the space inside or around a protein structure is occupied, this prevents other gases from interacting with the functionally active parts of such molecules (Takahashi et al., 2012).

Modification of the functions of ion channels can have important consequences at the level of the whole host organism. NO can regulate TRP channels via cysteine S-nitrosylation, or, indirectly, via cGMP protein kinase-dependent phosphorylation. Glucose deficiency and hypoxia (1% of

[28]Classifying acetaldehyde as a gas is somewhat arbitrary because this compound is in the liquid state at atmospheric pressure at temperatures below the boiling point of 20.28ºC.

O_2 in the gas mixture) activate the TRPM7 and the TRPC6 channel; the TRPA1 channel detects excessive O_2 concentrations and mild hypoxia (15% of O_2) in sensory and vagal neurons. TRPA1 also monitors other gases including hydrogen sulfide and carbon dioxide (Takahashi et al., 2012).The role of gasotransmitters in regulating ion channel activity with respect to somatic tissues and cells including the nervous system was summed up in a number of works (Althaus, 2012; Njie-Mbye et al., 2012; Pouokam & Diener, 2012; Takahashi et al., 2012; Wang et al., 2012; Althaus & Clauss, 2013; Peers et al., 2015; Oleskin & Shenderov, 2016). The therapeutic efficiency and safety of some gasotransmitters and/or their precursors was demonstrated in studies with model animals; this encouraged their use for treating human health problems (Bueno et al., 2013; Oleskin & Shenderov, 2016).

3.8.1. Nitric Oxide (NO)

Nitric oxide (NO) is a small short-lived signal molecule that can modify diverse proteins by binding to thiol groups and other amino acid sites (Farrugia & Szurszewski, 2014). In the human organism, NO is formed via both enzymatic and non-enzymatic reactions. There are three isoforms of endogenic nitric oxide synthases (NOSs) in the animal organism (three NOS enzymes): NOS1 (neuronal NOS, nNOS), NOS2 (inducible NOS, iNOS), and NOS3 (endothelial NOS, eNOS). These enzymes and some bacterial NOSs produce NO from L-arginine in a process involving oxygen and NADH and resulting in L-citrulline formation (Schreiber, 2006; Althaus, 2012; Althaus & Clauss, 2013; Lundberg & Weitzberg, 2013; Hezel & Weitzberg, 2015; Oleskin & Shenderov, 2016).

Bacterial NOSs (bNOSs) catalyze NO synthesis from arginine both *in vitro* and *in vivo.* They are present in various bacterial species (streptomycetes, bacilli, etc.; Schreiber, 2006; Gusarov et al., 2013), including human/animal pathogenic and symbiotic microorganisms that inhabit the gut (Sobko, 2006), the oral cavity (Hyde et al., 2014; Hezel & Weitzberg, 2015), and the vagina (Aleshkin et al., 2006). The classical L-

arginine-NO pathway coexists with the alternative nitrate-nitrite-NO pathway (Ivashkin & Drapkina, 2001; Makarov, 2010; Larsen et al. 2011; Lundberg & Weitzberg, 2013; Kim-Shapiro & Gladwin, 2014; Oleskin & Shenderov, 2016). The alternative pathway is characteristic of intestinal bacteria that obtain nitrate and nitrite from digested food (Sobka, 2006).

NO can protect bacteria from antibiotics. NO-dependent antibiotic resistance is due to chemical modification of toxic components and to mitigation of antibiotic-induced oxidative stress (Gusarov et al., 2009; Tinajero-Trejo et al., 2013). Oxidative stress is relieved by producing catalase to eliminate hydrogen peroxide (Schreiber, 2006).

When applied at nanomolar concentrations, NO predominantly performs regulatory functions, whereas its higher (micro- and millimolar) concentrations are toxic to both mammalian cells and microbial symbionts. Blood immune cells (macrophages) release high NO concentrations that exert a cytotoxic effect on tumor cells and other kinds of foreign cells. By interacting with protein FeS groups, NO binds to cytochrome hemes. Interaction of NO with molecular oxygen and superoxide radical yields toxic compounds, such as NO_2, N_2O_3, and especially $ONOO^-$(peroxynitrite) that inactivate the thiol groups of organic molecules and react with the tyrosyl residues of proteins and the nitrogenous bases of the DNA (Tinajero-Trejo et al., 2013; Robinson et al., 2014; Oleskin & Shenderov, 2016). Apart from immune cells, NO is synthesized by hepatocytes, vascular endothelium cells, and others. NO production enables the cells to destroy pathogenic protozoans, helminths (James, 1995), and bacteria (Chen et al., 2015).

NO-resistant bacteria contain structures, e.g., hemoglobins or respiratory and NADH-bound redutases, that inactivate NO by converting it to nitrate (Fang et al., 2012), or cause S-nitrosothiol formation in a reversible fashion (Laver et al., 2013).

During bacterial infection, pathogens neutralize NO by oxidizing it to nitrate with NO dioxygenase or reducing it to nitrous oxide or ammonia with various types of NO reductases (Medinets et al., 2015). Microbial cells are also capable of eliminating NO-caused damage. A mutant strain of *Mycobacterium tuberculosis* with an impaired DNA excision repair system (with a mutant *uvrB* gene) exhibited enhanced sensitivity to NO and

decreased virulence (Robinson et al., 2014). Potentially, a new generation of antibacterial preparations can be developed. They will disrupt NO detoxification and repair systems in pathogenic microor-ganisms and, therefore, facilitate the destruction of these pathogens by nitric oxide produced by the host cells in the inflammation area or intentionally delivered to the focus of infection. High NO concentrations stimulate biofilm formation in *Ps. aeruginosa*, which is attributable to the stressor effect of NO since biofilms are formed in response to stress (Barraud et al., 2006).

Pediococcus acidilactici (strains S2 and S3) and *Lactobacillus plantarum* (strain T119) isolated from fermented dairy items, pickled food, and silage produce cytotoxic NO concentrations (about 50 μM; Gündoğdu et al., 2006) that inactivate antibiotics, e.g., acridines, by nitrosylating them (Medinets et al., 2015).

The main targets of NO and related compounds are proteins that contain iron (guanylate cyclase, NOS enzymes, hemoglobin, and enzymes involved in the citric acid cycle as well as inprotein and DNA synthesis) and SH groups. NO also interacts with reactive oxygen species (ROS), resulting in the formation of highly toxic compounds exemplified by peroxynitrites (Ivashkin & Drapkina, 2001; Bowman et al., 2011; Tinajero-Trejo et al., 2013).

In *E.coli*, the main NO targets include Fe-S-containing regulatory proteins that influence the response to superoxide-induced stress. In *B. subtilus*, NO induces the genes that are responsible for the synthesis of flavohemoglobin, which is involved in detoxifying NO by converting it to NO_3^- or N_2O under aerobic or anaerobic conditions, respectively (Schreiber, 2006; Tinajero-Trejo et al., 2013).

As mentioned above, pico- or nanomolar NO concentrations exert a regulatory, and not a toxic, effect on biological systems. In mammals, NO is involved in regulating impulse transfer across synaptic clefts, regional blood flow, intestinal peristalsis, and water and electrolyte transport. NO influences the operation of the immune and cardiovascular systems and regulates energy metabolism (Ivashkin & Drapkina, 2001; Schreiber, 2006; Larsen et al., 2011; Lundberg & Weitzberg, 2013; Gusarov et al., 2014; Hezel & Weitzberg, 2015; Oleskin & Shenderov, 2016).

At low concentrations, NO behaves as a neurochemical both in the brain and in the peripheral nervous system. It is implicated in learning and cognition activities. Mice with a defective nNOS are characterized by elevated locomotive activity, virility that is retained for a long time, high fertility, and long-term depression (LTD). Male mice lacking neuronal isoform (NOS-1-/- or nNOS-/-)-encoding genes are more aggressive than wild-type males (Nelson et al., 1995). nNOS-containing mice are more resistant to experimental stroke caused by ligaturing the middle cerebral artery.

NO also affects the functions of ionotrophic glutamate receptors (iGluRs) and acid-sensitive ion channels (ASICs) that are present in various areas of the central nervous system and in other mammalian tissues. Dysfunctional ion channels pose the threat of neurological disorders. NO can modify iGluRs and ASICs either directly, by *S*-nitrosylation of cysteine, or indirectly, via cGMP protein kinase G (PKG)-dependent phosphorylation (Wang et al., 2012).

Recently, the traditional opinion that nitrate and nitrite contained in food can cause stomach cancer has been called into question (Bryan et al., 2012). Moreover, the application of drugs or dietary strategies for modifying NO metabolism in order to exploit the therapeutic potential of nitrates as NO sources has been discussed in the recent literature (Lundberg & Weitzberg, 2013; Sindler et al., 2014).

NO at low concentrations performs communicative and antioxidant functions in bacteria and is involved in biofilm dispersal regulation and in the expression of genes that are required for iron utilization.

NO as a signal molecule is likely to be implicated in the operation of quorum-sensing systems. Quorum sensing signals (acylated homoserine lactones, oligopeptides, furanones, and quinolones, see 1.3.3 above) activate processes that depend on high microbial population density. NO is similar to QS signals: its size is small, it accumulates extracellularly, and rapidly penetrates into the cell. Unlike other, more specific, signals, NO is capable of interacting with diverse targets (Schreiber, 2006).

Microbially produced NO exerts multifarious effects on eukaryotic organisms. In the flatworm *Caenorhabditis elegans,* NO synthesized by *B.*

subtilis and *E. coli* behaves as a transcription activator. Its effect on *C. elegans* enterocytes increases the flatworm's heat resistance and prolongs its life expectancy (Gusarov & Nudler, 2005). A similar mechanism may operate in higher animals, enabling the intestinal microbiota to slow down the host organism's aging process. The microbiota includes gram-positive bacteria of the genera *Lactobacillus, Streptococcus,* and *Lactococcus* that posess NO synthases (Yarullina et al., 2011; Oleskin & Shenderov, 2016). Both microbiota- and host-produced NO, can perform cyto-, vaso, and neuroprotective functions (Medinets et al., 2015).

Lact. plantarum probiotic strains are efficient NO producers. The probiotic strains-synthesized NO is rapidly degraded by *E.coli* and *Staph. aureus* both *in vitro* and in the intestines of test animals (Midtvedt, 2006).

Apart from synthesizing their own NO, intestinal bacteria, including probiotic strains (such as lactobacteria, bifidobacteria, and *Escherichia coli* strain Nissle1917), can stimulate NO formation by host epithelial cells (Zidek et al., 2010; Bueno et al., 2013; Hyde et al., 2014; Hezel & Weitzberg, 2015).

3.8.2. Carbon Monoxide (CO)

Carbon monoxide (CO) has long been considered as the most widespread air pollutant and a "silent killer" because of its high affinity for reduced iron in hemoglobin that transports oxygen to the tissues of the animal/human organism.

Endogenous CO was discovered in the human organism in 1950. Various plants and animals, including humans, have been revealed to synthesize CO as an intermediate product during heme degradation by oxygenases. There are three kinds of heme oxygenases (HO-1 to 3). The inducible (HO-1) and the constitutive (HO-2) enzymes are of paramount importance in terms of endogenous CO synthesis. HO-1 activity is induced by various stressors and widely spread in liver, kidney, and spleen cells as well as in aged erythrocytes. HO-2 is located in neurons, other brain cells, and the endothelial layer of blood vessels; the enzyme is activated by Ca^2-calmodulin, glucocorticoids,

and opioids (Gadalla & Snyder, 2010; Farrugia & Szurszewski, 2014; Oleskin & Shenderov, 2016).

CO is also formed by bacteria, including pathogens, plant and animal symbionts, and soil and marine species that contain heme oxygenases (King & Weber, 2007; Tinajero-Trejo et al., 2013; Oleskin & Shenderov, 2016). Some bacteria contain the specific *coo* operon that codes for CO dehydrogenase. This enzyme is responsible for the anaerobic metabolism of CO, which is the sole carbon source in a number of bacteria, for example, *Rhodospirillum rubrum* (Clark et al., 2006).

CO is a sufficiently stable molecule that easily enters cells because it readily crosses the cell membrane; inside a cell, it exerts its biological effects, including antiapoptotic, antiproliferative, anti-inflammatory, cytoprotective, and other activities, both at the CO generation site and at a sufficiently large distance from it (Farrugia & Szurszewski, 2014). CO also regulates ion channels/transporters in various subtypes of epithelial cells (Althaus, 2012; Althaus & Clauss, 2013).

Recently, convincing evidence has been presented that CO possesses all typical properties of a "gasotransmitter" with a broad biological action spectrum (Berne et al., 2012; Tinajero-Trejo et al., 2013). The protective influence of CO on the central nervous system was investigated in model systems. CO inhalation (up to 250 ppm) protects test animals against I/R brain injury and ischemic stroke (Wang et al., 2012; Zeynalov & Dore, 2009). The same CO concentrations prevent neurological damage (neuronal apoptosis) in a pig model of deep hypothermic circulatory arrest.

Pretreatment of primary cultures of cerebral granular neurons with CO (250 ppm) prevented apoptosis caused by oxidative stress or exogenous glutamate. The CO-dependent protection implicates activation of guanylate cyclase and, subsequently, mitochondrial ATP-sensitive K^+channels. This is accompanied by an increase in intracellular ROS level and stimulation of NO formation (Almeida et al., 2015). In hippocampal neurons, activation of membrane K^+ channels causes the extrusion of potassium ions from the cytoplasm and initiates apoptosis. CO can prevent apoptosis by directly inhibiting these channels (Almeida et al., 2015; Peers et al., 2015).

Nonetheless, studies with human neuroblastome cells (SH-SY5Y) revealed that long-term treatment with a CO donor (CO-RM-2) induced symptoms of cell injury caused by a decrease in antioxidant activity or NO formation inhibition. The ability of CO (at a concentration of 1,000 or 3,000 ppm; action time 40 min) to disrupt Ca^{2+}- dependent signaling pathways in SH-SY5Y cells and in the homogenate of the total brain tissue of rats appears to be due to its modulating effect on the target protein, plasmalemma Ca^{2+}ATPase (PMCA; Hettiarachchi et al., 2012). Intracellular H_2O_2 production in the brain tissue is increased at high CO concentrations, which is accompanied by enhanced production of hydroxy radicals and a decrease in the ratio between reductive and oxidative processes in mitochondria (Almeida et al., 2015). Hence, the action time and concentration of CO at the target site determine whether its effect is beneficial or detrimental.

CO is a physiological signal molecule regulating the functions of membrane channel proteins and transporters (Peers et al., 2015). The antimicrobial and anti-inflammatory effects of CO and CO-releasing molecules (CO-RMs), e.g., metalcarbonyl CO-RM-3 and glycinate, implicate the opening of K^+/Na^+channels in eukaryotic and bacterial cells. This decreases the protonmotive force and disrupts ion transport. The mechanisms of protection of the nervous and cardiovascular systems in the presence of CO-RMs have not been completely elucidated yet. Mitochondria represent the main target of CO. This does not rule out an additional effect of CO-RMs, the stimulation of ROS production in mitochondria. It was established that the CO released at low concentrations can produce a cardioprotective effect, due to its antioxidant properties. Recently, increasing attention has been paid to the use of hemeoxygenases, CO inhalation, and CO-RMs for treating various infection and inflammation processes as well as cardiovascular and, potentially, neurological problems (Smith et al., 2011; Wegiel et al., 2013; Berne et al., 2012; Almeida et al., 2015; Peers et al., 2015).

3.8.3. Hydrogen Sulfide (H_2S)

Hydrogen sulfide (H_2S) is a highly water-soluble gas that readily penetrates into cells. At a concentration of 1 ppm, it can be recognized because of its rotten egg odor; 4 ppm H_2S causes a headache; at still higher concentrations (500 ppm and above), H_2S can produce a lethal effect (Sitdikova & Zefirov, 2010; Gadalla & Snyder, 2010). The equilibrium ratio between its three forms (H_2S, HS^-, and S^{2-}) varies depending on the medium pH level. In the organism, this gas easily enters the cells via passive transfer across the membranes. Acute intoxication is due to H_2S binding to the iron of cytochrome *c* oxidase, which inactivates the enzyme and abolishes oxidative phosphorylation in mitochondria (Sitdikova & Zefirov, 2010). Despite its toxic effect, H_2S has recently been established to play a vital role in bacteria, plants, and invertebrate and vertebrate animals, including mammals.

H_2S synthesis is catalyzed by three enzymes: cystathionine-β-synthase (CBS), cystathionine-γ-lyase (CSE or CTH), and 3-mercaptopyruvate sulfurtransferase (3-MST) (Farrugia & Szurszewski, 2014). H_2S-synthesizing enzymes are expressed, to a different extent, in the cardiovascular, nervous, immune, urinary, respiratory, and GI system (Polhemus & Lefer, 2014; Oleskin & Shenderov, 2016).

The microbiota of the large intestine is one of the key factors implicated in the metabolism of S-containing compounds and the endogenous generation of H_2S in the human organism. A red meat-enriched diet stimulates H_2S synthesis by supplying the large intestine with a significant amount of sulfated proteins. The human large intestine contains considerable H_2S amounts that predominantly result from H_2S formation from inorganic, e.g., sulfates and sulfites, and organic (methionine, cysteine, taurine, sulfate-containing polysaccharides, and lipids) compounds (Carbonero et al., 2012; Olas, 2015). Sulfur-containing organic compounds, including those present in garlic, onion, and other foodstuffs, supply the organism with H_2S (Carbonero et al., 2012). Many prokaryotic species form H_2S in their natural habitat. The sources of microbial H_2S include, for example, *E. coli* strains that possess two relevant enzymes (L-cysteine transaminase and 3-MST).

The bacterial production of H_2S can also involve CBS or CSE (Shatalin et al., 2011). Some representatives of intestinal bacteria (*Prevotella*, *Bacteroides*, *Helicobacter*, *Peptococcus*, and *Akkermansia*) produce glycosyl sulfatases or similar enzymes that promote production of sulfates from sulfomucins (Carbonero et al., 2012).

Sulfate-reducing bacteria compete with methanogenic microorganisms for H_2 molecules both *in vitro* and *in vivo*. In the human large intestine, *Desulfovibrio vulgaris* is predominantly responsible for H_2S generation by reducing various sulfur-containing compounds, including sulfates and S-containing organic substances, e.g., cysteine. The bacteria of the large intestine ferment cysteine, yielding H_2S, ammonia, and pyruvate. If the oxygen content is low (under microaerophilic conditions), H_2S at micromolar concentrations can serve as an electron donor and an energy source. If food contains a limited amount of cysteine, endogenous and microbial CSE activity and, therefore, H_2S production are increased; conversely, enriching food in cysteine or the chemical/genetic suppression of CSE activity results in a decrease in H_2S production (Hine et al., 2015),

At high (millimolar) concentrations, H_2S is a highly toxic compound that causes a whole spectrum of pathological processes in the GI tract (Carbonero et al., 2012); it also produces genotoxic effects by damaging the DNA and induces inflammatory processes (Bannenberg & Vieira, 2009; Carbonero et al., 2012).

To reiterate, when applied at low (micromolar) concentrations, H_2S serves as an inorganic electron donor for mitochondria. H_2S is involved in regulating inflammation, apoptosis, cell proliferation, neuronal impulse transfer, and smooth muscle tone (Bannenberg & Vieira, 2009). Gut-produced H_2S is predominantly degraded by intestinal epithelial enzymes (Sitdikova & Zefirov, 2010). H_2S is excreted from the organism in its unbound form and as conjugated sulfate, mainly via the kidneys (Wang, 2012). The physiological effects of H_2S depend on its impact on various molecular targets including heme-containing proteins, ion channels, and signal proteins (Oleskin & Shenderov, 2016).

In the presence of glutathione, cysteine, or dihydrolipoic acid, H_2S is released from the lysate of cultured neurons and astrocytes at pH 8.0−8.4.

When excited, neurons take up sodium ions and excrete potassium ions, which results in increasing the intracellular potassium concentration and depolarizing the membranes of adjacent astrocytes. Depolarization causes activation of Na^+/HCO^{-3} co-transporters in the astrocytes. The influx of HCO^{-3} brings about cell cytoplasm alkalization. The main H_2S targets include ATP-sensitive potassium channels as well as calcium and chloride channels. There is sufficient evidence that the (neuro)modulatory effect of H_2S on cell functions and physiological processes is due to its interaction with several cell transporter systems. It was revealed that H_2S enhances the activity of transporter systems by facilitating the release of antioxidants that are required for protecting the systems against exogenous toxic substance-caused damage. The H_2S-transporter interactivity plays a major role in maintaining the redox potential of nervous cells. This is an additional mechanism of the neuroprotective and neuromodulatory activities of this gaseous substance. Of special note is the impact of H_2S on various types of K^+ channels that are essential for the transfer of ions in epithelial cells. H_2S is likely to indirectly affect Na^+ transfer by acting on the proteins of K^+ channels and transporter molecules (Althaus, 2012; Althaus & Clauss, 2013).

The influence of H_2S, a biological signal molecule, on neuronal activity in the hippocampus, cerebellum, cortex, and brainstem has been researched during the course of several decades. As a CNS neurochemical, H_2S is involved in cognitive processes and the operation of the memory system. In the peripheral nervous system, H_2S is implicated in regulating the GI tract, heart, and lung functions, pain perception, and inflammation (Duan et al., 2015).

H_2S is a neuromodulator and neuroprotector in various brain cells (Ishigami et al., 2009; Kimura, 2010; Gadalla & Snyder, 2010; Oleskin & Shenderov, 2016). At physiological concentrations, H_2S functions as a synaptic activity modulator. CBS, which is present in the cells of various brain areas, is responsible for the generation of H_2S. It activates transmembrane ATP-associated channels (in neurons both inside and outside the brain) via modulating glutamate-dependent *N*-methyl-D-aspartate receptors (Sitdikova & Zefirov, 2010). This gaseous modulator also

regulates the activity of serotonergic neurons and induces the release of corticotrophin-releasing hormone (Ishigami et al., 2009; Sitdikova & Zefirov, 2010). Two different forms of sulfur, acid-labile and bound sulfur, are stored in brain cells. Acid-labile sulfur is incorporated in the iron-sulfur centers of mitochondrial enzymes involved in oxidative phosphorylation. Significant amounts of bound sulfur are present in the cytoplasm of brain neurons and astrocytes. Cytoplasm alkalinization is a prerequisite for efficient H_2S conversion from the conjugated to the free form (Oleskin & Shenderov, 2016). Unbound H_2S is retained for a longer time in the brain tissue than in liver and heart cells (Ishigami et al., 2009).

In astrocytes, H_2S also influences the intracellular level of calcium that plays a major role in intercellular communication. The intracellular calcium level rapidly increases upon the addition of H_2S; subsequently, it slowly decreases. These effects of H_2S and various H_2S donors were revealed in astrocyte cultures and in the glia of hippocampal sections (Ishigami et al., 2009). H_2S was established to exert an influence on the operation of the peripheral nervous system, which involves modulating pain perception and transferring pain signals to the relevant brain areas (Sitdikova & Zefirov, 2010). The effects of H_2S are removed by NMDA antagonists.

To sum up, H_2S serves as a neuroprotector. The neurotoxic effect of glutamate on brain tissue cultures is partly due to inhibiting the entry of cystine into the cells. H_2S can mitigate the toxic effect by reversibly inhibiting cystine transfer by glutamate and, therefore, stimulating cystine influx into the cells (Kimura, 2010; 2012).

There is a supplementary pathway of synthesizing H_2S from D-cysteine, which involves 3-MST and D-amine oxidase. In contrast to the pathway of H_2S synthesis from L-cysteine, the D-cysteine-dependent pathway predominantly functions in the cerebellum and the kidneys. Studies with the primary cultures of cerebellar neurons revealed that the cerebellar tissue does not sustain hydrogen peroxide-induced oxidative stress if D-cysteine is available (Kimura, 2012). The discovery of the D-cysteine-dependent pathway of synthesizing H_2S provides foundations for a new therapeutic technique based on delivering H_2S to target tissues (Shibuya et al., 2013).

H_2S and *S*-adenosyl-methionine impede the increase in the glucocorticoid concentration of the blood plasma under stress. It was established that low H_2S concentrations neutralize reactive oxygen and nitrogen species (superoxide radical, hydrogen peroxide, peroxynitrite, hypochlorite, andothers) and reversibly inhibit the mitochondrial respiratory chain. It is the antioxidant effect of H_2S that is responsible for its neuro- and cardioprotective activities (Sitdikova & Zefirov, 2010).

An influence of H_2S on human behavior was suggested in studies with human subjects with seizures, psychiatric disorders, or abnormal electroencephalograms; most of the subjects lacked the enzyme (CBS) that is involved in H_2S synthesis. It was revealed that patients with Down syndrome, in contrast, are characterized by abnormally high concentrations of these enzymes in the brain tissue (reviewed, Oleskin & Shenderov, 2016). Further studies demonstrated H_2S involvement in a number of neurogenerative diseases. The H_2S content in the brain tissue was decreased by over 50% in Alzheimer patients (Duan et al., 2015), and this deficiency is apparently due to a drastic (70%) decrease in the concentration of *S*-adenosyl-methionine that activates the CBS enzyme. Depression is also characterized by a decrease in blood H_2S concentration (Duan et al., 2015). Nevertheless, abnormally high H_2S concentrations can exert toxic effects on neurons and be involved in the development of dementia. Autistic spectrum disorders are associated with increased excretion of H_2S metabolites (sulfate, sulfite, and thiosulfate) with urine and decreased blood sulfate content (Duan et al., 2015). Presumably, H_2S could be useful for patients with Parkinson's disease. H_2S prevented nervous cell damage and apoptosis in a model system in which a Parkinson's disease-like disorder was caused by administering the toxin rotenone to test animals (Sitdikova & Zefirov, 2010).

There is evidence that H_2S functions as a signal molecule in the visual system of mammals. H_2S synthesis-catalyzing enzymes (CBS and CSE) were detected in various kinds of eye cells, and H_2S was found to regulate glutamatergic neurotransmission during the signal transduction processes in this system. Further data on the regulatory influence of H_2S on ion channels and transporters will contribute to our understanding of the role of H_2S in

relation to the risk of development of ocular neuropathies (Njie-Mbye et al., 2012).

Even though the use of gaseous H_2S for therapeutic purposes is hardly feasible, chemical compounds that release H_2S in the human organism either rapidly (NaHS) or slowly (GYY 4137) can potentially be useful . This gives grounds for the suggestion that H_2S should be used for medical purposes (Pouokam & Diener, 2012). However, while the one-time use of NaHS provided protection for neurons from oxidative stress, the repeated administration of this substance produced a toxic effect on these cells (Ishigami et al., 2009). Nonetheless, H_2S treatment is considered an efficient therapeutic technique for a number of diseases (e.g., lung cystic fibrosis and kidney problems in patients with hereditary hypertension) that are characterized by enhanced Na^+influx into cells (Hine et al., 2015). In all likelihood, the employment of chemical donors or microbial producers of H_2S for medical purposes will hold much promise as a potential pharmacological approach to the treatment of neurodegenerative diseases.

3.8.4. Ammonia

Ammonia is one of the end products of degradation of proteins, peptides, urea, and various amino acids. NH_3 is predominantly formed by the intestinal microbiota and the cells of the GI tract, the kidneys, the liver, and the muscles. At least 4−10 g of NH_3 are daily synthesized in the intestines of adult human individuals. The amount of urea produced by conventional animals was found to be 20-30% higher than that synthesized by GF animals. The gut microbiota includes urease-containing microorganisms. GF animals lack gut urease activity. No labeled CO_2 was exhaled by GF rodents after administering carbon-labeled urea to them. In healthy human individuals, up to 7 g of urea are degraded daily by microbial ureases (amounting to 50% of the total pool of this compound; Richardson et al., 2013) and by those of fungi (*Candida albicans;* Burrus, 2012).

Among aerobes, Gram-negative intestinal bacteria of the genera *Proteus*, *Klebsiella*, and *Pseudomonas* as well as *E. coli* are among the most

active NH_3 producers; active NH_3-producing anaerobes also include the genera *Clostridia*, *Ruminococcus*, *Bacteroides*, and some lacto- and bifidobacteria. Peptidococci, ruminococci, coprococci, bifidobacteria, lactobacilli, clostridia, bacteroides, and some streptococci and enterococci exhibit significant urease activity.

Intestinal urease-producing microorganisms form ammonia that translocates to the liver via the portal vein and is reincorporated into urea. GI microorganisms also incorporate NH_3 into amino acids that are synthesized *de novo* using CO_2 or acetic, propionic, and other organic acids as carbon sources. Unless utilized in biochemical processes in the large intestine, microbially produced NH_3 rapidly passes through mucous membranes and spreads within the organism. For the most part, intestinal unbound NH_3 reaches the liver via the portal vein; in the liver, it is virtually completely converted into urea and glutamine via a series of biochemical reactions (the urea cycle). The unbound NH_3 content in the blood of healthy adults is approximately 35 mM (ca. 0.67mg/L). Unbound NH_3 circulates in the organism; it is excreted with urine and, to a lesser extent, with feces. Approximately 2–3 mg of ammonia are excreted per day with urine. Ammonia-derived metabolites are also excreted with urine or, alternatively, used for synthesizing amino acids and other biological molecules. Endogenous NH_3 formed in the brain and in peripheral tissues is not transferred to the liver; instead, it is transformed in these tissues into glutamine and alanine (Richardson et al., 2013).

Genetic disruption of the biosynthesis of urea cycle enzymes, liver and kidney dysfunction, excessive NH_3 formation in skeletal muscles caused by physical exertion or other kinds of stress, or an imbalance in the intestinal ecological system result in increasing NH_3 content in the organism to a toxic level (hyperammonemia). Shock and systemic hypoxia (oxygen deficiency) also increase the blood NH_3 level (Duan et al., 2015). Liver cirrhosis is associated with the formation of a direct bypass between the portal vein system and the bloodstream; this prevents the detoxification of harmful GI tract-produced compounds including NH_3 that reaches the bloodstream.

Increased NH_3 concentrations penetrate into the brain tissue, which is a major factor of pathogenesis of hepatic encephalopathy (HE). A mild form

of HE occurs in 80% of patients with liver cirrhosis. It manifests itself in fatigue, ache, muscle weakness, loss of appetite, nausea, vomiting, diarrhea, pain in the back, sides, or the abdomen, and motor and cognitive disturbances.

In the early 1970s, it was established that mutations that cause urea cycle disruption result in pathological changes in the brain of newborns. It was suggested that GABA is implicated in ammonia-induced toxicity in the nervous system. An increase in ammonia concentration in the brain tissue results in stimulating GABA-induced chloride channels in neurons and astrocytes. Evidence was presented that liver dysfunction-induced hyperammonemia is accompanied by changes in cell energy metabolism and in formation of excessive glutamine amounts in astrocytes. An increase in glutamine concentration in astrocytes disrupts neurotrans-mission processes. It is astrocytes that incorporate ammonia in glutamine molecules after it crosses the BBB. Hyperammonemia is also responsible for osmotic stress in the brain, which results in redistribution of cerebrospinal fluid and causes swelling in astrocytes and edema in the white matter as well as an increase in intracranial pressure (Cooper, 2012; Gorg et al., 2013; Ott & Vilstrup, 2014; Butterworth, 2015). High NH_3 concentrations can disrupt amino acid pathways, and brain energy metabolism; they can influence nitric oxide synthesis and brain signal transmission (Duan et al., 2015).

Ammonia also inhibits energy production in mitochondria, which seems to be due to ammonia's capacity to suppress ketoglutarate dehydrogenase activity and stimulate glycolysis (Ott & Vilstrup, 2014). An increase in ammonia concentration in the arteries affects the expression of a number of genes that code for neuroglial proteins. These proteins regulate cell growth, mitochondrial functions, and transfer of neuroactive amino acids (Butterworth, 2015). When applied at supraphysiological concentrations, NH_3 induces rapid release of glutamate from neurons. In humans, this results in the development of irritability, aggression, hyperexcitability, and movement disorders. In a study conducted with 49 male convicts and 52 control volunteers in China, it was established that the convicts were characterized by increased blood ammonia levels (Duan et al., 2015).

Hyperammonemia also promotes removal of GABA from neurons, which frequently manifests itself in somnolence or lethargy; these symptoms

may ultimately cause the development of a comatose state (Wang et al., 2012; Butterworth, 2015). Apart from the direct neurotoxic effects, high NH_3 concentrations increase the permeability of the BBB; modulate the serotonergic and dopaminergic systems of the brain; cause the accumulation of abnormal neurotransmitters, such as octopamine,in the brain; and result in glucose intolerance and increased urinary output calcium and phosphate concentrations (Galland, 2014; Butterworth, 2015). Systemic inflammation that constantly accompanies acute and chronic liver dysfunction represents an important risk factor in terms of encephalopathic complications. Hyperammonemia and the attendant neuroinflammatory response to liver cirrhosis cause microglia activation, monocyte recruitment, enhanced synthesis of proinflammatory cytokines (TNF, IL-1b, and IL-6), accumulation of ammonia, lactate, and manganese, and an increased permeability of the BBB.

Encephalopathy may result from the synergistic effect of ammonia and proinflammatory cytokines (Gorg et al., 2013; Butterworth, 2015). Astrocytes lose the capacity to adequately regulate their own volume, glutamine accumulation is increased, and a cascade of signaling processes is triggered. This results in enhanced Ca^{2+} accumulation and an increased formation of reactive oxygen and nitrogen species, which is due to the activation of NADPH oxidase and NOS (Gorg et al., 2013). Some patients suffering from hyperammonemia and attendant GI dysfunction (constipation or diarrhea) exhibit psychotic symptoms and movement disorders that resemble those typical of genuine autism spectrum disorders (Burrus, 2012; Wang et al., 2012; Frye et al., 2015; Oleskin & Shenderov, 2016).

The important role of microbial NH_3 with respect to the brain functions and life expectancy of HE patients is consistent with the fact that the patients' state is markedly improved after administering the antibiotic rifamycin, the prebiotic lactulose, and probiotics to them (Butterworth, 2015). The neuropsychic state, including cognitive capacities, is also improved by synbiotics, for example, by the *Bifidobacterium longum*-fructo-oligosaccharide combination (Galland, 2014). Neural inflammation, brain edema, and the resulting encephalopathy symptoms that are due to increased NH_3 concentrations in the brain tissue can be mitigated by minocycline, an

inhibitor of microglia activation, and *N*-acetylcysteine, as well as by mild hypothermia; all these techniques produce neuro- and hepatoprotective effects (Butterworth, 2015; Oleskin & Shenderov, 2016).

3.9. MICROBIOTA-HOST INTERACTION: THE ROLE OF NEUROPEPTIDES

Peptide neuromediators predominantly perform the function of neuromodulators: they increase or decrease the efficiency of other neurotransmitters in transferring the impulses across synapses. However, substance P and some other peptides also perform the neurotransmitter function *sensu stricto*: they transmit impulses across synaptic clefts.

Opioids (endophins, enkephalins, and dynorphins) bind to specific receptors that also bind morphine, heroin, and a number of other drugs and block the transfer of impulses, including those involved in pain perception. Opioids act on the posterior horns of the spinal cord as pain relievers and on the hypothalamus (causing euphoria); at high concentrations, they produce a soporific effect. Plausibly, they are involved in pain relief (analgesia) caused by acupuncture, a key component of traditional Chinese medicine in which thin needles are inserted into the body (Boldyrev et al., 2010).

There are 4 classes of opioid receptors. They are denoted as μ, δ, κ, and ORL-1 receptors that are coupled with G_s and G_i proteins. These receptors are present in the brain cortex, the striatum, the thalamus, the amygdala, the hypothalamus, the olfactory bulb, and other brain structures (Boldyrev et al., 2010). Endorphins, enkephalins, and dynorphins selectively bind to μ, δ, and κ receptors, respectively (Dubynin et al., 2010).

Importantly. a positive influence on work performance and mood (up to causing euphoria) is also exerted by other short peptides, including those produced by endocrinal glands. They are exemplified by thymus-synthesized *thymosins*. Apart from activating the immune system, thymosins also enhance positive emotions and stimulate loyal behavior and social interaction in primates (Dubynin et al., 2010).

A large number of peptides combine the functions of hormones and neurochemicals. Substance P is present in the hypothalamus, the amygdala, and the gray matter of the brain (which contain receptors for it); it regulates pain perception, anxiety development, stress, stimulates GI motility and the secretory activity of the pancreas and the salivary glands, and inhibits bile secretion. Substance P promotes blood vessel dilation, increases capillary permeability, stimulates mast cell degranulation, behaves as leukocyte attractant, causes smooth muscle contraction, and facilitates the release of prolactin, GI hormones, and inflammatory factors.

Neuropeptide Y functions as a neurotransmitter in brain cells and the peripheral nervous system and also produces a vasoconstrictor effect. In contrast to substance P, it mitigates anxiety and stress and relieves pain. Neuropeptide Y stimulates food intake and accumulation of lipids as energy substrates; the neuropeptide is also implicated in emotional behavior, memory system operation, and circadian rhythms.

Apart from regulating the vascular system, *vasopressin* is involved in learning and memorization processes. *Tachykinines* are associated with inflammation; they also produce a sedative effect. In addition to inhibiting growth hormone secretion by the pituitary gland, *somatostatin* decreases locomotive activity.

Cholecystokinin that stimulates the functioning of the gal bladder also regulates foraging behavior and pain perception. Enzymatic degradation of cholecystokinin yields a fragment that causes fear and anxiety in humans. Hypothalamus-produced *corticoliberin,* apart from impacting the pituitary gland and stimulating ACTH synthesis, influences the brain, increasing anxiety and locomotive activity. *Thyroliberin,* a hypothalamic neurohormone, activates emotional behavior and stimulates the respiratory center (Boldyrev et al., 2010; Dubynin et al., 2010).

The data concerning microbially produced neuropeptides are meaningful but still rather fragmentary (Fetissov et al., 2008; Holzer & Farzi, 2014). It was established that *Staph. aureus* synthesizes the autoregulator [Met]^5-enkephalin, a microbial opioid that functions as a neurochemical (Zagon & McLaughlin, 1992). Another opioid, β-endorphin,

is synthesized by some unicellular eukaryotes, such as the infusorian *Tetrahymena pyriformis* and the amoeba *Amoeba proteus* (Lenard, 1992).

To an extent, the boundary between hormones and neurochemicals is arbitrary and changeable. *Microbial endocrinology* is concerned with the operation and the functional roles of both classes of compounds in microbial systems (Lyte, 1993, 2010, 2011). Many chemicals combine both functions. As a hormone, insulin increases the permeability of plasma membranes for glucose, stimulates the formation of glycogen from glucose in the liver, and suppresses the activities of glycogen- and lipid-degrading enzymes. As a neurochemical, insulin is involved in transmitting information concerning feelings of hunger and satiety into the brain. In this capacity, insulin functions in combination with other neuropeptides (ghrelin, leptin, and peptide YY). It was established that insulin is produced by *E. coli* and the fungus *Neurospora crassa*, which contains a gene that is homologous to the insulin gene of mammals. In *N. crassa*, insulin is implicated in the regulation of carbohydrate metabolism (Lenard, 1992).

Microorganisms are capable of producing corticotropin (*Tetrahymena pyriformis*), somatostatin (*B. subtilis* and *Plasmodium falciparum*), progesterone (*Trychophyton mentagrophytes*), and α-factor (*S. cerevisiae*), a homologue of the gonadotropin-liberating hormone of higher animals (Lenard, 1992) that, apart from its hormone function, regulates brain activity (Dubynin et al., 2010).

Microorganisms also synthesize homologues of animal/human regulatory peptides that are widespread among symbiotic and pathogenic bacteria and fungi. Symbiotic *E. coli* strains synthesize homologues of leptin, insulin, ghrelin, peptide YY, neuropeptide Y, agouti-related peptide, orexin, α-melanocyte-stimulating hormone (α-MSH), adrenocorticotropic hormone (ACTH), oxytocin, and vasopressin (Fetissov et al., 2008).

A leptin homologue was detected in *Lactococcus lactis*. Microbial homologues of neuroactive compounds induce the synthesis of antibodies that cross-react with the neuropeptides of the host organism. Microbial peptides can modify animal and human behavior. The homologues of leptin, insulin, α-MSH, and ACTH that are synthesized by *Helicobacter pylori* can decrease appetite. A relationship between streptococcal infection and

anorexia nervosa (chronic suppression of appetite) was revealed, and the likely reason is that pathogenic streptococci produce leptin and gonadotropin-liberating hormone (Fetissov et al., 2008).

Apart from producing regulatory peptides and their homologues, the GI microbiota impacts their production by host cells. For instance, treating mice (starting from the weaning period) with antibiotics resulted in impoverishing their microbiota and changing its composition. Oxytocin, vasopressin, and BDNF gene expression in the brain was decreased, which was accompanied by cognitive activity disruption and decreased anxiety, compared to wild-type mice (Desbonnet et al., 2015). The gut mirobiota indirectly influences the formation of peptides GLP-1, GLP-2, and PYY by affecting the conversion of stem cells into enteroendocrine cells and, accordingly, altering the number of peptide-synthesizing cells (Mazzoli & Pessione, 2016).

Under the direct influence of the microbiota, GI enterochromaffin cells synthesize PYY and cholecystokinin; these two peptides in combination with ghrelin exert a satiety-causing effect. Administration of bifidobacteria-stimulating prebiotics results in decreasing the ghrelin level in the human organism. The ghrelin level is correlated with the gut microbiota composition in rats with different dietary regimens and physical activity levels. Ghrelin secretion is of considerable importance in terms of neurodegenerative diseases because ghrelin produces a protective effect that beneficially influences the mental state of patients with Alzheimer's and Parkinson's diseases (Westfall et al., 2017). Studies with rats suffering from ischemia revealed that adiministering ghrelin decreases the free oxygen radical level, protects mitochondria from oxidative damage, and prevents apoptosis. Experiments with a mouse Alzheimer's model demonstrate that ghrelin impedes amyloid accumulation in the brain and mitigates inflammation. Patients with Alzheimer's disease are characterized by a low ghrelin level in the organism. Accordingly, the gut microbiota's ghrelin-synthesizing capacity potentially enables using them for treating Alzheimer's disease as well as Parkinson's disease. Animal studies indicate that ghrelin counteracts the destruction of dopamine-producing neurons that is characteristic of Alzheimer's disease (Westfall et al., 2017).

Peptide-mediated host–microbiota communication is multidirectional, like that based on other neurochemicals. For instance, opiods modify the gut microbiota composition; in addition, the microbiota modifies the host's reponse to opioid administration. The development of host insensitivity to opioid analgetics depends on the microbiota (Liang et al., 2018).

Studies with the strain *Ps. aeruginosa* PAO1 (that produces the blue-green pigment pyocyanin) revealed that dynorphin and its analog U50,488 increased pyocyanin production and the antagonistic activity of *Ps. aeruginosa* with respect to *Lactobacillus plantarum* and *Lact. rhamnosum*, which form a part of the GI microbiota, and influenced the synthesis of virulence factors and biofilm formation in *Ps. aeruginosa* (Zaborina et al., 2007). Elevated opioid concentrations in the GI tract that are produced in response to stress activate the QS system of *Ps. aeruginosa*. This results in a decrease in colonial resistance of the intestine and, therefore, in an increase in *Ps. aeruginosa* abundance (Shpakov, 2009).

The opioid factor [Met]^5-enkephalin, which decelerates cell proliferation in vertebrate tissues, can inhibit the growth of *Ps. aeruginosa*, *Staph. aureus*, and *Serratia marcescens* (Zagon & McLaughlin, 1992). *Staph. aureus* possesses receptors to [Met]^5-enkephalin that is present in its culture liquid at a concentration of up to 1.6 ng/mL.

It was suggested that opioids had been performing their growth-modifying function millions of years before higher animals with their complex nervous system emerged (Zagon & McLaughlin, 1992).

Apart from the neurochemical action, peptides P and Y exert antimicrobial effects with respect to various gram-negative and gram-positive bacteria as well as fungi; these effects vary depending on the tested strain and the peptide concentration applied; the data presented in different works are partly discordant (Kowalska et al., 2002; Hansen et al., 2006; El Karim et al., 2008).

α- and β-defensins, α-melanocyte-stimulating hormone (α-MSH), and other peptides with neurochemical functions also exert an influence on the microbiota (de Freitas Lima et al., 2015; Shireen et al., 2015). For example, α-MSH suppresses the growth of *Staph. aureus* (Shireen et al. ,2015). The macrophage- and polynuclear leucocyte- produced peptide LL-37

(catelicidin) stimulates the quinolone-dependent QS system that is involved in virulence factor synthesis in *Ps. aeruginosa* and concomitantly enhances the tolerance of *Ps. aeruginosa* to the antibiotics ciprofloxacin and gentamycin (Strempel et al., 2013).

In addition to the presence of similar or identical mediators in neuronal networks and microbial cultures, microorganisms contain proteins that are functionally analogous and/or structurally homologous to neurochemicals-binding receptors. They are exemplified by the QseC/QseE type catecholamine receptors of a number of microorganisms (Clarke et al., 2006; Hughes et al., 2009) that was mentioned earlier in this work, the opioid receptor of the ζ type of *Staph. aureus* (Zagon & McLaughlin, 1992), and a homologue of the GABA receptor detected in the purple phototrophic bacterium *Rhodobacter sphaeroides* (Baker & Fanestil, 1991). A highly efficient GABA-binding receptor was isolated from *Pseudomonas* sp. Since bacteria belonging to the genus *Pseudomonas* are known to produce GABA, it seems likely that GABA can serve as a signal involved in cell-cell communication in this bacterial system (Lyte, 2010, 2014).

3.10. Microbial-Host Interaction: The Role of Purines

As mentioned at the beginning of this chapter, a large number of neurochemicals are multifunctional agents and fulfil signal functions in diverse taxa of animals, plants, and microorganisms, which provides justification for the use of the general term *biomediators* suggested by V. Roshchina (2010, 2016).

An important additional example is provided by *purines*, especially adenosine and its derivatives. Apart from being the precursors of nucleic acids, they "act as metabolic signals, provide energy, control cell growth, are part of essential coenzymes, contribute to sugar transport, and donate phosphate groups in phosphorylation reactions" (Fumagalli et al., 2017).

Purine regulatory effects in the animal/human organism are mainly due to their binding to

(i) G-protein-coupled P1 receptors that bind adenosine and also adenosine-5'-monophosphate (AMP); their subtypes, denoted A_1, A_{2A}, A_{2B}, and A_3, inhibit ($A_{1,\ 3}$) or ($A_{2A,\ 2B}$) stimulate adenylate cyclase activity and

(ii) P2 receptors including ligand-activated ion channels that exclusively bind ATP (P2X receptors with subtypes $P2X_{1\text{-}7}$) and G-protein-coupled P2Yreceptors (subtypes $P2Y_{1,\ 2,\ 4,\ 6,\ 11,\ 12,\ 13,\ 14}$) that bind ATP and ADP as well as uridine nucleotides (UTP, UDP, and UDP-glucose).

Purines are extracellular messengers targeting "secretory, exocrine and endocrine, endothelial, immune, musculo-skeletal and inflammatory cells" (Burnstock, 2017). In the nervous system, ATP behaves as a neurotransmitter: it is present in synaptic vesicles and released into the synaptic cleft when an action potential is generated across the neuronal membrane (Kudryashov, 2009). ATP and other purines predominantly operate as cotransmitters in synapses in which also other neurotransmitters are used, although ATP functions as a sole neurotransmitter in the part of the sympathetic nervous system that innervates submycosal arterioles in the gut (Fountain, 2013).

Among the effects of purines on the nervous system, of significant importance is the inhibitory action of adenosine and AMP on excitatory brain synapses via A_1 receptors. Overall, a sedative (tranquilizing) effect is produced. Purines prevent seizures and are involved in regulating initial sleep stages. Caffeine and theophyllin are A_1 receptor antagonists and, therefore, act as psychomotor stimulants in most human individuals (Kudryashov, 2009; Boldyrev et al., 2010; Dubynin et al., 2010).

Purines are also of significant importance in terms of neurodevelopment. For instance, Ca^{2+} release-controlling purinergic receptors (P2Y1) help developing neurons (neuroblasts) reach their location in the neocortex (Fumagalli et al., 2017).

As for the immune system, release of ATP and other purines associated with cell death is perceived as a "danger signal" and stimulates inflammation[29]. Adenosine chemotactically attracts neutrophils, via A_1 and A_3 receptor activation, towards inflammatory stimuli (Barletta et al., 2012). However, purines can both activate and inhibit the immune system, depending on the chemical micro-environment (the presence of chemokines) and the receptor type(s) involved (*Cordis EU Research Results*, 2013).

Importantly, both purines and their receptors are widely spread in unicellular organisms. Suffice it to mention that P2X-type receptors are present in the amoeboid cells of *Dictyostelium discoideum* (where receptor DdP2XA modulates vacuole contraction) and in the unicellular green alga *Ostreococcus tauri* (Fountain, 2013).

The purine pool in the human/animal organism is partly supplied by microbial symbionts; for instance, purines are actively produced by lactobacilli. Recent work indicates the important role of purine compounds in terms of bacterial infection. Not only symbiotic but also virulent *E. coli* strains as exemplified by UPEC (uropathogenic *E. coli*) produce purines, and inactivation of some of the genes involved in purine synthesis (the *purN* and *purT* genes) impedes the UPEC invasion of human bladder cells (Andersen-Civil et al., 2018).

To sum up, the involvement of the symbiotic and opportunistic microbiota in a wide variety of physiological, biochemical, immune, and behavioral processes depends on a large number of neuroactive chemicals including biogenic amines, amino acids, SCFAs, gasotransmitters, peptides, and purine nucleotides. These low molecular weight agents serve as nutrients, effectors, cofactors, and, most important within the context of the host-microbiota axis, signal molecules. Some of them are useful metabiotics (Shenderov et al., 2017; Oleskin & Shenderov, 2019), i.e., small-size molecules that represent microbial structural components, metabolites, or signals whose chemical structure enables them to influence the host organism with its microecological, nervous, and immune system.

[29] In plants, extracellular ATP sensed by the plant-specific DORN1 receptor is implicated in regulating the response to wounding (Cao et al., 2014).

Chapter 4

THEORETICAL AND PRACTICAL IMPACT OF THE BIOSOCIAL PARADIGM IN MICROBIOLOGY IN CONNECTION WITH THE MICROBIOTA-HOST SYSTEM

It follows from all the above that research on the social interactions and structures in microorganisms is of considerable theoretical and practical importance. This research provides the foundations for a fruitful interaction of microbiology with the social sciences and the humanities.

4.1. IMPLICATIONS OF THE POPULATION ORGANIZATION AND COMMUNICATION-CENTERED PARADIGM IN MICROBIOLOGY IN TERMS OF PHILOSOPHY, THE SOCIAL SCIENCES, AND THE HUMANITIES

Taken together, the results of recent studies within the framework of the population organization and communication-centered paradigm (POCCP) in modern microbiology provide convincing evidence that microbial

populations are heterogeneous, i.e., they include different cell types. Moreover, cells in many microbial populations are functionally differentiated and engage in contact, distant chemical, and, presumably, distant physical communication. These communication facilities help the population maintain its integrity in the absence of a single controlling center and enable microbial cells to display various forms of social behavior ranging from aggression to cooperation. As coherent systems, microbial populations are characterized by supracellular-level structures exemplified by the matrix. Their development is comparable to a multicellular organism's ontogeny (Yerusalimsky, 1952). Microbial populations form a part of various ecosystems and of the whole biosphere.

The biosocial features of microbial populations are comparable to those of animal communities and even of human society. Many microbial low molecular weight substances are identical, structurally similar or functionally analogous to neurochemical factors involved in regulating human social behavior (Shenderov, 1998, 2011).

POCCP promotes the interaction between microbiology and other subfields of biology, especially ethology (behavior research). Thanks to POCCP, it has become possible to apply key terms of animal ethology, e.g., affiliation and cooperation, to studies with unicellular organisms that display analogous behavioral phenomena (Smirnov et al., 1982; Smirnov, 2004; Oleskin, 1993, 1994, 2001, 2009, 2012).

POCCP helps overcome the barrier between microbiology and *cytology*. Many concepts suggested by microbiologists apply to the cells of animal or plant organisms, while originally cytological terms apply to microbial signals as exemplified by the microbial peptides that were denoted as cytokines, in an analogy to eukaryotic tissue-produced factors (Kaprelyants et al., 1999). The term extracellular matrix is widely used by cytologists, apart from microbiologists. Like the extracellular matrix of animal tissues, the microbial matrix often contains fibrillar structures. The similarity between the matrix of animal tissues and that of microorganisms is highlighted by the fact that they contain common chemical components, such as sialic acids.

Microbial biosocial systems form a part of more complex systems that may include both unicellular and multicellular organisms. Some quorum sensing signals facilitate communication among different components of such multispecies systems.

As for the human organism-inhabiting commensal and parasitic microbiota, its constant interaction with the host via many signal agents enables it to be highly responsive to the physical and even the emotional state of the host. Suffice it to mention (see 3.1.1) that catecholamines and other neurochemicals produced by the host under stress strongly influence the growth rate and other characteristics of many microbial inhabitants. Since the state of human individuals is under the influence of their interaction with other people, the microbiota is expected to respond to the psychological climate in human society that depends on the social situation and even on political developments. Therefore, the area of research dealing with microbiota-host communication signals is of *biopolitical* interest.

Of philosophical importance is the interpretation of the human being as a "superorganism" with the microbiota representing a special multifunctional organ. The self of a human individual proves to be dependent on what traditionally was not considered as a part of the self (Rees et al., 2018). The realization that the human being "is not a unitary entity but a dynamic and interactive community of human cells and microbial cells" (ibid.) has far-reaching consequences that are beyond the scope of biology per se. Actually, a new philosophical paradigm is taking shape. It is based on the idea that the microbiota exerts a strong influence not only on the physical health of human individuals but also on their mental state, social behavior, and even ethical norms and esthetic preferences. This is the reason why our knowledge of the human microbiota is so important for the social sciences and the humanities. Even the traditional distinction between the natural and the social sciences is called into question (Rees et al., 2018): the human microbiota that is to be investigated by natural scientists (microbiologists along with endocrinologists and neurologists) actually influences the aspects of the human being that are traditionally considered in terms of the humanities.

The present work is focused on the operation of the *brain-gut-microbiota* axis; recent data on this subject are expected to promote the development of psychology, especially as far as research on memory, behavior, human character, and temperament is concerned. The microbiota directly or indirectly impacts pain perception, emotions, cognition, stress resistance, personal features, and social behavior, which is of obvious psychological importance. In Chapter two (2.1), it was emphasized that different human "bacteriotypes" (with different *Bacteroides: Prevotella: Ruminococcus* ratios in the GI microbiota) are presumably correlated with different psychological traits. Therefore, they are of relevance to traditional psychological classification systems that subdivide people into types based on their temperaments or other personal features. The psychologically meaningful notion of the Unconscious as well as the Shadow concept put forward by Karl Gustav Jung, are to be reconsidered in terms of the impact of the microbiota on the host organism, including the direct or indirect (immune and endocrine system-mediated) effect of symbiotic and parasitic microorganisms on the nervous system and behavior in health and disease.

Currently, "the discipline of psychology is on the cusp of a significant paradigm shift, moving away from CNS-centric approaches toward a more holistic conceptualization of health and disease which integrates other body systems", including, naturally, the microecological system of the human organism (Ganci et al., 2019).

The POCCP in microbiology actually creates a new basis for integrating our knowledge in various branches of the life sciences as well as for bridging the gap between them and the social sciences. This integrative potential of the POCCP should be fully realized in the future.

Importantly, research on population organization and communication in microorganisms is not only of theoretical (psychological, sociological, and philosophical) interest. The POCCP holds much potential value in terms of innovative *biomedical* and *biotechnological* developments.

4.2. Implications of the Population Organization and Communication-Centered Paradigm in Microbiology in Biomedical Terms

Important recent biomedical developments are based on the therapeutic and preventive effects of the natural microbiota of the human organism. The microbiota that represents a complex orchestrated ensemble of communicating microorganisms is responsible for providing the organism with indispensable organic compounds ranging from vitamins to hormones and neurotransmitters.

4.2.1. Practical Applications of Probiotics (Psychobiotics)

To reiterate, *probiotics* are functional food products and drug preparations based on purposefully selected live microorganisms that suppress the development of potential pathogens, stimulate the immune system, and supply the host organism with nutrients and regulatory substances. Some probiotic microorganisms can form relatively stable populations in the gut.

Probiotics including their subclass denoted as *psychobiotics* that specifically influence the brain and psyche, receive much attention from researchers around the globe. Recent studies with animal models and clinical tests have enabled selecting a sufficient number of symbiotic human organism-inhabiting microorganisms that efficiently produce low molecular weight neuroactive substances or their complexes. The BASs can be used to treat depression, anxiety, and other mental problems, including neurodegenerative diseases. These psychobiotics ameliorate the microecological system of the GI tract and regulate the operation of the microbiota-gut-brain axis.

From the practical viewpoint, the promising area or research referred to as *microbial endocrinology* can be construed as research and development activities aimed at using “neuroactive molecule-producing probiotics as

therapeutic agents for the treatment of neurogastroenteric and/or psychiatric disorders" (Mazzoli & Pessione, 2016). Even though much research is still to be conducted in order to elucidate the role of the human gut microbiota in the development of psychiatric diseases, the microbiota already "represents an attractive target for novel interventions" (Ganci et al., 2019).

4.2.2. Practical (Medical) Importance of Data on Microbial Sociality and Communication

Apart from probiotics, the *sociomicrobiology* concept discussed at the beginning of this work is of direct relevance to potentially pathogenic microorganisms. The virulence of some bacteria including the gonorrhea pathogen varies depending on their social organization visualized in the architecture of their colonies (Shapiro, 1988). The collective behavior of the swarmers of pathogenic *Proteus* species promotes their migration inside the human organism via the urinary tract (Oleskin, 2001).

Data on microbial cell–cell communication are currently gaining in importance in biomedical terms. As mentioned above, the production of virulence factors in many opportunistic pathogens is subject to regulation by quorum-sensing signals and, therefore, requires a sufficiently high population density. Their QS systems can potentially be targeted by a new generation of drug preparations (Shenderov, 2011). The operation of these QS systems can be suppressed by (Shenderov, 2017; Shendrov et al., 2017)

- QS receptor antagonists including trans-isomers of fatty acids, L-carbohydrate isomers, and lectins;
- Inhibitors of N-acylhomoserine lactone-dependent QS systems such as halogenated furanones;
- Inhibitors of histidine kinases that form a part of peptide-dependent QS systems;
- QS signal degradation-catalyzing enzymes including microbial acylases, lactonases, and proteases exemplified by bifidobacterial serpins;

- Synthetic analogs of QS signals;
- Molecules of microbial, plant, and animal origin that disrupt QS-dependent communication (lactones, lectins, polyphenols, etc.).

Additional information concerning prospective QS systems-targeted drugs is contained in Table 8.

Table 8. Low molecular weight compounds of microbial origin that can be used as new-generation drugs for suppressing the operation of the QS systems of potential pathogens

Type of QS systems-inhibiting drugs	Examples
Protein synthesis inhibitors	Antibiotics that inhibit ribosome-dependent protein synthesis, antimicrobial peptides
QS receptor antagonists	Microbial trans-isomers of fatty acids, bacteriocines
Inhibitors of signal transduction in peptide-dependent QS systems	Histidine kinase inhibitors
Inhibitors of signal transduction in N-acylhomoserine lactone-dependent QS systems	Microbial halogenated furanones
QS signal-degrading enzymes	Microbial acylases, lactonases, and proteases

(According to: Shenderov, 2017)

Serious problems are caused by the heterogeneity of microbial populations and, more specifically, by *microbial heteromophism* (see 1.3) in a clinical setting. Diagnosing infectious diseases presents considerable difficulties because of this bacterial phenomenon. Making a diagnosis should not only depend on detecting typical morphological forms of pathogens; account should be taken of the pathogens' morphologically aberrant variants, especially when examining native pathological material (Vysotsky & Kotlyarova, 1999).

Unfortunately, microbial heterogeneity and constant genetic changes also make microbial populations more adaptive and resistant to drug preparations; therefore, Pechurkin et al. (1991) doubted whether drugs

would ever be obtained to which pathogens will not be able to develop resistance.

In more general terms that apply both to pathogens and useful symbionts, "we must now additionally consider that the gut microbiota, particularly in the case of drugs taken orally, can both impact the metabolism of the drugs and be a crucial effector/mediator of drug response" (Long-Smith et al., 2020, p.17.2).

4.2.3. Biomedical Implications of Microbial Biofilms

As we mentioned above (1.3.9), almost any microorganism can form biofilms under natural conditions. Unfortunately, the biofilms of pathogenic microorganisms pose serious threats. If bacteria succeed in forming biofilms inside our body, they may become invulnerable to antibiotics and cause chronic infection, e.g., in a surgical wound, in the lungs, or in the urinary tract. "Biofilm formation is an important aspect of many, if not most, bacterial diseases, including native valve endocarditis, osteomyelitis, dental caries, middle ear infections, ocular implant infections, and chronic lung infections in cystic fibrosis patients" (Jefferson, 2004, p.63). Biofilms overgrow catheters, contact lenses, and joint and intraocular implants. They cause gingivitis, bacterial vaginosis, and other infections (Jacubovics et al., 2013). As far as such harmful biofilms are concerned, "knowledge of the environmental cues, genetic elements, and molecular mechanisms that are involved in biofilm formation is necessary for a rational design of strategies to eliminate biofilms or to prevent biofilm formation" (Harmsen et al., 2010, p.253).

Fortunately, our modern-day knowledge enables us to overcome some of the pathogenic biofilms-caused problems (Saha et al., 2018). The following strategies of combating biofilms are practically used currently:

- Preventing biofilm attachment to surfaces by covering them with biofilm-repelling materials as exemplified by silver ion-containing substances

- Destroying the biopolymer-containing basis (matrix) of a biofilm, including the DNA that strengthens the matrix and can be degraded with DNases
- Introducing antimicrobials- or bacteriophages-containing lipid membrane vesicles (liposomes) into the biofilm matrix
- Using antibodies against pathogenic microorganisms or their toxins
- Using signal molecules such as nitric oxide that stimulate biofilm dispersal
- Applying surfactants including those of bacterial or fungal origin, as exemplified by *B. subtilis*-produced surfactin that efficiently degrades the biofilms of potential pathogens (*E. coli*, *Salmonella enterica*, *Proteus mirabilis*, etc.) growing on vinyl catheters inserted into the bladder (Saha et al. 2018).

4.2.4. Microbiota Transplantation

Fecal microbiota transplantation (FMT) from healthy donors to individuals with disrupted microbiota is a promising procedure. Of note, FMT is not a novel idea, its use has been recorded over the millennia (Lynch et al., 2019).

The donor's physiological and also neuropsychological features can influence the recipient's health state as well as psychological and behavioral traits. The data of animal studies discussed above (in Chapter two) should be supplemented with the following example.

The microbiota of healthy male mice was transferred to young female mice that were genetically predisposed to type 1 diabetes, an autoimmune disease in which the Langerhans islets of the pancreas are damaged by inflammation. The symptoms of diabetes were improved in the recipients; their symbiotic microbiota composition was normalized and the level of testosterone, the male hormone, was increased. The inflammation of Langehans islets and the production of autoantibodies to islet antigens were decreased (Markle et al., 2013).

FMT was successfully used to treat recurrent *Clostridioides difficile*-caused infections both in model studies and in the clinical setting, "but various studies have demonstrated heterogeneity in therapeutic outcomes in different diseases such as IBD and irritable bowel syndrome" (Lynch et al., 2019, p.657).

Ameliorating the GI microecological system by FMT improves digestion and beneficially influences the brain and behavior. Presumably, this procedure can be used for treating many mental diseases including autistic specrum disorders, Tourette's syndrome, epilepsy, and Parkinson's disease (Evrensel & Ceylan, 2016; Westfall et al., 2017; Liang et al., 2018). For instance, transplanting the GI microbiota of healthy donors to individuals with Parkinson's disease improved their locomotive behavior and ameliorated other symptoms (Westfall et al., 2017).

Unfortunately, FMT can also cause serious problems. First, identifying a completely healthy microbiota donor presents difficulties. Second, microorganisms including symbionts contain a large number of genes that are responsible for the synthesis of various toxins and other virulence factors as well as for resistance to antimicrobial compounds. Therefore, FMT may result in spreading pathogenicity and antibiotic resistance genes-carrying micorganisms in the human population.

Healthy microbiota transplantation procedures include autotransplantation. The microcenosis of a young individual is frozen, stored for a long time, and transplanted to the same individual at a different age for the purpose of treating various diseases, promoting physical and mental health, improving the operation of the immune system, and rejuvenating the organism (Shenderov, 2001).

4.2.5. Microbiota, Neurochemicals, and Nutrition

Recent progress in research on the microbiota and its interaction with the human organism has lent much weight to nutrition science including its new subfield, *nutritional psychiatry*. It is based on the idea of using the diet for the purpose of preventing and treating mental diseases. The diet is as

important for neurology and psychiatry as it is for cardiology, endocrinology, and gastroenterology (Sarris et al., 2015; Shenderov et al., 2016).

The Western-type diet is high on calories and low on valuable nutrients including folic acid, other vitamins of the B group, vitamin D, S-adenosylmethionine, N-acetylcysteine, zinc, magnesium [30], and dietary fibers. This contributes to the current spread of combined diseases (comorbidities) that affect both the organism's physiological state, including the functioning of the GI system, and the individual's mental health. Serious problems may be caused by a lack of nutritional cofactors and phytochemicals that protect the organism from oxidative stress associated with oxygen radicals (Sarris et al., 2015). The westernized diet alters the GI microbiota, decreasing the number of fiber-degrading microorganisms and increasing the concentration of animal protein and lipid decomposers (Shenderov, 2008; Rowland et al., 2017).

Ameliorating the microbiota and supplementing it with useful probiotics requires purposeful diet modification. Suffice it to mention that "strategies to replace missing microorganisms will likely fail over time if they are not supported by dietary alterations to retain supplemented microorganisms" (Lynch et al., 2019, p.657).

Since there are important interindividual differences in GI microbiota composition, it is imperative that individually designed ("customized") diets be developed, for the purpose of optimizing the functioning of the microbial consortium because "there may be no single, one-size-fits-all diet and… differential human responses to dietary inputs may rather be driven by unique and quantifiable host and microbiome features" (Kolodziejczyk et al., 2019, p.742).

[30] These substances are of significant neurochemical and immunological importance. S-adenosylmethonine influences a human individual's mood. N-acetylcysteine modulates the activity of the immune system and possesses anti-inflammatory, antioxidant, and neuroprotective properties. Zinc impacts hippocampal neurogenesis by stimulating factor BDNF expression. Folic acid and zinc deficiency is typical of people suffering from depression; a lack of vitamin D in the maternal organism increases the risk of schizophrenia in the offspring. All the above biochemical factors should be applied in combination to increase their efficiency, and this is characteristic of a healthy diet (Sarris et al., 2015).

The primary colonization of the gut by microorganisms during the perinatal period and the first 3-4 years of an individual's life is of paramount importance in health terms, especially with respect to the education and maturation of the immune system, as emphasized in the aforementioned "Old Friends" hypothesis (El Aidy et al., 2015; Kerry et al., 2018). Therefore, special attention should be given to the mother's and the young infant's diet.

Of note in the context of the *microbiota–gut–brain* axis are such prebiotics as oligosaccharides, polyunsaturated fatty acids (particularly, ω-3 fatty acids), dietary fibers, and polyphenols (Shenderov, 2001; 2014). ω-3 fatty acids and oligosaccharides can be successfully used for treating patients with mental problems (Liang et al., 2018), especially if comorbidities involving both the somatic and mental health state are to be treated. "A notable example is hepatic encephalopathy <see above, 3.8.4, in the context of NH_3 effects, O.A>, for which manipulation of the gut microbiota by lactulose or rifaximin results in clinical meaningful benefits" (Lynch et al., 2019).

Of much promise is the health-promoting strategy that combines an innovative probiotics-, prebiotics-, and metabiotics-enriched diet with more traditional psychiatric techniques including psychotherapy.

Of direct relevance to diet therapy is the fact that food products and additives contain BASs, including those of microbial origin, that impact the whole "triangle" comprising the microbiota, the nervous system, and the immune system. These BASs include *nootropics* that stimulate brain activity, cognition, and creativity, as well as produce other positive psychological effects (Dubynin et al., 2010; Shenderov et al., 2017). For instance, GABA mitigates anxiety and improves sleep quality. Oral administration of GABA or GABA-supplemented food/beverages (containing about 50–100 mg of GABA) has positive effects on human physical and mental health. These effects include (i) relieving psychological stress in people who perform arithmetic tasks and in acrophobic subjects exposed to heights and (ii) increasing the ability to perform prioritized planned actions (Mazzoli & Pessione, 2016). It should be re-emphasized

that, apart from food, GABA is also supplied by microorganisms that contain glutamic acid deaminase (GAD; Strandwitz, 2018; Cani et al., 2019).

Of relevance is also ferulic acid (trans-4-hydroxy-3-methoxycinnamic acid, FA) that is contained in seed plants (rice, wheat, and oats), vegetables (tomatoes and carrots), and fruits (pineapple and orange). Plants with a high FA content were traditionally used in Chinese medicine as anti-inflammatory drugs. FA is a strong antioxidant that can be used for treating neurodegenerative diseases, obesity, and diabetes. FA stimulates the proliferation of the stem cells of the nervous system. Chronic administration of FA to mice relieved Alzheimer's-specific behavioral symptoms, reduced the number of pathological amyloid Aβ fibrils, mitigated neuroinflammation, and alleviated oxidative stress (Wesfall et al., 2017). Apart from food, large FA amounts are synthesized by the GI microbiota that produces the necessary esterase enzyme. The probiotic strain *Lactobacillus fermentum* NCIMB 5221 displays high FA-synthesizing activity. Therefore, its application is a reasonable alternative to FA administration in the form of drugs or to an FA-enriched diet (Westfall et al., 2017).

Many traditional herbal medicines, e.g., *Hypericum perforatum*-based preparations that improve memory and mitigate depression- and anxiety-like symptoms in model studies with rats, have beneficial effects on physical and mental health; this is suggestive of their positive action on brain neurochemistry and GI microbiota. The use of such drugs is recommended for the purpose of treating diabetes and its CNS complications (Thakur et al., 2019).

A potentially useful food additive could be prepared by mixing several neuroactive amino acids, e.g., glutamic acid, an excitatory neurochemical, and GABA, an inhibitory neurochemical. When mixed at an appropriate ratio, these two neurochemicals could help adjust a patient's brain activity level during the premorbid and the initial stage of chronic mental diseases.

However, BASs (and nootropics) should be used with caution. Their excessive concentrations can produce negative effects (Mazzoli & Pessione, 2016). For instance, high glutamate concentrations may induce apoptosis in brain and heart cells. This poses a threat to one's health and may even cause

a heart attack or a stroke. Likewise, despite the useful effects of GABA, only its moderate concentrations should be administered to patients. It should be noted that high GABA concentrations (300–720 nmoles per 1 g of dry weight) are present in many plant foods including brown rice germs and sprouts, spinach, barley and bean sprouts. Fermented foods are characterized by still higher GABA levels that are released by bacteria during fermentation (Mazzoli & Pessione, 2016; Strandwitz, 2018).

4.3. Role of Neurochemicals in Microbiota-Host Interactivity

The biomedical implications of the POCCP also include the functions of neurochemicals within the framework of the *microbiota-gut-brain* axis in connection with their roles in the microbial consortium as well as in the host nervous and immune system. Of special medical interest are the following points: (i) Impact of neurochemicals on the microbiota in health and disease and (ii) Microbial contribution to the host pool of neurochemicals in relation to the operation of the immune system and the nervous system.

4.3.1. Impact of Neurochemicals on the Microbial Consortium

The host microbiota specifically responds to neurochemicals that are produced by various pathogenic, opportunistic or symbiotic microorganisms and by host cells including immunocytes. It should be re-emphasized that many biogenic amines, amino acids, peptides, and SCFAs produce stimulatory or inhibitory effects on the growth of a large number of human organism-inhabiting microorganisms (Anuchin et al., 2008; Malikina et al., 2010; Oleskin et al., 1998a, b; Lyte, 1993, 2010, 2011, 2013a,b, 2014, 2016; Lyte & Ernst, 1992, 1993; Shenderov, 2013a, b; Oleskin & Shenderov, 2013, 2016; Oleskin et al., 2010, 2014, 2016, 2017a, b). Neurochemicals are released, and expected to produce their effects, in various microecological

niches of the human body, including the airways, the vagina, and especially the GI tract.

Neurochemical release by various microbes and host tissues is enhanced by inflammation [31] : inflammation-damaged nerve terminals release catecholamines, intestinal epidermal chromaffin cells produce serotonin, and immunocytes in the inflammation area actively liberate serotonin and histamine. Therefore, it appears that the nonpathogenic (symbiotic) microbiota is "interested" in maintaining chronic inflammation in the GI tract because it provides the microbiota with a stimulatory neurochemical "cocktail" (Anuchin et al., 2008). If the intensity of this inflammation is sufficiently low, it could be regarded as a quasi-normal state which stimulates the development of many beneficial bacteria. This quasi-normal state corresponds to homeostatic conditions under which inflammatory and regulatory signals are integrated, resulting in "controlled inflammation compatible with tissue immunity" (Belkaid & Hand, 2014).

Such local permanent low-intensity inflammation seems to be a prerequisite for the maintenance of a sufficient immunological tolerance of the GI tract (Rogovsky, 2015).

The neurochemical "cocktail" is partly prepared by immunocytes. It should be re-emphasized that various types of immunocytes are equipped with genes controlling the synthesis of the key enzymes of the synthetic pathways of biogenic amines, amino acids, and SCFAs. They are exemplified by tyrosine hydroxylase that enables immunocytes to synthesize catecholamines. It seems likely that the activation and migration of immune cells during inflammation could promote the growth of various microbial inhabitants of the ecological niches of the human organism. Unfortunately, as pointed out in Chapter three, catecholamine-stimulated microorganisms include potentially enteropathogenic species (Lyte, 1993, 2010, 2011, 2013a, b, 2014, 2016). Papadoxically, an infection-elicited immune response may result in releasing factors that intensify the infection by

[31] Inflammation is "a localized reaction that produces redness, warmth, swelling, and pain as a result of infection, irritation, or injury. Inflammation can be external or internal" (Shiel, 2019).

boosting the growth and also virulence of pathogens and creating a typical clinical vicious circle.

The terms "microbiota" and "microbial consortium" are to be used in the broadest sense, so as to include, apart from bacteria, also eukaryotic microorganisms. These are exemplified by fungi. Researchers are only beginning to elucidate their role in the *microbial consortium-immune system-nervous system axis*. The above text provides data that opportunistic fungal symbionts such as representatives of the genus *Candida* react to neurochemicals including dopamine, serotonin, histamine, and GABA (see Chapter three) that may be released both by human (e.g., immune and nervous) and bacterial cells.

Some host-produced signals influence the virulence of opportunistic pathogens. Of note are the microbial effects of peptide neurochemicals. Immunocytes including macrophages and polynuclear leucocytes release catelicidin (peptide LL-37). In *Ps. aeruginosa*, catelicidin activates the quorum-sensing system that uses quinolone as the autoinducer. The system is responsible for producing virulence factors. It is also involved in the tolerance of *Ps. aeruginosa* to antibiotics (profloxacin and gentamycin, reviewed, Oleskin et al., 2016).

Opiods as neuromodulators function as pain relievers and "pleasure substances"; they are released under inflammatory stress. As mentioned in Chapter three, their representative dynorphin exerts a stimulatory influence on *Ps. aeroginosa* virulence and biofilm formation, via activation of the quinolone-dependent quorum-sensing system, and potentiates the antagonistic activity of *Ps. aeruginosa* with respect to important probiotic species such as *Lact. plantarum* and *Lact. rhamnosum*, which form a part of the GI microbiota. To reiterate, the opioid factor $[Met]^5$-enkephalin slows down the growth of *Ps. aeruginosa*, *Serratia marcescens*, and especially *Staph. aureus* that expresses $[Met]^5$-enkephalin receptors (reviewed, Oleskin et al., 2016).

Microorganisms including pathogens respond to many other neurochemicals and hormones. The pathogen *Burkholderia pseudomallei* specifically responds to the peptide hormone insulin, and this seems to be one of the reasons why *B. pseudomallei*-dependent infection is characteristic

of many patients with insulin-dependent type I diabetes that regularly use insulin as a drug. Insulin-mediated communication between the host and the pathogen is bilateral because *B. pseudomallei* produces its own insulin (reviewed, Oleskin et al., 2017a).

In addition to peptide neurochemical/neurohormonal factors, steroid hormones may produce specific effects on the microbiota including, notably, fungi. The pathogenic fungi of the genera *Coccidioides* and *Candida* possess estrogen receptors. Estradiol[32] stimulates the virulence of the prokaryote *Chlamydia trachomatis* (Lyte & Freestone, 2009).

Since some microorganisms produce steroid hormones (Lenard, 1992; Oleskin et al., 1998b) it is potentially possible to treat health problems caused by insufficient hormone levels in the organism with microbial steroid hormone producers that should be considered a new generation of probiotics. Importantly, estrogens influence the dopaminergic and serotonergic systems of the brain and, therefore, they can be used for treating mental diseases such as schizophrenia (Kulkarni & Gavrilidis, 2011).

4.3.2. Immunological and Neurophysiological Implications of Microbially Produced Neurochemicals

The immune and nervous system are under the influence of both host-produced and microbial neurochemicals. Some probiotic bacteria including lactobacilli produce immunocyte activity-decreasing neurochemicals exemplified by acetylcholine. Such probiotics potentially hold much value as remedies for autoimmune diseases in which immunocytes are overactivated. Nonetheless, the same probiotic strains can be harmful for patients with insufficiently active immune cells because they may pose the threat of promoting infectious processes and the growth of malignant tumors.

The above considerations also concern catecholamines and their precursor DOPA that are synthesized by many symbiotic and potentially

[32] Estradiol is the most important hormone of the estrogen group that also includes estrone and estriol.

pathogenic microorganisms. Catecholamines produce complex and partly self-contradictory effects that involve a large number of receptors. However, in many situations, they predominantly exhibit anti-inflammatory and immunosuppressive activity. This activity is to be expected with the norepinephrine- and/or dopamine-producing probiotic strains of *Lact. helveticus* and *Lact. delbrueckii* subsp. *bulgaricus* (Oleskin et al., 2014a, b). As mentioned above, catecholamines cause a shift from the Th1-mediated cellular to the Th2-dependent humoral immune response. Such probiotics may cause allergic processes, atopic dermatitis, and other Th2 helper-dependent disorders. However, the same probiotics can help us treat Th1-dependent problems including diabetes (type 1).

Lactic-acid bacteria-based probiotics efficiently synthesize neuroactive amino acids including GABA. It seems likely that the immunomodulatory effects of lactobacilli are partly dependent on their neuroactive amino acids. In particular, the lactobacilli-produced GABA should inhibit the production of proinflammatory cytokines and, therefore, contribute to the anti-inflammatory activity of the lactobacilli (Auteri et al., 2015).

Apart from synthesizing neuroactive substances, GI microbiota representatives including probiotics influence their production by host cells such as imunocytes and enterocytes; they also affect neurotransmitter receptor gene expression. The probiotic strains of *Lactobacillus rhamnosus* stimulate GABA production in the CNS (van de Wouw et al., 2017).

If we switch from probiotics to potential pathogens, e.g., the food spoiler *Bacillus cereus*, it should be emphasized that the catecholamines (and DOPA) produced by them (Oleskin et al., 2010) may considerably aggravate the infectious process. Apart from promoting secondary infection that could be caused, e.g., by catecholamine-stimulated enteropathogenic *E. coli* strains (Lyte, 2013a, b, 2016), microbial catecholamines should exert a systemic immunosuppressive effect that could be life-endangering under these conditions. It is not surprising, therefore, that *B. cereus* may cause rapidly progressing infections in organs and tissues that are distant from the GI tract. *B. cereus* can penetrate into a traumatized eye and cause fulminating panopthalmitis that completely destroys it (Shamsuddin et al., 1982). In contrast, the immunostimulatory effects of serotonin and histamine

that are synthesized by probiotic lactobacilli, e.g., by *Lact. reuteri*, promote the improvement of the clinical and anatomical symptoms of colitis (Gao et al., 2015).

It should be re-emphasized that:

i. the microbiota can synthesize a variety of neurologically and immunologically relevant *peptide factors* (see 3.9) such as leptin, insulin, ghrelin, peptide YY, neuropeptide Y, agouti-related peptide, orexin, α-melanocyte-stimulating hormone (α-MSH), adrenocorticotropic hormone (ACTH), oxytiocin, and vasopressin. Microbially produced neuromediator homologues cause B lymphocytes to produce antibodies that cross-react with host peptides (reviewed, Oleskin et al., 2016).
ii. some neuromediators are recognized by microorganisms as quorum-sensing autoinducers or their analogs (see 3.1.1). This seems to account for the stimulatory effect of catecholamines on microbial growth; the effects actually implicate bacterial adrenoreceptors such as kinases QseC and QseE in *E. coli* (Clarke et al., 2006; Hughes et al., 2009). Serotonin is the signal of one of the quorum-sensing systems of *Ps. aeruginosa* (Knecht et al., 2016).
iii. communication between the microbiota and the host using QS signals is bilateral because host cells, including those of the immune system, react to some of them. 3-oxo-dodecanoyl-homoserine lactone, a major QS signal of *Ps. aeruginosa*, inhibits TNF-α and IL-12 synthesis by immunocytes and stimulates the production of the proinflammatory γ-interferone as well as of IL-8. Cytolysin, the autroinducer of the *cyl* operon of *Enterococcus faecalis*, has been revealed to produce toxic effects on neutrophils, macrophages, epithelial cells, and erythrocytes (Kaper & Sperandio, 2005).

The third partner involved in the microbiota-immune system communication is the nervous system including the brain. Administering neurochemicals to animals produces complex multidirectional effects that often are distinct from those caused by direct interaction between the

neuromediator and the immune system. This is exemplified by the *in vivo* effects of dopamine and serotonin. Activating postsynaptic dopamine and presynaptic serotonin receptors in the brain stimulates the activity of the immune system. In contrast, activating presynaptic serotonin and postsynaptic dopamine receptors in the brain inhibits the immune system (Idova et al., 2012). Microbial neuroactive peptides, apart from affecting the immune system operation, can modify animal and human behavior via their effects on the CNS. For instance, the homologues of leptin, insulin, α-MSH, and ACTH that are synthesized by *Helicobacter pylori* (the pathogen involved in gastric and duodenal ulcer development) can decrease appetite (Fetissov et al., 2008).

4.4. Biotechnological Implications of the Biosocial Paradigm in Microbiology

Of significant importance are the biotechnological applications of POCCP. Biotechnology can be defined as the industrial employment of biological processes and agents; it is based on obtaining highly efficient microbial forms as well as animal or plant cell cultures with desired features (Egorov et al., 1987). This actually is in conformity with a more recent definition of biotechnology as "any technological application that uses biological systems, living organisms, or derivatives thereof, to make or modify products or processes for specific use" (UN Convention on Biological Diversity, 2013).

4.4.1. Microorganisms as Neurochemicals-Producing Biofactories?

The potentially promising biotechnological idea of converting neurochemicals-synthesizing microorganisms into "biofactories" that produce neurochemicals, their precursors, and metabolites presents serious

difficulties. The concentrations of such microbial neurochemicals typically are too low to be used for biotechnological industrial production. Nevertheless, it is to be hoped that modern efficient selection techniques including, notably, genetic engineering will enable us to create microbes that *overproduce* valuable neurochemicals and related substances.

Successful attempts to develop such overproducers can be illustrated in the example of neuroactive amino acids-producing microorganisms. Screening for efficient GABA producers and optimization of their cultivation conditions enabled obtaining GABA-overproducing microorganisms. Impressive results were obtained with neuroactive amino acids-producing *Lactobacillus* and *Lactococcus* strains that were isolated from Italian cheese (Siragusa et al., 2007), Chinese adzuki beans (Liao et al., 2013), and fermented cod bowels (Lee et al., 2010). These strains produced over 1 millimole/L of GABA. Apart from GABA, *L. brevis* cultivated in a medium with seaweed enriched the medium in other neuroactive amino acids, such as taurine, glycine, and β-alanine (Lee et al., 2010). The cultures of lactobacilli and bifidobacteria that were isolated from human individuals in the Central Region of Russia, exhibited a comparable efficiency in producing GABA. For instance, the strain *Bifidobacterium adolescentis* 150 produced up to 5.6 g/L, i.e., ~ 50 mM GABA (Yunes, 2017). The microbial producers of GABA are of special interest because GABA is sufficiently widely used for medical and especially psychiatric purposes. It is known that GABA, a neuron excitation-inhibiting neuromediator, produces many beneficial effects (see subsection 3.6.3).

Bacteria also produce sufficiently high concentrations of other neuroactive amino acids that can be used in functional food items and drug preparations for therapeutic and preventive purposes. The probiotic strain *Lact. casei* K_3III_{24} releases micromolar concentrations of glutamic acid and taurine into the medium (Oleskin et al., 2014 a, b). Importantly, taurin improves vision, in addition to other beneficial effects. The culture of *Lact. brevis* BJ20 that was grown on a seaweed-containing medium, considerably enriched the medium in neuroactive amino acids, such as taurine, glycine, β–alanine, and GABA (Lee et al., 2010).

The bacteria *E. coli, Bacillus cereus, Lact.* spp., and others form catecholamines and, still more important, their precursor DOPA (Shishov et al., 2009; Oleskin et al., 2010, 2014a, b). It should be re-emphasized that DOPA crosses the BBB; in the brain, it is converted to dopamine and thereupon to norepinephrine. Dopamine and norepinephrine regulate important brain processes. DOPA is used as a remedy for diseases that are characterized by lowered dopamine levels in functionally important brain areas. Importantly, a decrease in dopamine content in the *substantia nigra* of the brain is typical of Parkinson's disease (Dubynin et al., 2010). Screening human symbiotic microorganisms for efficient DOPA producers could be an important biotechnological project that has relevance to the potentially important idea of using psychobiotics for amelioration of the operation of the human brain.

In contrast to the tested bacteria, the yeast *S. cerevisiae* accumulates neurochemicals such as catecholamines, DOPA, and serotonin inside its cells without releasing them into the culture liquid (Malikina et al., 2010; Oleskin et al., 2010). This fact has some practical implications because humankind has been using yeast culture liquid as wine and beer since time immemorial. If preparing a beverage involves separating (by filtering or centrifugation) the culture liquid from the yeast cells, then the beverage is expected to contain no neurochemicals. However, if yeast cells (without prior heating) directly form a part of the beverage, the human organism is exposed to the effects of the neurochemicals that are liberated from yeast cells during the digestion process.[33]

These data indicate that microorganisms can be considered important producers of neuroactive compounds that impact human physical and mental health. This provides the foundations for target-oriented biotechnological developments and the production of customized functional food with predictable physiological and psychological effects. Current research is providing us with new options for subtly manipulating human behavior by modifying the diet, which includes introducing neurochemically active

[33] Heating leads to the degradation of biogenic amines and related compounds.

substances-producing microorganisms or their components and metabolites into the human GI tract.

This is of obvious biopolitical importance in the present-day world. It should be stressed that *biopolitics* is construed as an interdisciplinary area of research dealing with political implications of biology (Somit & Peterson, 1998, 2011; Masters, 2001; Oleskin, 2012). One of its subfields is focused on the impact of BASs, including those of microbial origin, on the brain and social behavior. Currently, new strategies of manipulating human behavior by modifying the diet are being developed; this may be achieved by introducing neurochemically active microorganisms, their metabolites, and signal molecules into the GI tract.

Apart from obtaining neurochemical overproducers, genetic engineering enables us to develop other projects that are aimed at ameliorating microbiota-host interactions. Suffice to mention the idea of obtaining genetically modified probiotic strains that produce immunomodulatory substances. Genes that enable such probiotics to synthesize lipoteichoic acids, anti-inflammatory interleukin-10, and other immunomodulators should be inserted into their DNA (Kerry et al., 2018).

4.4.2. Ecosystem Biotechnology

The biotechnological applications of POCCP also include biotechnological projects in which the microbial producers of important chemicals are cultivated in natural or artificial associations or whole ecosystems. Of relevance are also industrial developments enabling long-term storage of viable microbial cultures for industrial purposes and the retention of their biotechnologicaly important gene pool.

Instead of genetically modifying microorganisms, biotechnologists can attempt to achieve their goals by using mixed cultures. In order to convert starch to ethanol in a one-stage process with an *E. coli* culture, the genome of this bacterium should be supplemented with the α-amylase gene. However, the same process can be carried out with a mixed culture containing, apart from *E. coli*, a natural α-amylase-producing

microorganism (Oleskin & Samuilov, 1992). Many combined cultures possess significant advantages over pure single species (single strain) cultures conventionally used for biotechnological purposes (Pandhal & Noirel, 2014):

- Mixed cultures can carry out more complex process than pure cultures; apart from the conversion of starch to glucose, of note are processes aimed at obtaining ethanol from xylose and degrading wood lignocellulose; mixed cultures can also carry out multistage chemical transformation processes that are necessary for biotechnological drug production.
- Many mixed cultures are more efficient than pure cultures in synthesizing biotechnologically important products; if the cultures of the main producers of drug preparations, food additives, biofuel, etc. are supplemented by other cultures with additional key enzymes, this may significantly increase the product yield
- Many mixed cultures grow on relatively inexpensive and readily available substrates
- Such cultures are frequently characterized by a high stability that was achieved, e.g., in a balanced five-species cellulose-degrading mixed culture (Pandhal & Noirel, 2014)
- Mixed cultures often exhibit an increased adaptability to new environmental factors (including a changed medium composition) and an enhanced resistance to extraneous microorganisms.

The hopes of biotechnologists are also pinned on new techniques of cultivating mixed cultures and microbial associations. For instance, such cultures/associations can be grown in microfluid chambers. Their spatial configuration promotes successful cooperation among association components while limiting their competition. Microbial associations can also be cultivated in aqueous droplets embedded in the oil phase (Pandhal & Noirel, 2014).

Complex biotechnological processes enable biogas production from organic materials that often represent agricultural or municipal waste products. The success of such biotechnological projects depends on cooperation among microbial populations that specialize in the following biogas production stages (Oleskin & Samuilov, 1992; Pandhal & Noirel, 2014):

- Hydrolyzing biopolymers to monomers;
- Converting monomers to organic acids (acidogenesis);
- Degrading the organic acids to SCFAs typically dominated by acetate (acetogenesis);
- Producing methane as the main combustible biogas component (methanogenesis).

Although of paramount importance, this biotechnological process presents serious difficulties in terms of establishing a balanced cooperative microbial sytem because the microorganisms that carry out different process stages differ in growth rates, metabolic requirements, optimum pH values, etc.

Biogas production should be considered an example of ecosystem biotechnology also because it is environment-friendly and produces a triple positive effect in ecological terms: (i) this process eliminates potentially environment-endangering organic waste products; (ii) it results in producing economical biofuel, and (iii) organic waste products are converted during the fermentation process into a mineral fertilizer. Generally, ecosystem biotechnology holds much potential in environmental terms. For instance, it promises to limit the industrial use of petrochemicals as dangerous pollutants. In particular, using "chemical processes for the synthesis of various aromatics using petroleum-derived benzene, toluene and xylene (BTX) as the starting materials can be replaced by biobased sustainable and environmentally friendly processes using renewable non-food resources" (Huccetogullari et al., 2019).

4.4.3. "Omic" Technologies

Molecular genetics-based "omic" technologies are currently acquiring increasing importance. *Metagenomics*, i.e., direct analysis of all genomes in a given sample (Boddu & Divakar, 2018), seems to hold much promise for the future. Metagenomics has proved to be significantly more efficient than 16 S rRNA sequencing, a method used in the 1990s for quantitative analysis of the composition of the gut microbiome. "Metagenomic sequencing … utilizes taxonomically informative gene tags to target and amplify genomes of interest, analysis of this data allows researchers to determine the composition and function of different microbiomes" (Huang et al., 2019). As far as probiotics are concerned, an important requirement (see Chaper two, 2.6) is that they should survive at low pH values that are characteristic of the upper GI tract.

The impact of metagenomics is illustrated by the results of screening the metagenome libraries of the microbial consortia of the plankton and the rhizosphere (the area around plant roots) of the Tinto (Red) River in South-West Spain. Its mining industry-polluted water is characterized by an extremely acidic environment; the water color is red because of the presence of iron oxides. 15 genes responsible for the resistance of microbial cells to medium acidification were cloned and expressed in the bacteria *B. subtilis* and *Pseudomonas putida* that were enabled to survive under acidic stress (Guazzaroni et al., 2013). In light of this data, it seems feasible to optimize the features of useful microorganisms, including their capacity to synthesize neroactive compounds, by means of "omic" technologies.

"Omic" technologies are of considerable interest with respect to mixed cultures and more sophisticated microbial associations that are established in terms of ecosystem biotechnology. These technologies enable predicting the possible functions of the microbial species and strains that form a part of such associations. They make it possible to evaluate their efficiency, applicability to specific projects, and other biotechnologically important features by reconstructing microbial proteins and metabolic pathways within the framework of *metatransciptomics* and *metaproteomics* (Pandhal & Noirel, 2014).

However, the currently available databases for microbial genomes, epigenomes, and metabolomes are incomplete, and this, unfortunately, limits the potential of "omic" technologies (Boddu & Divakar, 2018).

Microorganisms display various forms of collective behavior, engage in contact and distant cell-cell communication, and form supracellular systems that can be considered as biosocial systems, by analogy to animal social groups (families, schools, etc). Microbial cells in biosocial systems tend to be morphologically differentiated and functionally specialized. Research on population organization and communication in microorganisms promotes the interaction between microbiology and cytology, as well as between microbiology and ethology. This research has important philosophical and social implications, since it enables us to consider the human brain, behavior, and political activity from an innovative viewpoint that places emphasis on the role of the microbiota and its communicative signals. The new microbiological paradigm (POCCP) is also of significant importance for medicine and biotechnology.

CONCLUSION

The present work that can be considered both a monograph and a student guidebook is focused on the social organization and communication of microorganisms. It also addresses the biomedical, philosophical, and psychosocial implications of the interaction of the GI tract-inhabiting microbiota with the host nervous and immune system. Special attention is given in this work to the functions of neurochemicals, including catecholamines, serotonin, histamine, acetylcholine, agmatine, neuroactive amino acids, SCFAs, gasotransmitters, peptides, and purines, in terms of the ongoing host-microbiota dialogue. The work is intended for biologists dealing with various branches and subfields of the modern-day life sciences, healthcare workers, sociologists, psychologists, and philoso-phers, as well as for all those interested in a complex interdisciplinary area of research involving biology, medicine, sociology, psychology, ethology, and ecology.

The structural organization of microbial biosocial systems such as colonies and biofilms and their interaction wth the host organism are considered in *network organization* terms in several sections of this work. It is emphasized that microbial network structures may be both useful and harmful with respect to the physical and mental well-being of human individuals. The work also emphasizes the fact that the detrimental impact of network structures is not inevitable. There are methods and techniques aimed at preventing the negative influence of networks and enhancing their

positive effects. Whenever decentralized networks, e.g., those of the microbiota, interact with centralized hierarchies, as exemplified by the CNS of the human host, an important role is assigned to intermediary structures (Oleskin, 2014, 2016). Such a role is normally performed by the immune system of the organism that limits the development of microbial network structures and regulates it in order to promote the host's physical and mental health.

The network structure concept does not only apply to the interaction between the host organism and the microbiota that forms decentralized distributed network structures. Not only microorganisms but also researchers can organize their teams as decentralized network structures. Therefore, the network organization of microbial biosocial systems including those inhabiting the human organism is of considerable interest in terms of its applications to human social structures that can use analogous organizational principles. A potentially applicable originally microbial principle is that of *merging individual cells into coherent supracellular matrix-embedded structures* as exemplified by microbial colonies or biofilms.

An analog of a coherent biofilm in human society is a structure composed of human individuals that are united by their common ideas, values, and behavioral norms; taken together, these unifying factors are analogous to the biofilm matrix. Individual differences among network structure members seem less important against the background of the network-consolidating immaterial matrix made up of ideas and values. In practice, the extrapolation of microbial network structures to human society implies the consolidation of a nonhierarchical flat team of people by means of psychological techniques that promote the dominance of group-level goals, values, and creative work over individual differences. While bacterial cell envelopes partly merge into the biofilm matrix, interpersonal boundaries in a close-knit creative team tend to lose their importance; moreover, the personalities of networked team members tend to become psychologically similar. This is what Vasily Nalimov and other scholars in the field of transpersonal psychology called *personality merging.*

Partial temporary personality merging may take place during brain storming sessions in which special emphasis is placed on group values and group identity symbols. Group consolidation can be promoted by techniques that make good use of archaic, biological evolution-molded behavioral trends and needs. An ancient technique of strengthening a group's integrity is based on regular collective meals. It is recommendable to organize a social breakfast, lunch, or party for the whole network structure during or after a creative work session. Such techniques enable coordinating network members' behavior and synchronizing their activity rhythms even in the absence of a hierarchical structure and a permanent leader (Oleskin et al., 2017d).

It is within this context that the present work is expected to perform an important additional function. It is to encourage creatively-minded social reformers, especially young enthusiasts, to establish a new decentralized networked center that would specialize in the currently popular interdisciplinary area of research dealing with *Microbial Communication, Neurochemicals, and Probiotics.* This network structure could be of direct relevance to *restorative medicine* that mainly deals with patients during recuperation or remission periods; special emphasis is often placed upon non-surgical and drug-free treatment strategies. The main professed aims of restorative medicine also include prevention or amelioration of disabilities and the improvement of the life quality of the disabled (Oleskin, 2018).

The potential importance of the new networked research center is primarily due to the fact that microorganisms, including useful probiotic strains, produce a wide spectrum of neuroactive substances that influence the human brain. Research on the mechanisms of this influence is of obvious medical and psychological importance.

In the authors' opinion, the structure of choice for this innovative networked center is a *hirama (High-Intensity Research and Management Association).* This is a creative decentralized team that is set up for carrying out an interdisciplinary project (Oleskin, 1996, 2014; Oleskin & Masters, 1997). The project is subdivided into several subprojects. For example, a project concerned with *Microbial Communication, Neurochemicals, and*

Probiotics can be broken down into the subprojects on the following subjects (Figure 17):

- Social Organization and Communication of Microorganisms
- Impact of Microbial Products on the Brain
- Probiotics

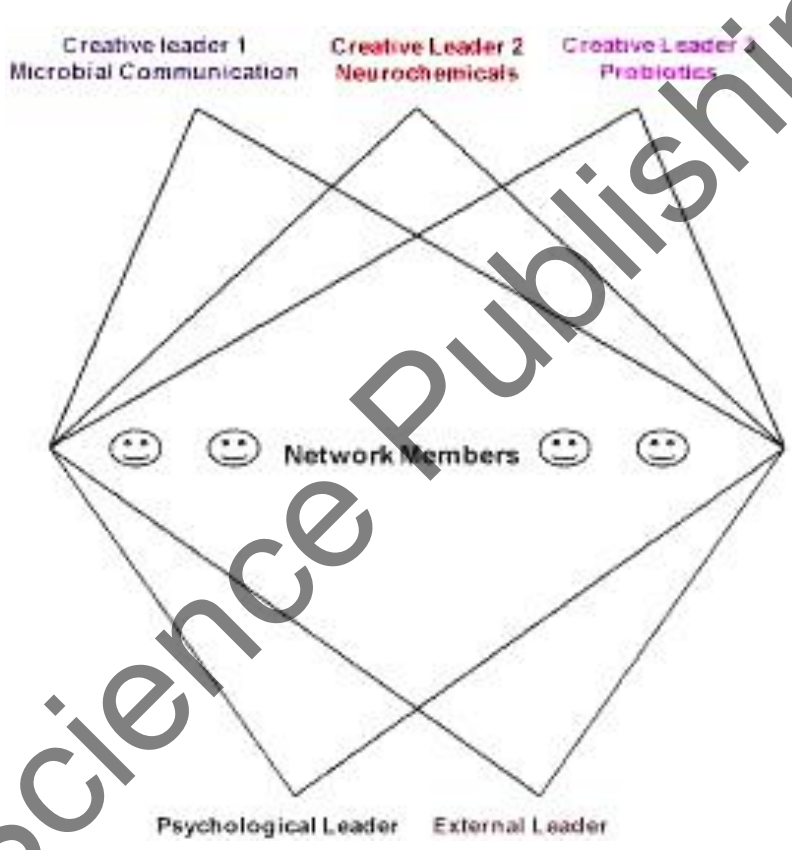

Figure 17. A pilot network structure for a team conducting research on *Microbial Communication, Neurochemicals, and Probiotics.* According to: Oleskin, 2018.

However, despite subdividing the project into subprojects, the network is not subdivided into parts. Its members work, in parallel, on several (ideally on all) subprojects. Only one person, the *partial subproject leader*, is attached to a particular subproject. The person collects ideas on this

subproject, which are also generated by other network members. A partial leader responsible for coordinating work on a particular subproject can be assisted by several experts on the same subproject. They interact with unspecialized network members that are more numerous in many hiramas (see: Oleskin, 2014, p.16). A hirama also has a *psychological leader*. The psychological leader creates an atmosphere that promotes efficient work on all subprojects and helps other partial leaders interact with one another, mitigating or preventing internal conflict. "In addition, a hirama typically includes an *external leader*... The individual with this role is responsible for propagandizing hirama-promoted ideas, establishing contacts with other organizations, and shaping the group's pastime and leisure activities, thus contributing to the development of informal loyal relationships among members" (Oleskin, 2014, p.17).

In this book, the term *biopolitics* has been used several times. Bopoltics in its widest sense can be construed as *the totality of all kinds of interactions between the life sciences and politics*. Indisputably, the data and concepts of present-day biology exert a strong influence on political theory (political philosophy and poltical science) and political practice, including the actions and decisions of political leaders and ordinary citizens/subjects of all countries of the world in relation to the state of the environment and the whole biosphere[34] (Foucault, 2003; Somit & Peterson, 2011; Oleskin, 2012).

The present work should convey the message to the audience that the innovative population organization- and communication-centered paradigm in microbiology in conjunction with its neurophysiological ramifications actually forms a part of present-day biopolitics. It incorporates biologically based technologies aimed at manipulating human behavior for political, military, or commercial purposes. Such technologies, in all likelihood, will be developed in the future; they are still in their infancy at present. However, it has already become possible to produce specialized microbial cultures that colonize the GI tract and other niches in the human organism and exert an influence on the human body and mind. By applying customized probiotics and substances stimulating their development, such as prebiotics, new

[34] Discussing the various specific meanings of the term biopolitics is beyond the scope of this work (see: Oleskin, 2012).

target-oriented diets can be designed, e.g., for political leaders. Presumably, different political leader types, as exemplified by democratic and authoritarian leaders, will require different kinds of functional personalized microbiota-containing food items.

It is to be hoped that this subfield of modern biopolitics will prove to be useful to multitudes of people whose activities actually shape the course of history and whose physical and mental health, social behavior and even political participation to a great extent depend on the harmonious operation of the whole consortium of our important invisible friends.

GLOSSARY

Agonistic Behavior. Comprises all conflict-related forms of social behavior.

Aggression. In ethology, the term means approaching an opponent and inflicting damage on him/her or at least generating stimuli that cause him/her to submit (Tinbergen, 1968).

Autoprobiotics. Frozen GI microbiota components of human individuals; their long-term storage enables recolonozing the GI tract of these individuals whenever necessary, in order to treat them for dysbiosis or to rejuvenate their organism.

Autoregulators (Autoregulatory Substances). Microbial metabolites that are released by a cell population, or its part, into the medium. Many autoregulators are not utilizable in terms of constructive or energy metabolism but perform major communicative functions and, therefore, influence the physiological state and the reproductive potential of the cells involved (El'-Registan, 1988).

*Affiliation. S*ocial behavior involving an individual animal's tending to approach and remain near conspecifics (*Dewsbury*, 1978), particularly those belonging to the same family or social group

Bacteriotypes (Enterotypes). Putative classification of human individuals into three bacteriotypes (entrotypes), depending on the dominance of the genera *Prevotella*, *Bacteroides* or *Ruminococcus* in the gut microbiota (Arumugam et al., 2011; Clarke et al., 2014).

Biogenic Amines. A group of nitrogen-containing organic compounds performing neurochemical and/or hormonal functions and serving as signals in cell systems. They include catecholamines (dopamine, norepinephrine, and epinephrine), serotonin, histamine, octopamine, tyramine, etc.

Biofilms. "Matrix-enclosed microbial accretions that adhere to biological or non-biological surfaces" (Hall-Stoodley et al., 2004, p.95).

Biopolitics. Totality of all kinds of interactions between the life sciences and politics. (Oleskin, 2012). One of its subfields is aimed at using biologically based technologies of manipulating human behavior for political, military, or commercial purposes.

Biosocial Systems. Systems composed of biological individuals or their groups that are characterized by communication, affiliation, and cooperation among them. Biosocial systems consist of individuals/ groups of the same species (homotypic systems) or, alternatively, belonging to several species (heterotypic systems, or associations).

Biotechnology. Industrial employment of biological processes and agents that is based on obtaining highly efficient microbial forms as well as animal or plant tissues and cell cultures with desired features (Egorov et al., 1987). Biotechnology is "any technological application that uses biological systems, living organisms, or derivatives thereof, to make or modify products or processes for specific use" (UN Convention on Biological Diversity, 2013).

Cheaters (Free Riders, Defectors). Individuals (including microbial cells) that benefit from the goods produced by cooperators without engaging in cooperative behaviors. Such noncooperators do not spend their resources and possess competitive advantages over "honest cooperators" (Özkaya et al., 2017).

Communication. Exchanging information and obtaining it from other living organisms (Nikolaev, 2000).

Contact Communication. Communication based on direct contact between livng organisms, e.g., microbial cells.

Cooperation. Interaction between two or more individuals for the purpose of solving a problem or carrying out a task. Alternatively, cooperation

is defined from the viewpoint of a whole group (biosocial system): cooperators contributing to the collective good are contrasted with cheaters (free riders) exploiting it (Hochberg et al., 2008, modified).

Distant Chemical Communication. Distant information transmission among living organsms based on signal molecules.

Distant Physical Communication. Distant information transmission among living organisms involving electromagnetic and/or acoustic waves or other physical communication channels.

Dysbiosis. Microbiota disruption (in the GI tract) manifesting itself in a decrease in the number of useful microorganisms and impoverishment of taxonomic diversity of the microbiota, which is frequently accompanied by an increase in the number of potential pathogens.

Ecosystem Biotechnology. Biotechnological developments based on the cultivation of the microbial producers of important chemicals in natural or artificial associations or whole ecosystems. Of relevance are also industrial projects aimed at long-term storage of valuable microbial strains and the retention of their gene pool.

Enteric Nervous System (ENS). The semi-autonomous part of the nervous system located in the intestinal wall.

Enteroendocrine Cells (EECs). Gut mucosa cells that produce hormones including regulatory peptides.

Ethology. A field of biology dealing with animal behavior. Many ethological concepts are applicable to the behavior of free-living (microbial) cells as well as cells within the tissues and organs of multicellular organisms.

Fecal Microbiota Transplantation (FMT). A therapeutic or preventive procedure based on transferring the microbiota of healthy donors to recipients with disrupted GI microbiota.

Gasotransmitters. Gaseous substances, such as nitric oxide, carbon oxide, hydrogen sulfide, and, probably, other gases that perform nerochemical functions.

Gastro-Intesinal (GI) Microbiota. A complex organized consortium of communicating microorganisms ("the microbial organ") that supplies the host organism with indispensible organic substances from vitamins

to hormones and neurochemicals and also performs many other vitally important functions.

Germ-Free (GF) Animals. Animals raised under aseptic (germ-free) conditions.

Heteromorphism. Formation, in a microbial population, of abnormal cell types, including cells with disrupted division and defective cell walls as well as cell wall-lacking forms (oval or spherical cells of the spheroplast or protoplast type), filamentous, giant, and miniscule cells such as L forms.

Hierarchy. "Applied to social organization, hierarchy is synonymous with rank order and involves the concept of social dominance. Individuals in a group yield to others in contention for something, such as food and mate, according to a more or less linear order" (Immelmann & Beer, 1989, p. 131).

HIRAMA (High-Intensity Research and Management Association). A creative decentralized networked team that is set up for carrying out an interdisciplinary project (Oleskin, 1996, 2014, 2018; Oleskin & Masters, 1997); ths project could be concerned, for instance, with *Microbial Communication, Neurochemicals, and Probiotics*.

Intestinal Immune System. Composed of immune cells in the gut-associated lymphatic tissue (GALT).

Isolation (Avoidance). Conflict-mitigating behavior that does not directly involve aggression and implies avoiding a potential opponent.

Loyal Behavior. All kinds of friendly interactions among individuals; loyal behavior helps consolidate a biosocial system.

Matrix. Biopolymer substances that bind together and envelop the cells of a microbial colony or biofilm.

Metabiotics. Biologically active substances that are produced by symbiotic (probiotic) microorganisms and exert a positive influence on various physiological processes and activities (Shenderov et al., 2017, p. 27).

Microbial Endocrinology. The area of research dealing with the role of hormones and neurochemicals in communication among microorganisms and in the host–microbiota dialogue (Lyte, 2010, 2011, 2013a, b; 2016).

Microbial Metabolome. Low molecular weight (< 1500 Da) metabolites of microbial origin.

Microbiome. Total genome of all microorganisms, e.g., of the GI microbiota of the human organism.

Microbiota-Gut-Brain Axis. Incorporates the whole gut microbiota, the enteric, parasympathetic, sympathetic nervous system, and the CNS; of paramount importance is the interaction of these systems with the endocrine and immune system.

Network Structure. A biosocial system or, in human society, an organization, characterized by a lack of centralized hierarchy, partial and/or temporary leadership, and, in many structures of this type, broad overlapping roles/functions of the individuals it comprises. In a broader sense, a network structure is any sets of items, which are called vertices or nodes, with connections between them, called edges or links (Newman, 2012).

*Neurochemicals. S*ubstances that transmit messages between nervous cells (neurons) or from a neuron to a muscular or glandular cell (that carries out the neuron's command) and/or modulate the efficiency of impulse transmission. In this work, we do not pay special attention to the differences between *neurotransmitters* that directly transmit impulses across the synaptic cleft between nervous cells and *neuromodulators* that modulate neurotransmitter effects; the more general term *neurochemicals* is mostly preferred throughout this work.

Neuropeptides. Peptide neucrochemicals that often perform neuromodulatory functions by altering the efficiency of impulse transmission across synapses that use other agents as neurotrans-mitters.

Nutritional Psychiatry. A recently developed subfield of psychiatry that is based on using the diet, including food-associated microorganisms and their products, for the purpose of preventing and treating mental diseases (Sarris et al., 2015).

"OMIC"Technologies Molecular genetics-based technologies including *metagenomics*, i.e., direct analysis of all genomes in a given sample, as well as *metatransciptomics* and *metaproteomics* that enable reconstructing microbial proteins and metabolic pathways.

Population Organization and Communication-Centered Paradigm (POCCP) in modern microbiology. A subfield of microbiology that focuses on cell-cell interactions and signal exchange in the microbial world as well as on the structure and functioning of microbial colonies and biofilms.

Prebiotics. Soluble dietary fibers and other organic agents that stimulate the growth of useful microotrganisms.

Probiotics. Live microorganisms that, "when administered in adequate amounts, confer a health benefit on the host" (FAO/WHO, 2006).

Psychobiotics .Live microorganisms that, when administered in adequate amounts, confer a health benefit on patients with psychiatric problems (Cryan & Dinan, 2012).

Quorum-sensing (QS) systems. Signaling systems that control, in a cell density-dependent fashion, many important microbial processes including bioluminescence, synthesis of antibiotics and enzyme complexes, intercellular transport of genetic information (transformation and conjugation), cell aggregation, protein secretion, biofilm and gas vesicle formation, sporulation, virulence factor production, etc.

Short-chain fatty acids (SCFAs). Saturated unbranched fatty acids with short carbon chains. Of paramount importance in biological terms are SCFAs with two to four carbon atoms in the chain, i.e., acetic, propionic, and butyric acid, or, according to their anion names, acetate, propionate, and butyrate.

Socal behavior. Any behavior that affects another individual's (cell's) evolutionary fitness (Ulvestad, 2009).

Sociomicrobiology. The subfield of microbiology that is concerned with communication and collective behavior in microorganisms (Sekowska et al., 2009).

Swarming. Group migration away from high-density areas (Foster, 2010) that involves highly motile swarmer cells.

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