Anaerobic Microorganisms from Human Microbiota Produce Species-Specific Exometabolites Important in Heath and Disease

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Abstract: Potential role of small molecules of microbial origin (SMOMs) in human homeostasis is under intensive research. Today the data on chemical structure of microbiota’s metabolites are still limited as well as their mechanisms of participation in the systemic inflammation and regulatory processes in health and disease. We have previously shown that in sepsis the levels of some phenolic acids in the blood increase tens of times, while some other metabolites are reduced or entirely disappear. It is known that various diseases, especially critical ill state, usually associated with maximal shifts in the composition of indigenous anaerobic microflora. In this study the attempt was made to evaluate the potential input of pure culture of different species anaerobic bacteria in "in vitro" setting in human metabolome. The main objective of the present study was to determine individual contributions of anaerobes in the cumulative pool of low-molecular-weight phenolic and carboxylic acids. The production of these metabolites by predominant species of human anaerobic flora has been studied by gas chromatography–mass spectrometry (GC–MS) analysis. It was demonstrated that, along with lactate, bifidobacteria and lactobacilli produced in vitro considerable amounts of phenyllactic and p-hydroxyphenyllactic acids. Clostridium spp. produced lactic and phenyllactic acids, as well as 2-hydroxybutyric acid. C. sporogenes, alternatively to C. perfringens, produced great quantities of phenylpropionic and p-hydroxyphenylpropionic acids. Bacteroides spp. turned to be powerful producers of succinic and fumaric acids and greatly contributed to the production of lactic acid. Eubacterium lentum produced lactic, phenyllactic, and succinic acids at high concentrations. The species-specific differences in the selective production of particular metabolites between indigenous and conventionally pathogenic human anaerobes may be useful for determining the composition of biocenosis and microbial associations, and can be used as well as in the design of novel probiotics.

Keywords: Anaerobes, microbial metabolites, phenyllactic acid, p-hydroxyphenyllactic acid, phenylpropionic acid, lactic acid, succinic acid, probiotics.

1. INTRODUCTION

The presence of abundant mutualistic and facultative pathogenic microorganisms is associated with their active metabolic activity and presumable interference of their metabolic products with host metabolism. The critical physiological role of substances produced by normal microflora has been widely recognized and resulted in the development and common use of probiotics - preparations based on live cultures of the normal human gut beneficial microflora and/or their metabolites - to sustain health and combat disease, which is supported by a considerable body of published scientific evidence in last decade. Meanwhile, there are multiple blank spots in our knowledge of commensal or facultative pathogenic human microflora exometabolites, and available data are very scarce.

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Published data on low-molecular-weight metabolites of anaerobes, volatile short-chain fatty acids (SCFAs) and their effect on human immune responses have been reviewed [1, 2]. Most recent evidence indicates that normal intestinal microbiota may impact immune responses in a positive way. GPR43 receptors for SCFAs were identified in intestinal epithelial cells [3, 4]. SCFAs were established to affect the production of prostaglandins and cytokines, key regulators of cellular immune response [5] via stimulation of neuropeptide PYY and 5-HT release in the ileum and colon through receptors localized in neuroendocrine and intestinal mast cells [6]. At the same time high concentrations of SCFAs, in particular propionate and butyrate, were demonstrated to exhibit toxic effects. Whereas low concentrations of SCFAs delay apoptosis of polymorphonuclear neutrophils and extend their functional activity, higher concentrations of these acids accelerate apoptosis [7]. It has also been reported that butyric and propionic acids produced by bacteroids have an antiphagocytic effect, when at high concentrations [8].

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Cytotoxic action was similarly established in other low-molecular-weight metabolites of anaerobic bacteria at high concentrations, thus not being limited to SCFAs only [9]. Enhanced production of SCFAs, as well as succinic and lactic acids in the presence of excess substrate was found in probiotic bacteria [10, 11], which gains special importance in view of the fact, GPR91 receptors for succinic acid were also identified in mammalian cells, with predominant expression in kidney and intestinal epithelium [12].

There is growing evidence of specific physiological and pathophysiological role for particular bacterial metabolites such as low-molecular-weight phenolic acids. Specific anti-carcinogenic and anti-inflammatory effects of microbial phenolic acids and their impact on the functioning of human colon epithelial cells were explored in a unique study [13]. It was shown that, depending on the chemical structure, microbial phenolic acids produce either antioxidant or toxic action on mitochondria [14].

There are multiple reasons to suggest much greater role of microbial metabolites in human homeostasis, as: they provide the dynamic equilibrium co-existence of microbiota and host organism, being involved in both - maintaining healthy status homeostasis [15] and pathogenesis of infectious [16] and noninfectious diseases [17].

In earlier studies substantial qualitative and quantitative differences for particular microbial metabolites were established in comparative evaluation of blood serum specimens from healthy volunteers and patients with sepsis [17, 18], thus predetermining further studies involving identification of microorganisms producing these metabolites.

In the set of consecutive studies it was established that all most common causative agents of sepsis in humans, both aerobic and facultative anaerobic bacteria, such as staphylococci, enterobacteria, enterococci, and others - produce low-molecular-weight phenolic acids, although with varying intensity [15, 18]. Most of these compounds are known as metabolites of exclusively microbial origin. The extent of colonic exposure to some phenolic compounds, i.e., particular concentrations of phenylacetic, phenylpropionic, p-hydroxyphenylacetic, p-hydroxyphenylpropionic, dihydroxycinnamic, benzoic and other acids was determined in healthy individuals by some researchers [19]. Certain compounds, carboxylic acids in particular, such as succinic, lactic, fumaric and other acids are equally inherent in metabolism of both prokaryotic and eukaryotic cells. Taken into account that more than 95% of gut microflora is represented by true anaerobes, and that interactions of host-microbial metabolites are so poorly known, there is a strong incentive to learn more about metabolic products of anaerobic microorganism. Thus, the main objective of the present study was to determine individual contributions of anaerobes from facultative pathogenic human microflora in the cumulative pool of low-molecular-weight carboxylic and phenolic acids at different durations of incubation.

2. MATERIALS AND METHODS

2.1. Microorganisms

The following strains of Eubacterium lentum ATCC 43055, Bacteroides fragilis ATCC 25285, B. thetaotaomicron ATCC 29741, Clostridium perfringens ATCC 13124, and C. sporogenes ATCC 19404 were used in this in-vitro setting. Additionally Bifidobacterium bifidum and Lactobacillus fermentum strains isolated from bifidumbacterin and Lactobacterin preparations manufactured by the NPO Mikrogen (Ministry of Public Health of the Russian Federation) were included into the study. The purity of studied strains was checked with BD BBL CRYSTAL anaerobe ID system (Beckton Dickinson). Prior to the experiment, all microbial cultures were grown in Schaedler broth (Beckton Dickinson) in glass test tubes in anaerostats placed into a thermostat at 37°C in a standard three-component atmosphere containing 80% N₂, 10% CO₂, and 10% H₂. After incubation, all strains were plated onto Schaedler agarized medium (Beckton Dickinson) at a tenfold dilution and incubated in an anaerostat in a standard atmosphere. Afterwards samples for colony-forming units (CFU) determining procedure were selected. The inoculate (0.1 ml) was placed in glass test tubes containing 8 ml of Schaedler broth (nine samples per strain were examined). All test tubes were placed in an anaerostat. Three samples of each strain were harvested for GC-MS examination after 24, 48, and 96 h of incubation, respectively and CFU determination. After 24–48 h of incubation, the estimated number of microorganisms was 8 x 10⁸–2 x 10⁹ CFU and changed thereafter only insignificantly.

After selecting the samples for CFU determination, all test tubes were centrifuged for 15 min at 1400 g, the supernatant was subjected to gas chromatography–mass spectrometry (GC-MS) analysis to identify metabolites of the strains tested. Schaedler broth,
which was incubated together with test tubes under the same conditions served as a control.

2.2. Preparation of Samples for GC-MS Analysis

The samples of bacterial cultures were centrifuged for 15 min at 800 g; the internal standard for GC-MS analysis (10 μl of ethanol solution of 400 ng D₅-benzoic acid) was introduced into the supernatant (1 ml). Then, each sample was treated with diethyl ether (2 x 1 ml) at pH 2; the ether extract was evaporated to dryness at 40°C. For obtaining trimethylsilyl (TMS) derivatives of compounds, the residue was treated with 20 μl of N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) (Fluka) at 80°C for 15 min. The resulting sample was dissolved in 80 μl of hexane and analyzed by the GC-MS method. Control samples were subjected to similar procedures.

2.3. GC-MS Analysis

Qualitative and quantitative composition of compounds in microbial culture samples was analyzed using an Agilent 6890/5973 gas chromato-mass spectrometer (Agilent Technologies, USA) in the full scan mode [20]. The components were separated by chromatography on a HP5MS quartz capillary column, 0.2 mm in diameter, 25 m in length, with a film thickness of 0.33 μm. The carrier gas was helium; the flow rate was 24 ml/min; the flow rate through the column was 1.2 ml/min. The temperature conditions during analysis were as follows: temperature in the evaporator 280°C; the initial temperature in the column thermostat was held at 80°C for 4 min; then, the temperature was increased to 240°C at a rate of 7°C/min and then to 320°C at a rate of 15°C/min, and was held at this level to the end of the analysis. The volume of a sample injected was 2 μl; the total time of analysis was 35 min; and the time of the detector operation delay was 4 min. A list of compounds detected, retention times, and typical ions in the mass spectra are presented in Table 1. The quantity of a compound was estimated by comparing the area of its peak with the area of the peak for a known amount (0.4 μg) of the standard compound, TMS-derivative of D₅-benzoic acid (retention time 10.45 min), taking its molecular mass into account. Mass spectrometry data for the identification of compounds were obtained using the database NIST-02. The content of the i-th component was calculated by the formula:

\[
c_i = \frac{S_i \cdot M_i \cdot m_i \cdot I_{\text{max}}}{S_0 \cdot M_{\text{std}} \cdot V_s \cdot I_i},
\]

where \( c_i \) is the concentration of the i-th component (μg/ml), \( M_i \) is the molecular mass of the TMS-derivative of the i-th component, \( m_i \) is the mass of the standard introduced (0.4 μg), \( I_{\text{max}} \) is the height of the most intensive peak in the mass spectrum of the i-th component, \( S_i \) is the area of the peak of the i-th component, \( M_{\text{std}} \) is the molecular mass of the TMS-derivative of the standard (231), \( V_s \) is the volume of a sample (1 ml), and \( I_i \) is the height of the major ion in the mass spectrum of the TMS-derivative of the i-th component.

The content of individual compounds in samples was determined in triplicates. The results were recalculated in μg/ml and were well reproducible. The statistical significance of difference was estimated by the Student’s t-tests. The data shown represent the means ± standard error of means (S.E.M.).

3. RESULTS

We examined the content of 32 low-molecular-weight products, among which are lactate and dicarboxylic acids, phenolic acids, and indole derivatives (Table 1). It was found that some low-molecular-weight substances that are believed to be of microbial origin are not the products of anaerobes vital activity, since they were not detected in the nutrient medium after the cultivation. Among these are \( p \)-hydroxybenzoic, \( p \)-hydroxyphenylacetic, \( o \)-hydroxyphenylacetic, \( p \)-hydroxyphenylcinnamic, 1- and 3-indoleacetic acids, as well as the benzyl alcohol and \( N \)-acetyltyrosine. Therefore, these compounds were not included into further consideration. The data are presented only for compounds with documented production into liquid nutrient medium during cultivation for 24, 48, or 96 h.

**Bifidobacteria**

It is known that bifidobacteria actively utilize carbohydrates to form mainly acetic and lactic acids in the molar ratio 3: 2 and do not produce butyric and propionic acids and carbon dioxide [10, 21]. There’s very little published evidence indicating that bifidobacteria can produce phenyl derivatives of lactic acid. It was found in our experiments that, along with lactic acid (61 μg/ml), bifidobacteria produce great amounts of phenyl-lactic (80.3 μg/ml) and \( p \)-hydroxyphenylactic acids (38.23 μg/ml) (Figure 1), and produced amounts of these two phenolic acids exceed the amount of lactic acid.

It was also established that \( B. \) *bifidum* (~0.150 μg/ml) produces 2-ketoglutaric acid in trace amounts.
Table 1: List of Compounds Tested and their Mass-Spectral and Chromatographic Characteristics

<table>
<thead>
<tr>
<th>No.</th>
<th>Compound</th>
<th>Retention Time of TMS-Derivative, Min</th>
<th>Main Ion, m/z (Relative Intensity)*</th>
<th>Additional Ion, m/z</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>D$_2$-Benzoic acid</td>
<td>10.45</td>
<td>184 (999)</td>
<td>110</td>
</tr>
<tr>
<td>2</td>
<td>Phenol</td>
<td>6.92</td>
<td>151 (999)</td>
<td>166</td>
</tr>
<tr>
<td>3</td>
<td>$p$-Cresol</td>
<td>8.45</td>
<td>165 (999)</td>
<td>180</td>
</tr>
<tr>
<td>4</td>
<td>Benzyl alcohol</td>
<td>8.55</td>
<td>91 (999)</td>
<td>165</td>
</tr>
<tr>
<td>5</td>
<td>Benzoic acid</td>
<td>10.51</td>
<td>179 (999)</td>
<td>105</td>
</tr>
<tr>
<td>6</td>
<td>$p$-Hydroxybenzoic acid</td>
<td>18.27</td>
<td>267 (999)</td>
<td>223</td>
</tr>
<tr>
<td>7</td>
<td>2,4- Dihydroxybenzoic acid</td>
<td>20.75</td>
<td>355 (999)</td>
<td>281</td>
</tr>
<tr>
<td>8</td>
<td>3,4- Dihydroxybenzoic acid</td>
<td>20.84</td>
<td>193 (846)</td>
<td>370</td>
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<tr>
<td>9</td>
<td>Phenylacetic acid</td>
<td>11.71</td>
<td>164 (172)</td>
<td>91</td>
</tr>
<tr>
<td>10</td>
<td>$p$-Hydroxyphenylacetic acid</td>
<td>18.04</td>
<td>179 (256)</td>
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<tr>
<td>11</td>
<td>2-Hydroxyphenylacetic acid</td>
<td>15.22</td>
<td>179 (999)</td>
<td>147</td>
</tr>
<tr>
<td>12</td>
<td>Phenylpropionic acid</td>
<td>14.02</td>
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<td>$p$-Hydroxyphenylpropionic acid</td>
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<td>179 (999)</td>
<td>192</td>
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<td>14</td>
<td>Cinnamic acid</td>
<td>16.29</td>
<td>205 (999)</td>
<td>131</td>
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<td>15</td>
<td>$p$-Hydroxyphenylicinnamic acid</td>
<td>22.22</td>
<td>219 (999)</td>
<td>293</td>
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<td>16</td>
<td>Phenyllactic acid</td>
<td>17.12</td>
<td>193 (999)</td>
<td>147</td>
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<tr>
<td>17</td>
<td>$p$-Hydroxyphenyllactic acid</td>
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<td>179 (999)</td>
<td>147</td>
</tr>
<tr>
<td>18</td>
<td>Phenylpyruvic acid</td>
<td>16.85</td>
<td>147 (999)</td>
<td>293</td>
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<td>$p$-Hydroxyphenylpyruvic acid</td>
<td>20.01</td>
<td>147 (999)</td>
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<tr>
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<td>N-Acetyltirosine</td>
<td>23.09</td>
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<td>o-Hydroxyphenylacetic acid</td>
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<tr>
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<td>$p$-Hydroxybenzaldehyde</td>
<td>13.45</td>
<td>179 (999)</td>
<td>194</td>
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<td>23</td>
<td>1-Indolacetic acid</td>
<td>22.20</td>
<td>130 (999)</td>
<td>247</td>
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<tr>
<td>24</td>
<td>3-Indolacetic acid</td>
<td>22.80</td>
<td>202 (999)</td>
<td>319</td>
</tr>
<tr>
<td>25</td>
<td>Succinic acid</td>
<td>12.05</td>
<td>147 (999)</td>
<td>148</td>
</tr>
<tr>
<td>26</td>
<td>Fumaric acid</td>
<td>12.68</td>
<td>245 (999)</td>
<td>147</td>
</tr>
<tr>
<td>27</td>
<td>2-Ketoglutaric acid</td>
<td>17.50</td>
<td>147 (798)</td>
<td>347</td>
</tr>
<tr>
<td>28</td>
<td>2-Hydroxyglutaric acid</td>
<td>16.98</td>
<td>129 (692)</td>
<td>147</td>
</tr>
<tr>
<td>29</td>
<td>2-Hydroxybutyric acid</td>
<td>8.22</td>
<td>131 (784)</td>
<td>147</td>
</tr>
<tr>
<td>30</td>
<td>Lactic acid</td>
<td>6.82</td>
<td>117 (696)</td>
<td>145</td>
</tr>
<tr>
<td>31</td>
<td>Malic acid</td>
<td>15.58</td>
<td>147 (367)</td>
<td>233</td>
</tr>
<tr>
<td>32</td>
<td>Homovanillic acid</td>
<td>20.15</td>
<td>209 (338)</td>
<td>326</td>
</tr>
</tbody>
</table>

*Relative intensity of MS-peaks from NIST-02 Database.

**Lactobacilli**

It is known that almost half the carbon content of final products of fermentation is contained in lactic acid [10, 21]. We also found that, along with lactic acid (~132 μg/ml), lactobacilli produce phenyllactic and $p$-hydroxyphenyllactic acids (about 13.7 μg/ml) (Figure 2); i.e., they produce similar low-molecular-weight substances as bifidobacteria. As in bifidobacteria, the production of metabolites tends to increase significantly up to 48 h of cultivation, and after that remains at achieved level or continues to grow very slowly.

We also found that *L. fermentum* utilized benzoic, 2,4- and 3,4-dihydrobenzoic, and cinnamic acids, diminishing their concentrations to negligible amounts.

**Clostridia**

We examined two species of clostridia *Clostridium perfringens* and *Clostridium sporogenes*. A common
Figure 1: Low-molecular-weight exometabolites of *Bifidobacterium*.
Phenolic acids: phenyllactate (PHL) and *p*-hydroxyphenyllactate (*p*-HPL) (A) and lactate (LAC) (B) identified during a 24–96-h incubation of *B. bifidum*. The concentrations for the zero time correspond to the content of these acids in the starting medium.

Figure 2: Low-molecular-weight exometabolites of *Lactobacillus*.
Phenolic acids: phenyllactate (PHL), and *p*-hydroxyphenyllactate (*p*-HPL) (A) and carboxylic acids (B) identified during a 24–96-h incubation of *L. fermentum*. The concentrations indicated for the zero time correspond to the content of these acids in the starting medium. Lactate (LAC), succinate (SUC), and 2-hydroxybutyrate (2-HBUT).

Feature of these two species is the production of lactic (26.3–48.3 μg/ml) and 2-hydroxybutyric acids (8.5–15.2 μg/ml) (Figure 3). The production of other metabolites varies depending on species. Thus, *C. sporogenes*, as distinct from the other anaerobes studied in this work, produces high levels of phenylpropionic (~182.7 μg/ml) and *p*-hydroxyphenylpropionic acids (~129.5 μg/ml), while *C. perfringens* does not; however, in contrast to *C. sporogenes*, it produces small amounts (2.4 μg/ml) of 2-hydroxyglutaric acid.

Some distinguishing features in the metabolism of two clostridial species were revealed. Thus, the maximum production of exometabolites occurred within the first 24 h of incubation; then the production of exometabolites decreased, partially due to a high metabolic activity with the involvement of own clostridial vital activity products. The production of lactic acid in *C. perfringens* is twice as high as in *C. sporogenes* and is comparable with that in bifidobacteria.

Clostridia produces phenyllactic acid only within the first 24 h of incubation and in insignificant amounts as compared to bifidobacteria and lactobacilli. *C. sporogenes* produces about 10.2 μg/ml of phenyllactic acid, and after achieving this threshold the level of the acid falls to the control value, while *C. perfringens* produces 2.2 μg/ml within 24 h without any substantial changes during further incubation. *C. sporogenes*, as
distinct from *C. perfringens*, produces 4.0 μg/ml of *p*-hydroxyphenyllactic acid, and thereafter the concentration of the acid falls to the control value. We also found that *C. sporogenes*, as distinct from *C. perfringens*, utilized benzoic and 2-hydroxyphenylacetic acid and *p*-hydroxybenzaldehyde, reducing their concentrations to negligible amounts.

**Bacteroids**

We studied two species of bacteroids: *Bacteroides fragilis*, the most common causative agent of nonclostridial anaerobic infections in humans, and *Bacteroides thetaiotaomicron*, a representative of gut microflora of healthy humans. It is known that predominating final fermentation products in bacteroids are acetic and succinic acids; other acids are also produces, though in lesser amounts [21].

Data from our study confirm that both bacteroid species produce considerable amounts of succinic acid (363.6–420.3 μg/ml) (Figure 4). The production of this acid reaches its maximum by 48 h in *B. fragilis* and by 96 h in *B. thetaiotaomicron*. Besides, it was found that *B. fragilis* and *B. thetaiotaomicron* produce fumaric and lactic acids and small amounts of phenylacetic acid, 3.7 and 4.2 μg/ml, respectively, after 48-h incubation. *B. fragilis* production of these acids exceeds production by *B. thetaiotaomicron* by 1.5–2 times. It should also be noted that the concentration of malic acid, fumarate, and lactate after a 48-h incubation of both bacteroid species increases and then falls to control values.

**Eubacteria**

These bacteria showed the highest metabolic activity among the anaerobes studied. It is known that, during the cultivation of eubacteria yields glucose fermentation products, such as acetic, lactic, and succinic acids [21]. In our experiments, the highest metabolic activity in *Eubacterium lentum* was observed by 96 h of incubation with considerable amounts of...
both carboxylic (lactic acid about 197.6 μg/ml, succinic acid 186.4 μg/ml) and phenolic (phenyllactic acid about 137.2 μg/ml, \( p \)-hydroxyphenyllactic acid about 25.8 μg/ml) acids detected (Figure 5). In addition, *E. lentum* produced small amounts of 2-ketoglutaric acid (about 77 ng/ml).

4. DISCUSSION

Based on the data of *in vitro* experiments, the distinguishing features of the metabolism of some anaerobic representatives of human microflora were derived. These finding deserve special attention in view of modern approaches to biological role of low-
molecular-weight compounds of microbial origin. New data on physiological significance of exometabolites, in particular dicarboxylic and phenolic acids, dictate the necessity of new studies in order to elucidate specific patterns of exometabolites production by various microorganisms. We found a species-specific variability in the production of carboxylic and phenolic acids by anaerobic bacteria.

In a rich nutrient Schaedler medium all monocultures of anaerobes were producing lactic acid. The highest level of lactic acid production was seen in Eubacteria, while the lowest - in Clostridia and bacteroids; bifidobacteria and lactobacilli are intermediate producers of lactic acid. Following species-specific features in lactic acid production, all studied anaerobes were arranged in decreasing quantitative lactate production order as follows: E. lentum > L. fermentum > B. bifidum > C. perfringens > B. fragilis > C. sporogenes > B. thetaiotaomicron. Considerable amounts of phenolic acids were produced by all species except for bacteroids. In a similar manner – from highest to lowest phenolic acids producers, anaerobes are arranged as follows: C. sporogenes > E. lentum > B. bifidum > L. fermentum > C. perfringens.

Succinic acid turned to be the predominant final metabolic product in bacteroids. Specific impacts of this metabolite on numerous physiological phenomena in human organism have been established in a number of studies, such as stimulation of angiotensin biosynthesis [12], activation of hemopoiesis [22], and modulation of cell viability [23]. Intracellular accumulation of succinic and fumaric (another metabolite of anaerobes) acids, contributes to switching from aerobic metabolism to anaerobic [24]. Presumably, the overproduction of succinic acid by bacteroids could initiate the development of particular symptoms. In health bacteroids as members of microbial associations are known to behave as friendly mutualists in whatever habitats throughout the human body, i.e. on the mucosa of colon, oral cavity, vagina etc. However, when B. fragilis invades a new tissue as a monoculture, it turns into one of the most aggressive and most common causative agents of nonclostridial anaerobic human infections, such as appendicitis, peritonitis, subdiaphragmatic abscesses, nonclostridial anaerobic infections of soft tissues, abscesses of pararectal fat, infectious endocarditis, etc. [25, 26].

Unlike most other anaerobes, bacteroids are capable of utilizing the products of their own metabolism. Thus, the accumulation of malic and lactic acids during the growth of bacteroids in medium was maximal by 48 h and then decreased many -folds by 96 h. Similar feature – utilization of own metabolic products, e.g., phenyllactic and p-hydroxyphenyllactic acids, was also noted in C. sporogenes. C. sporogenes is also present in the feces of infants and adults (as member of microbial associations). In a monoculture, this microorganism occurs in bacteremia, infectious endocarditis, CNS infections, pleural effusions, abscesses, and other purulent infections [25, 27-29].

Bifido- and lactobacteria also produced phenyllactic and p-hydroxyphenyllactic acids, although there was a gradual accumulation of these products in cultivation medium between 24 and 96 h, without any evident signs of utilization. Bifidobacteria are found normally in the oral cavity, colon, and female reproductive tract, and their high colonic concentration is viewed as indicator of health, particularly in infants, thus Bifidobacteria are considered to be nonpathogenic and friendly useful for humans. Lactobacilli are also present at high concentrations in similar habitats and are thought not to be involved in pathological processes. It is generally accepted that accumulation of lactic acid during bifido- and lactobacteria growth inhibits their further propagation. Presented findings allow to suggest some correlation between pathogenicity potential and the ability to utilize the final products of own metabolism, i.e. of carboxylic acids by bacteroids and of phenolic acids by clostridia, which is not the case for bifido- and lactobacteria, unable to use their final metabolic products.

The greatest diversity of metabolic products was found in E. lentum; by 96 h of growth, the utilization of neither carboxylic nor phenolic acids occurred. The production of lactic and phenyllactic acids in this microorganism is comparable to that in lacto- and bifidobacteria taken together. The production of p-hydroxyphenyllactic acid in eubacteria is also comparable to that in lacto- and bifidobacteria. Along with bacteroids, E. lentum participates in the formation of succinic acid pool. Besides, E. lentum produces 2 species-unique 2-hydroxyhexanionic and 2-hydroxy-3-methylbutanionic acids, not found in other microorganisms studied. The biological implications of such very high metabolic activity of eubacteria is not yet clear. However, important to indicate very common presence of eubacteria in multiple pathological processes not directly related to infection. Thus, there is definite evidence of eubacteria involvement in metabolism of steroids [30, 31]. Besides, these bacteria degrade
hydroxybutyric acid is inherent to the organism of the host. The production of 2-acid of microbial origin specific regulatory functions in findings and published data allow to attribute to succinic documented in ischemic tissues perfusates [40]. Our receptor [12] and much higher than the concentrations exceeds significantly the binding constant for the incubation can overcome the 3 mM level, which this metabolite in culture medium during prolonged unique producers of succinic acid. Concentrations of production of anaerobes -[malic acid} – for Fusobacterium species produce 2-hydroxybutyric acid as an exometabolite [43]. The accumulation of 2-hydroxyglutaric acid is characteristic for some neurometabolic diseases [44, 45].

SUMMARY

Thus, the evidence obtained supports the phenomenon of species-specific production of low-molecular-weight metabolites by anaerobes of the human microflora. In a healthy state a close cohabitation of different species within gut flora provides balanced metabolic process up to final products, which are not toxic or harmful to the host thus maintaining mutualism or symbiosis.

Thereby, provided data can be useful in the development of novel probiotic preparations and well as in qualitative and quantitative evaluation of microbial associations in norm and pathology.

Presumably, with increasing levels of exometabolites their impact on various bodily organs and systems will also be growing. It has been demonstrated in earlier studies [17, 18, 46], that levels of some phenolic acids in critically ill patients (especially in sepsis) significantly exceed established physiological thresholds. It is obvious that such novel approaches and their potential capacity will not be ignored in the development in new diagnostic modalities of the future, targeted not only at the detection of particular components of microbial associations, but also to comprehensive evaluation of associated metabolic shifts. Microbial metabolites can also find their use in the future sophisticated therapy of different pathological conditions, as deviation from health in many cases are associated with shifts in microbiome and metabolite environments.

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REFERENCES


[38] Li XN, Panikh S, Shu Q, et al. Phenylbutyrate and phenylethylacetate induce differentiation and inhibit proliferation...
Anaerobic Microorganisms from Human Microbiota Produce Global Journal of Pathology and Microbiology, 2013, Vol. 1, No. 2

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http://dx.doi.org/10.1158/1078-0432.CCR-0747-3

http://dx.doi.org/10.1016/j.jnutbio.2007.08.002

http://dx.doi.org/10.1016/j.jhep.2007.03.016

http://dx.doi.org/10.1590/S0100-879X2001000500010


http://dx.doi.org/10.1016/S0748-7456(97)00245-x


http://dx.doi.org/10.1007/s10545-007-0487-0


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