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The role of probiotic microorganisms in the control of health and fertility of soil

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Abstract

The method of microbial diagnostic based on gas chromatography – mass spectrometry of fatty acids, hydroxy acids and fatty aldehydes – was used for the study of the soil microbial community. Mass spectrometry of microbial markers (MSMM) method permits simultaneous determination of more than a hundred microbial fatty acids *in situ* in clinical, biotechnological or environmental samples without precultivation and biochemical test materials and primers. Some beneficial probiotic bacteria in soils microbial community such as *Acetobacter diazotroficus*, *Bacillus* sp., *Bifidobacterium* sp., *Clostridium* spp., *Lactobacillus* sp., *Rhodococcus* sp. and other are discussed in this article. Soil conditions *in situ* as well as physiological features of these microorganisms allows to present the following trophic chain: hydrocarbons (plant's exudates and residues) → products of their oxidation by actinobacteria (nocardia, rhodococci or mycobacteria) → free aminoacids and biomass proteins (metabolism products of nocardia, rhodococci or mycobacteria) → products of their fermentation by clostridia (or propionibacteria)→ volatile fatty acids (acetic, propionic, isobutiric, butyric et all.) and H_2 and CO_2. This syntrophic association may be the basis for agricultural ecosystem and can support the compounds of soil's health and fertility production. Questions concerning the potential effects on soil biofertilization (humus preservation, formation of water-stable aggregates) in agricultural systems are considered.

Keywords: probiotic bacteria, soil, biofertilization, fatty hydroxy acids biomarkers.

Introduction

The soil of agrocenosis is inhabited by a complex community of microorganisms, also referred to as the microbiota, which are believed to have an important role in health and fertility of soils. This concept came to environment researches from medical practice. A probiotic has been defined as "a preparation or a product containing viable, defined microorganisms in sufficient numbers, which alter the microflora in a compartment of the host and by that exert beneficial health effect in this host" [9]. A prebiotic has recently been (re)defined as "a selectively fermented ingredient that allows specific changes, both in the composition and/or activity in the gastrointestinal microbiota that confers benefits upon host well-being and health" [7]. Finally, a synbiotic is the combination of a probiotic and a prebiotic [4].

Anaerobic bacteria of g.g. *Bifidobacterium* and *Lactobacillus* as the most known genus with pro-biotic properties for the human health [3;9] were the first ones in our consideration of bacterial communities of soils. Carbohydrate degradation has been extensively studied in a variety of different *Bifidobacterium* species [11] and selected

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exopolysaccharide-producing strains of lactic acid bacteria [6] for definition of their role in synthesis of (potential) prebiotics. In environment, including in the soil, a bifidobacteria are more widespread, than lactobacilli since these bacteria prefer neutral pH values for the metabolic activity and as an organic substrate – easily oxidizing carbohydrates. *Bifidobacterium* is a "useful" genus for the soils, thanks to its ability to producing of a number of enzymes, amino acids and regulators of plants' growth [1]. The species of bacteria such as *Bifidobacterium longum* [13], *Lactobacillus rhamnosus* and other lactic acid bacteria [6] are known as bacteria bringing benefit thanks to the metabolites. These anaerobic species of bacteria occur of various habitats including in the soil [12], but their probiotic effects it isn't discussed.

In this context the purpose of this article was consideration of aerobic and anaerobic structures of microbic cenosis of different soils types with allocation of bacteria - probiotics and their contribution in the preservation of soils' Corg in agrocenosis and formation of water-stable soil aggregates.

Material and Methods

Characteristics of 326 soil samples of different types were analyzed. Podzols, soddy podzols and sandy soddy podsols, meadow-gley podsols, meadow-brown, brown forest, gray forest, podzolic, chernozems leached and typical chernozems (middle- and south taiga, forest-steppe, steppe and south steppe zones) and chestnut soils were studied in the territory of the Russian Federation and agricultural alluvial calcareous soils (Calcic Fluvisols Oxyaquic) of Konya province (Çumra area) – in the territory of Turkey.

The samples of soils were selected in different physiographic conditions taking into account their anthropogenous use: virgin and bare fallow, a deposit (are excluded from crop rotations of 5 and more years) and an arable land (the soils which are in intensive agricultural use – grain crop rotations and maize monoculture).

The mixed samples were prepared in field conditions from 5-7 separate samples of a soils layer of 0-10 cm. Selection was made in chessboard order the soil drill by diameter of 25 mm, the volume of 200-250 cm^3.The mixed sample dried at the room temperature and, having been placed in a plastic bag, were stored to the analysis in a deep freeze. The determination of organic substance content and soil pH were carried out by standard techniques.

The gas chromatography – mass spectrometry (GC-MS) analysis was provided by chromate-mass-spectrometer AT-5973 D (Agillent Technologies, USA) detection with a special program was designed to permit selective accumulation of specific ion signals from microorganism marker compounds. The areas of markers peaks were integrated automatically (mass-phragmentography) and supervised manually under regular programs of the device using internal standard. Then these data were input into account program prepared in electronic EXCEL tables. The method allows defining the bacteria species of number more than 10^3-10^4 cells/g of soil. The methodology of a molecular method of gas chromatography-mass spectrometry (GC-MS) was in detail presented by [5, 8].

Anaerobic components of microbial communities were studied by cultivation on the selective medium BACTEC PLAS in anaerobic bottles (USA) in which the gas phase consisted of carbon dioxide and molecular nitrogen in the ratio 1:1. Further the structure of anaerobic community was studied by the gas chromatography (GC) method, and volatile fatty acids of anaerobic bacteria were studied on the gas chromatograph GC-14 A ("Shimadzu», Japan).

Systematization and generalization of an experimental material was executed by statistical methods in MS Excel.

Results and Discussion

Data about soils of farmland (the arable land, the bare fallow, the deposit and the virgin) for studying of the general tendencies conditionally are divided into two groups: soils with < 6,9 (podzols, soddy podzols and sandy soddy podsols, meadow-gley podsols); II - soils with pH > 7,0 (chernozem leached, the chestnut soil, etc.). The analysis of data showed that bifidobacteria were present at the quantities more 5% in arable land and deposit. Quantities of bacteria of this genus is twice lower in the virgin, and in the bare fallow its presence is noted only in soils with pH< 6,9. In agricultural alluvial calcareous soils (Calcic Fluvisols Oxyaquic) *Bifidobacterium* sp. are defined under *Beta vulgaris saccharifera*, in number from 0,5 to 2,2% at the depth from 10-20 cm up to 50-60 cm, respectively. Bacteria of g. *Lactobacillus* sp. were identified only in the acid soils of an arable land and bare fallow

(Table 1). In separate research of poor sandy soddy podsols (the Humus = 0,8%) lactobacilli dominate (13%) in NPK + lupine, and a bifidobacterium – NPK + straw (17%). *Bifidobacteria* and *Lactobacillus* were not observed in concentration higher than 10^3 after barley cultivation on these soils but bifidobacteria dominated (12%) after the following culture in a crop rotation (*Zea mays* L.). Lactobacilli weren't identified in the rhizosphere soil of the maize. However, in the rhizoplane of the maize which was grown up in a long-term monoculture (65 years) on the chernozem leached, quantity of lactobacilli reached 2,6% whereas bifidobacteria – were ten times less. Thus the quantity of specific bacteria depends on the crop cultivated in the rotation, i.e. from roots' exudates which initiate and modulate dialogue between roots and these soil microbes.

Table 1. Quantity of *Bifidobacterium* sp. and *Lactobacillus* sp. as a part of microorganisms' community* soils of different agricultural uses, %

Bacteria	An arable land		Bare fallow		Deposit		Virgin	
	I	II	I	II	I	II	I	II
Bifidobacterium sp.	5,4	7,3	3,59	---	6,4	5,1	2,6	4,3
Lactobacillus sp	1,4	---	0,1	---	---	---	---	---

* defined bacteria and micromycetes; I – for soils with < 6,9 (are podzols, soddy podzols and sandy soddy podsols, meadow-gley podsols); II - for soils with pH > 7,0 (chernozem leached, the chestnut soil, etc.) --- - the species isn't identified

The quantity of these species of probiotics didn't correlate with the quantity of Corg. in the studied soils. However the content of Corg correlated with the total number of microorganisms in all samples irrespective of type of the soil of r^2=0,8-1,0 (it is noted, direct dependence in soils pH less than 6,9 and inverse relationship at pH more than 7,0). Significant positive correlative dependences were established between number of an anaerobic bacterium *Clostridium* sp. and humus content (r^2 = 0,58), and also of aerobic actinobacteria *Nocardia* sp. (r^2 = 0,59), which capable to decomposition of polymeric carbohydrates in soils. Clostridia dominated in different types of soils, but in a higher quantity in that ones which weren't exposed to intensive agricultural use. *Rhodococcus* dominated in different types of soils, including the arable land. Associative diazotroph *Acetobacter diazotrophicus* dominated in the arable land. The quantity of nocardia reached dominating sizes only in the deposit soils (Table 2). Aerobic bacteria – *Rhodococcus* both *Mycobacteria* and anaerobic – *Propionobacteria* dominated in the community of bacteria in the long-term field experiment (65 years) of maize monoculture. Corg in the soil didn't change for this long-term of cultivation.

Table 2. Quantity of *Clostridium pasteurianum* and *Nocardia* sp. as a part of microorganisms' community soils of different agricultural uses, %

Bacteria	An arable land		Bare fallow		Deposit		Virgin	
	I	II	I	II	I	II	I	II
Clostridium pasteurianum	2	3	9,8	10	13	3,3	7,8	2,6
Nocardia sp.	0,5	0	3,3	---	0,7	13	0,7	0,5
Rhodococcus rhodochrous	18	---	13	20	8	---	32	6,8
Acetobacter diazotrophicus	9,7	9	4,6	4,1	4,1	3	3,5	7,7

Apparently, bacteria are united in aerobic and anaerobic consortium in which are in trophic interrelation in chain: hydrocarbons (plant's exudates and residues) → products of their oxidation by actinobacteria (nocardia, rhodococci or mycobacteria) → free aminoacids and biomass proteins (metabolism products of nocardia, rhodococci or mycobacteria) → products of their fermentation by clostridia (or propionibacteria) such as volatile fatty acids and H_2 and CO_2. It promotes creation of oxidation-reduction conditions for formation of humus substances in different types of soils and to its preservation at the expense of enough of acetic and propionic acid for further assimilation of these acids by other aerobic subdominants acetobacteria in particular. The autochthonic organic substance (humus) thus isn't exposed to degradation. Thus the protective role of soil microbiota is developed by preservation of the major factor of its fertility – quantity of humus.

The quantity of water-stable units is one of the most important characteristics of soils for its fertility quantity differences in taxonomical structure between microbic communities that inhabit aggregates of different size and the difference from the bulk soil were shown. On the total number of microorganisms in community of units of essential distinctions wasn't noted (Table 3).

Table 3. Structure of bacteria community of units with a diameter 0.002 and 1-2 mm from the bulk soil (Kursk' chernozem)

Bacteria, cells/g × 10^6	Nucleus, <0,002	Nucleus, 1-2	Soil
Acetobacter diazotrophicus	9,71	7,54	18,61
Actinomadura roseola	4,21	4,48	3,50
Agrobacterium radiobacter	14,86	17,62	1,77
Bacillus sp.	3,54	6,40	12,63
Bacillus subtilis	1,54	2,54	3,21
Bacteroides hypermegas	0,09	0,00	0,20
Bacteroides ruminicola	0,78	0,84	2,20
B. fragilis	0,00	0,14	0,00
Bifidobacterium sp.	12,27	8,66	2,17
Butyrivibrio 1-4-11	1,18	1,21	1,16
Butyrivibrio 7S-14-3	30,38	27,47	18,26
Clostridium difficile	0,28	0,00	0,00
C. propionicum	7,06	1,52	0,96
C.pasteurianum	10,09	9,45	0,00
C.perfringens	0,06	0,05	0,20
Corynebacterium sp.	1,20	1,65	2,41
Cytophaga sp.	0,74	0,58	3,45
Desulfovibrio sp.	0,96	0,00	0,00
Eubacterium lentum	10,00	5,37	6,83
Eubacterium sp.	0,04	0,05	0,04
FeRed	2,52	0,09	0,00
Methylococcus sp.	0,00	0,99	4,97
Micrococcus sp.	10,83	12,95	12,38
Mycobacterium sp.	2,64	1,89	0,00
Nitrobacter sp.	7,22	6,14	14,76
Nocardia carnea	2,03	3,70	2,24
Nocardia sp.	157,87	8,54	121,17
Pseudomonas fluorescens	2,09	1,13	6,38
P. vesicularis	0,34	0,16	2,27
P.putida	0,71	0,57	6,34
Propionibacterium sp.	8,40	12,91	5,99
Pseudonocardia sp.	3,87	4,22	3,75
Rhodococcus terrae	3,50	10,10	15,84
R.equi	3,65	5,42	3,99
Ruminococcus sp.	101,97	76,80	89,34
Sphingobacterium spiritovorum	1,84	1,05	3,87
Sphingomonas capsulata	0,87	0,55	2,31
Staphylococcus sp.	3,73	3,61	3,00
Streptomyces sp.	18,00	20,34	21,03
Wolinella sp.	0,00	0,00	2,03
Xanthomonas sp.	0,92	0,86	6,17
Total number	442	284	406

The number and the variety of the anaerobic species which concentrate in units were approximately twice more in comparison with the soil. It is more characteristic for units of the soil of diameter < 0,002 mm. Additional four species of anaerobic bacteria were observed in the nucleus of water-stable units of such size which aren't found in a soil sample in concentration higher than 10^3 cells/g of a substratum. These are two species of clostridia – *Clostridium difficile* and *C. pasteurianum*, sulphate- reducing bacterium *Desulfovibrio* sp. and also the anaerobic Gr $^-$ iron reducing bacteria described earlier as a Gr $^-$ species, reducing of Fe (III) in kaolin (5). The maintenance of one more species of clostridia –– is 7 times higher. As a result, concentration of clostridia in water-stable units with a diameter <0,002 mm exceeded that in the soil by 17 times as a whole. Only two additional anaerobic species of bacteria is revealed (there is no sulphate-reducing *Desulfovibrio* sp. and *Clostridium difficile*) for units with a diameter of 1-2 mm. As a result the number of anaerobic clostridia in these units was 6,5 times higher in comparison with their quantity in the soil *Bifidobacterium* sp. is 6 times higher in a nucleus of the unit of smaller

diameter, than in the soil, and is 4 times higher in the units of the bigger size. High needs of bifidobacteria in carbohydrates which these bacteria decompose with formation, except milk acid, CO_2, ethanol and/or acetic acid and the high contents (10%) CO_2 demands for growth in the medium are known [1]. Thus, considerable concentration of the anaerobic bacteria in nucleus of water-stable units which take part in anoxic stages of carbon, nitrogen, sulfur and the iron cycles providing the trophic and energetic relationship and formation of specific organic substances was shown.

The clostridia, which are producers of various volatile fatty acids (VFA), apparently, have the main role in formation of specific organic substance. The increased concentration of a sulphate-reducing *Desulfovibrio* sp. in the units of <0,002 ($9,0 \times 10^5$ cell /g) in comparison with the soil assumes its participation in anaerobic oxidation of metabolites' clostridia. Absence of *Desulfovibrio* in the units of 1-2 mm testifies that there were no thermodynamic conditions for anaerobic process of a sulphate-reduction in this microcosm yet. The quantity of iron reducing bacteria in units of <0,002 mm it was rather great ($25,2\times 10^5$ cells/g). The quantity of this species decreases in 25 times with increase of the particles size to 1-2 mm. The iron reducing bacteria participate in the process of anaerobic reduction of Fe (III) and, therefore, in change of Fe (III)/Fe (II) ratio and oxidation-reduction conditions in the aggregates. So reorganization of the metabolic status of microbial community towards domination of anoxic condition became apparent. Besides, iron as the polyvalent cation, can cause a recharge of a dispersed particles' surface, creating special conditions of their coagulation. Therefore, the value of iron reducing bacteria in formation of nucleus of water-stable aggregate can be shown both in the metabolic relation of functioning of a microbiocenosis, and in formation of physical and chemical conditions of aggregate stability.

Mycobacterium sp. from aerobic species was found in number of $3\cdot\times10^6$ cells/g in water-stable aggregate and wasn't identified in the soil. This bacterium can form a pseudo-mycelium with a growth on firm substrate, possesses hydrophobic mycolic acids and by that increases water-stable nucleus of an aggregates and its resistance to processing by ultrasound. Essential excess of number (in 7-8 times) was noted also for an aerobic bacterium *Agrobacterim radiobacter* (14,9 -17,6 $\times10^6$ cells/g) in nucleus aggregates in comparison with 1,8 $\times10^6$ cells/g in soil. It is known that for this culture growth on media with carbohydrates is accompanied by plentiful formation of extra cellular polycarbohydrate slime. Apparently, the biogenesis gel of this culture participates in stabilization process of microbic consortium structure and aggregate fraction. It is possible to say that pro-biotic properties of these anaerobic (*Clostridium difficile*, *C. pasteurianum*, *C. propionicum*. *Desulfovibrio* sp. and iron reducing bacteria) and aerobic (*Mycobacterium* sp., .*Agrobacterim radiobacter*) species consist in increase of aggregate stability of the soil.

Further biochemical opportunities of anaerobic consortium of the unit with the diameter of 1-2 mm were studied. The analysis of the structure of anaerobic community showed significant growth of such species: *Eubacterium nodatum*, *E. moniliforme*, *Clostridium* spp., *Enterobacter aerogenes*(10^9 cells/g) and *Fusobacterium*, *Enterococcus*, *Ruminococcus* (10^8 cells/g).

Metabolites of anaerobic consortium contained 8 types of VFA among which unbranched acids dominate: bytiric (3,8 mM/ml), propionic (1,8 mM/ml) and acetic (2,4 mM/ml) acids – products of carbohydrates fermentation by primary fermentative bacteria. Isovaleric acid also was produced in rather large number (1,0 mM/ml) - product of an anaerobic fermentation of proteins and aminoacids.

Secondary anaerobic bacteria (H_2 utilizers) use metabolites of primary anaerobic as electron donors in an oxidation-reduction chain. That is, it is possible to assume that in the energetic metabolism anaerobic ecosystems of aggregate nucleus are based on hydrogen. A layered microbial architecture as presented in Figure 1 has been proposed when carbohydrates are the primary substrate.

At infringement of soil structures an oriented movement of bacteria in a direction of a gradient pH and also electron donors and acceptors changes take place. Bacteria cells are capable to measurement of these gradients, "remember" what level is ecologically optimum for their ability to live and purposefully aspire to returning in the adapted habitats. Apparently, a substratum - substratum signals of their metabolites also play great value in this process.

Conclusion

Anaerobic bacteria of g.g. *Bifidobacterium* and *Lactobacillus* as the most known genera with pro-biotic properties for the human health didn't correlate with quantity of Corg. in the studied soils. Bacteria of g. *Lactobacillus* sp. were identified only in acid soils of an arable land and bare fallow. In separate research on poor

sandy soddy podsols (the humus = 0,8%) lactobacilli dominated (13%) in the variant of NPK + lupine. The bifidobacteria were present at the quantities more 5% in arable land and deposit. Lactobacilli weren't identified in the rhizosphere soil of the maize. However, in the rhizoplane of the maize quantity of lactobacilli reached 2,6% whereas bifidobacteria – were ten times less. Thus the quantity of specific bacteria depended on the crop cultivated in the rotation: from roots' exudates.

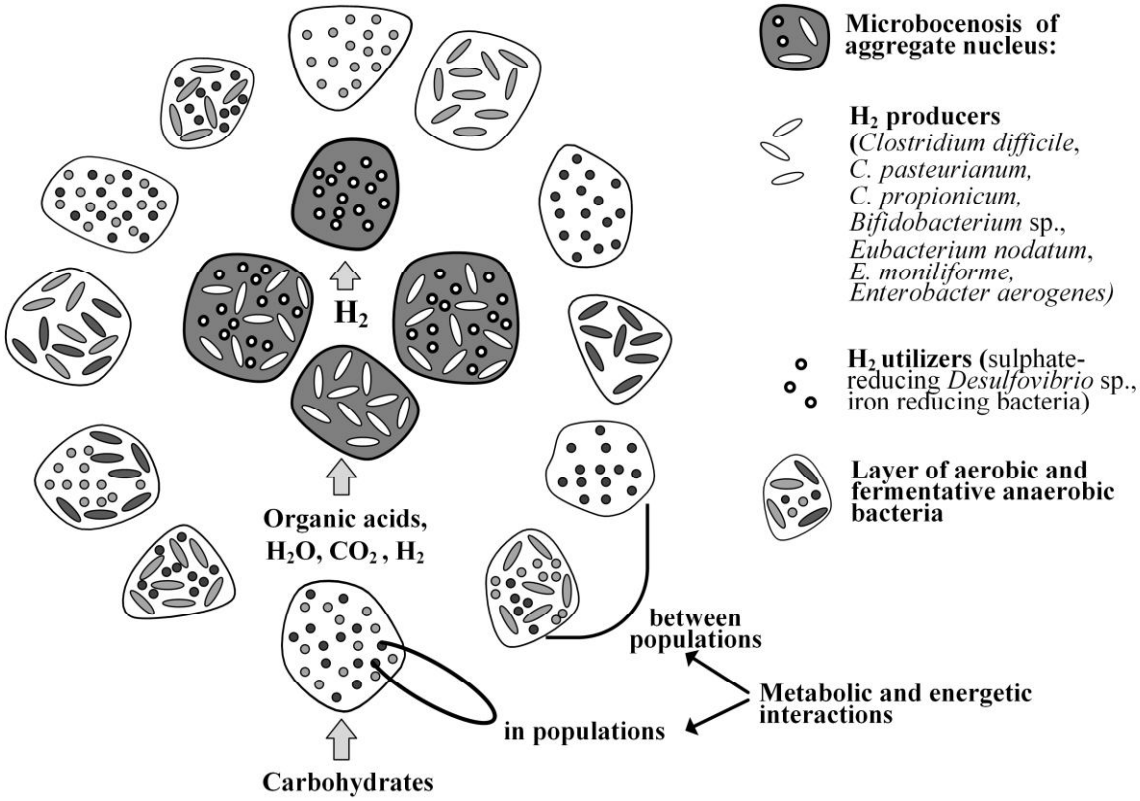

Figure 1. Schematic representation of architecture of water-stable aggregate

Associative diazotroph *Acetobacter diazotrophicus* dominated in the arable land. The quantity of nocardia reached dominating sizes only in the deposit soils. Aerobic bacteria – *Rhodococcus* both *Mycobacteria* and anaerobic – *Propionobacteria* dominated in the community of bacteria in a long-term field experiment (65 years) of maize monoculture. Corg in the soil didn't change for this long-term cultivation. Significant positive correlative dependences were established between number of anaerobic bacteria *Clostridium* sp. and humus content (r^2 = 0,58), and also `of aerobic actinobacteria *Nocardia* sp. (r^2 = 0,59).

Apparently, bacteria are united in aerobic and anaerobic consortium in which the following products are linked by trophic interrelation in the chain: hydrocarbons (plant's exudates and residues) → products of their oxidation by actinobacteria (nocardia, rhodococci or mycobacteria) → free aminoacids and biomass proteins (metabolism products of nocardia, rhodococci or mycobacteria) → products of their fermentation by clostridia (or propionibacteria) of volatile fatty acids and H_2 and CO_2. It promotes creation of oxidation-reduction conditions for formation of humus substances in soils of different types and for humus preservation at the expense of sufficient acetic and propionic acid content for further assimilation of these acids by other aerobic subdominants, by acetobacteria in particular. The autochthonic organic substance (humus) thus isn't exposed to degradation. So the protective role of a microbiota of the soil - the preservation of a major factor of its fertility – high quantities of humus

Considerable concentration of the anaerobic bacteria which take part in anoxic stages of the cycle of carbon, nitrogen, sulfur and iron providing the trophic and energetic relationship and formation of specific organic substance in nucleus of water-stable units was shown. The clostridia, which are producers of various volatile fatty acids (VFA), apparently, have the main role in formation of specific organic substance. Pro-biotic properties

of anaerobic (*Clostridium difficile*, *C. pasteurianum*, *C. propionicum*. *Desulfovibrio* sp. and iron reducing) bacteria and aerobic (*Mycobacterium* sp., .*Agrobacterim radiobacter*) species consist in increase of aggregate stability of the soil. *Bifidobacterium* sp. in this anaerobic consortium participates in creation of specific organic substance (milk acid, ethanol and/or acetic acid), and also CO_2. Pro-biotic role of this species in forming of soil nucleus aggregates was revealed.

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References

1. Biology of the prokaryotes.1999.Lengeler. J. W, Drews G., Schlegel H.G. (Eds.) Georg Thieme Verlag .Thieme,Stuttgart, New York, p.p.371-375
2. Canfield, D.E., Rosing, M.T., Bjerrum, C. 2006. Early anaerobic metabolisms. Phil. Trans. R. Soc. B. 361.1819–1836.
3. Gibson G.R, Roberfroid MB. 1995. Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. J. Nutri. 125.1401–1412.
4. Gibson, G. R., Probert, H. M., Loo J. Van, Rastall R. A., Roberfroid, M. B. 2004. Dietary modulation of the human colonic microbiota: updating the concept of prebiotics. Nutr. Res. Rev.17. 259-275.
5. Osipov, G.A., Turova, E.S. 1997. Studying species composition of microbial communities with the use of gas chromatography-mass spectrometry. Microbial community of kaolin. FEMS Microbiol Rev. 20.437-446.
6. Pepe O., Ventorino V., Cavella S., Fagnano M., Brugno R. 2013. Prebiotic Content of Bread Prepared with Flour from Immature Wheat Grain and Selected Dextran-Producing Lactic Acid Bacteria. Appl. Environ. Microbiol. 79. 12. 3779-3785.
7. Roberfroid, M. 2007. Prebiotics: the concept revisited. J. Nutr.137. 830-837.
8. Shekhovtsova, N.V., Osipov, G.A., Verkhovtseva, N.V., Pevzner, L.A. 2003. Analysis of lipid biomarkers in rocks of the Archean crystalline basement. In: Proceedings of SPIE. 4939. 160-168.
9. Schley PD, Field CJ. 2002. The immune-enhancing effects of dietary fibres and prebiotics. Br. J. Nutr. 87.221–230.).
10. Schrezenmeir, J., de Vrese, M. 2001. Probiotics, prebiotics, and synbiotics–approaching a definition. Am. J. Clin. Nutr.73. 361-364.
11. van den Broek, L. A., Hinz, S. W., Beldman, G., Vincken, J. P., Voragen, A. G. 2008.*Bifidobacterium* carbohydrases—their role in breakdown and synthesis of (potential) prebiotics. Mol. Nutr. Food Res. 52.146-163.
12. Veith B, Herzberg C, Steckel S, Feesche J, Maurer KH, Ehrenreich P, Baumer, S, Henne A, Liesegang H, Merkl R. 2004. The complete genome sequence of *Bacillus licheniformis* DSM13, an organism with great industrial potential. J Mol Microbiol Biotechnol. 7. 204–211.
13. Viborg, A.H, Katayama, T., Hachem, M.A., Andersen, M. CF., Nishimoto, M., Clausen, M. H., Urashima, T., Svensson B., Kitaoka, M. 2014. Distinct substrate specificities of three glycoside hydrolase family 42 β-galactosidases from *Bifidobacterium longum* subsp. *infantis* ATCC 15697. Glycobiology.24. 208 - 216.