Probiotics for Environmental Sanitation: Goals and Examples

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Abstract

Bacterial probiotics are well-known paramedical tools to improve health and nutrition. These traditional applications are based on abilities of the probiotics to degrade complicated organic compounds and to suppress hazardous microorganisms in the intestinal tract. During the last decades, probiotic bacteria have been used also for sanitation of water for aquacultures. The chapter surveys possible application of probiotics in the environmental remediation. More specifically, utilization and sanitation of terrestrial wastes, especially manure and farm litter, with probiotic bacilli are discussed.

Keywords

Probiotic • Bacteria • Bacilli • Manuring • Remediation • Sanitation

1 Introduction

It is well known that the improvement of modern technologies results in an increase of production of wastes from all the industrial fields, including agriculture and farming.

In particular, cattle breeding, swine breeding, poultry, and any animal husbandry are related with increasing output of pollutants which include manure, guano, and bedding and litter mixed with the animal excrements. All these wastes contain organic matter enriched with biogenic elements (P, N, etc.), and as well, usually these pollutants are infected with some harmful enteric bacteria from the digestive tract of the animals. Historically, traditions of the European agriculture suggested utilization of the wastes for manuring, i.e., fertilization of soils with rich organic matter of dung and litter. Old traditional methods of natural digestion or composting proved to be insufficient, since it does not keep pace with the modern industrial scale of accumulation of the wastes. Increasing bulk of accumulating manure and litter threatens environment in total, and what is more, they serve as a possible source of spreading enteric bacteria (Dolgov 1984; Kisil 2007; Lysenko 2007).

Some modern biotechnologies suggested solution of the mentioned problems that involve new bacterial strains and/or microbial associations which are able to accelerate the organic matter recycling (Kovalev and Glazkov 1989; Zenikov 2006). One of these approaches consists of a microbial methane-containing gas mixture, known as biogas that is used in the same households as a source of energy (Ilyin 2005; Mironov et al. 2006). Another approach is related to the selection of microbial agents to speed up the decomposition process (Podgornov et al. 2009).

An additional problem in the utilization of these wastes is their sanitation. Manure and litter can contain not only enteric bacteria but, as well, some other hazardous groups of microorganisms. Natural digestion of the wastes does not provide a guaranty of disinfection. Introduction of antibiotics in the environment to suppress harmful microorganisms is not rational, and in some countries it is prohibited by laws and regulations to avoid appearance of

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antibiotic-resistant bacterial forms in the environment (Kümmerer 2008; De Knecht 2010). On the other hand, chemical treatment of waste is expensive, it does not provide complete disinfection, and, what is more, it prevents an active natural degradation of wastes (Gehan et al. 2009). Thus, the most promising utilization of organic pollutants combined with sanitation could be provided by those microbial strains which inhibit pathogenic bacteria. In this regard, significant potential interest is the search of helpful strains among probiotic cultures because of their ability both to degrade complex organic compounds and to suppress hazardous intestinal organisms (Li and Ni 2000).

Thus, study on utilization of litter and sanitation of polluted environment by bacterial probiotic strains is a modern upcoming trend of environmental microbiology and biotechnology.

2 Bacterial Probiotics for Nutrition and Sanitation

2.1 Definition of the Term

By the end of the nineteenth century, physicians and biologists proved that the intestinal microorganisms and their balance determine not only the presence/absence of infectious intestinal diseases but also the total human health. In recognition of the importance of the intestinal microbiota, there was even a popular term to describe it as an additional "invisible organ of the body" (Bogadelnikov and Vyaltseva 2011). In fact, different kinds of intestinal microorganisms may produce both positive and negative effects on metabolic processes in the host organism, and thus composition and stability of the microbiota shall determine health and growth of the host organism as a whole. The importance of the intestinal microbiota has led to artificial direct introduction of additional helpful microorganisms into the alimentary system. The cure with microbial cultures is especially important in case when a host organism is in short or limited with usual components of its intestinal microbiota (e.g., the host is a gnotobiont (Tlaskalová-Hogenová et al. 2011)). The intestinal microorganisms can be refunded with the help of additional bacterial mixtures ("bacterial cocktails"), the composition of which is selected by medical microbiologists for each patient individually. Health and weight of people and animals are highly dependent on the gut bacteria. A human being himself/herself cannot break down even cellulose of cucumber or chitin of mushrooms – all these processes are accomplished by intestinal bacteria. It was shown that intestinal bacteria affect also the nerve system and mood (Bravoa et al. 2011).

Microorganisms of intestinal biota are responsible for several different activities, namely:

1. Splitting of complex chemical compounds, foremost – ones which are not digestible or destroyed by the host – to easily digestible compounds.
2. Synthesis of vitamins, essential amino acids, and other valuable compounds.
3. Providing a constant presence of defensive bacteria which suppress any incoming dangerous pathogenic microorganisms.
4. Suppression of pathogenic forms can be carried out both directly by the intestinal microbiota and by strengthening the host immune system. The last thesis includes data showing that intestinal bacteria can enter the bloodstream and transferred inside the host organism. In this case, they can increase the total immune defense with no manifestation of a disease (Berdichevsky 2001).

Live microbial cultures, which are supplemented to enhance the intestinal microbiota in respect of the mentioned four activities, are called "probiotic(s)." Perhaps, the term "probiotic" in the literal sense of this word ("for life") is not the best choice, but it is accepted for decades (Parker 1974). The current variations in the definitions of probiotics depend on the position of the expert and the specific intended use: nutrition and feeding or medical purposes.

Accordingly, these are the following generalized definitions of the term "probiotic":

- Food supplement containing live bacteria or yeast that completes normal gastrointestinal microflora, given especially after its depletion caused by preliminary infection or effect of an antibiotic drug (American Heritage Dictionaries 2007)
- Useful bacteria used to colonize the intestinal tract (Jonas 2005)

Thus, probiotics are generally considered as a therapeutic tool to enhance the normal gastrointestinal microbiota. In livestock, they are used for the same purpose, but keep in mind primarily the increase in production (Core 2004). Application of probiotics to improve quality of the environment, for environmental remediation and sanitation, is clearly in progress in the last decades only. In this chapter these different applications of bacterial probiotics are compared.

2.2 Bacterial Probiotics for Medical Purposes

In the medical and paramedical literature, probiotics are suggested as a tool for treatment or prevention of any disease. The most known and most common probiotic bacteria belong to (1) bifidobacteria (genus *Bifidobacterium*), (2) lactic acid bacteria or lactobacilli (genus *Lactobacillus* and some species

Table 1 Microbial composition of probiotic preparations (information from official descriptions of preparations)

Name of preparation	Microorganisms/additions
Bifidumbacterin	*Bifidobacterium bifidum*
Lactobacterin	*Lactobacillus plantarum*
Colibacterin	*Escherichia coli* M-17
Bactisporin	*Bacillus subtilis* (*Paenibacillus subtilis*) 301
Bactisubtil	*B. cereus* Ir583t
Sporobacterin	*B. subtilis* 534
Bifilong	*B. bifidum*, *B. longum*
Bificol	*B. bifidum* 1 and *E. coli* M-17
Acilact	*L. acidophilus* 100 AIII, NKI, K3III24
Acipol	*L. acidophilus*/polysaccharide of kefir
Linex	*L. acidophilus*, *B. infantis*, *Enterococcus faecalis*
Biosporin	*B. subtilis*, *B. licheniformis*
Bifidumbacterin forte	*B. bifidum*/activated charcoal
Bifiliz	*B. bifidum*/lysozyme
BifiDoc	*B. bifidum*/complex of organic acids
Hylac forte	Complex of *E. coli* metabolites
Enterol	*Saccharomyces boulardii*

which were initially included into this genus), and (3) bacilli (genera *Bacillus* and *Paenibacillus*) capable of producing lactic acid (Table 1). Since the first two mentioned groups are endogenous to the human intestinal microbiota, they were used as probiotics initially and most commonly in the form of some soured or fermented milk beverages (kefir, yogurt, etc.) and later as preparations in the form of bacterial lyophilized biomass. One of the beneficial properties of probiotics is a good fixation to the epithelium of the intestinal mucosa that ensures a stable colonization and competitive inhibition of adhesion of pathogenic microorganisms.

An example of a well-studied probiotic for humans is represented by bifidobacteria (Pikasova 2009). Indeed, they are active producers of lactic and acetic acids and suppress the spoilage and enteric bacteria (*Salmonella*, etc.) both via competition for substrates and with acidification. In addition, bifidobacteria produce metabolites which have a direct inhibitory effect on some pathogens (Ivanova 2010).

The most recent publications showed that the medical role of probiotics is much wider than the opposition of intestinal infectious diseases; they provide the state of the organism as a whole (Million et al. 2013; Murphy et al. 2013). A similar suggestion was published by the founder of immunology E. Metchnikoff in 1903 (Metchnikoff 2010). He believed that aging is an accumulated result of putrescent products of the intestinal microbiota and suggested that any “old age” and senile decrepitude are just consequence and effect of the harmful agent assemblage in the host body, and a scale of this accumulation is proportional to the time of the life. E. Metchnikoff made some investigations and statistics for centenarians and concluded that all humans could live at least up to 150 years. He was enough logical to assume that this harmful agent is presented by putrefactive bacteria in the intestines, and as well, he proposed to include lactobacilli with the sour milk products into the diet as a method to suppress intestinal spoilage and, accordingly, to increase longevity.

Publications of different scientists throughout the years highlighted some possibility of bacteria to shift from the intestine into the body. These works were analyzed by B. Berdichevsky (2001) who examined the “autoinfection” not only as a seeming pathogenic process but also as a common symbiotic defense against other incoming microorganisms. He showed that bacteria, which had been labeled with tritium (^3H), penetrated from the rat intestinal tract into the kidneys and translocated into the newborn skin on the healing up wound.

Thus, the intestinal microbiota not only provides digestion of complicated compounds and inhibits pathogens, but it also changes the state of the host organism. Possible bacterial spreading from the intestinal tract into the host body is a basis for new sight on role of probiotics.

2.3 Probiotics in Aquaculture Sanitation

Similar to the application for human health and nutrition, probiotics are widely used to increase the livestock by improving balance of the digestive animals. For cattle, the lactic acid bacteria were used historically in silage. Particular attention was paid to the application of probiotics in aquacultures where they generally provide a more rapid increase of production than, for example, in cattle (10 % versus 3 %). In 1980 K. Yasuda and N. Taga proposed to use probiotic bacteria both as a food additive for fish and as an agent against fish diseases (Yasuda and Taga 1980). Typical well-known probiotics for aquaculture are represented by bacteria of the genera *Lactobacillus*, *Vibrio*, *Bacillus*, and *Pseudomonas*, and they provide major advantages due to the suppression of pathogenic bacteria. The application of bacterial probiotics replaced the use of antibiotics. For example, application of probiotic *Carnobacterium divergens* for Atlantic salmon and rainbow trout decreased the number of pathogenic *V. anguillarum* and *Aeromonas salmonicida* from 10^5 to 10^2 CFU (colony-forming units)/g of feces in 3 days (Balcázar et al. 2006; Verschuere et al. 2000).

In 1992, bacterial strain *Vibrio alginolyticus* was used as a probiotic in shrimp farming in Ecuador, and its application increased profit in the shrimp larvae production up to four times (Grifftih 1995). In contrast, chemical disinfectants and antimicrobial compounds gave little result in the disease control and resulted in the emergence of some new pathogens resistant to these agents. Thus, the problem was solved with introduction of new component into the ecosystem, where new probiotic habitants affected as bacterial antagonists. Food and Agriculture Organization (FAO) has already recognized that application of probiotics is one of the main methods for improving production and development of aquaculture while maintaining environmental safety.

There is a fundamental difference in the use of probiotics for aquaculture compared with any animal breeding: the introduced bacteria act not only on the digestive tract but also on the whole water environment. Pathogenic microorganisms in aquatic environments can be located not only in the digestive system of the host organism (shrimp, fish) but inhabit and remained in high concentrations outside. These bacteria can easily penetrate into the host organism with coming water. That is why the use of probiotics is particularly important for filter-feeding invertebrates (shrimp) and for the fish in the larval stage.

Industrial aquaculturing is affiliated with risks of diseases of fishes, shrimps, etc., and with deoxygenation of water resulted in loss of yield. Massive development of putrefactive bacteria in shrimp ponds leads both to decrease of the shrimp crop and to their death. Usually, this mortal suffocation is accompanied with change of color of the bottom sediments (pond soils) from yellow/brown to black that is an indicator of anaerobic conditions produced via sulfide iron production. In these cases, the added probiotic bacteria inhibited the hazardous anaerobic bacteria (Farzanfar 2006) and provided sanitation of the ponds (Mayer et al. 2012). Application of probiotics and biodegrading microorganisms to the pond is a sustainable approach to minimize the environmental impact of aquaculture. These experiments showed that the strain *Paracoccus pantotrophus* 768 is able to reduce undesirable waste compounds and had a positive impact on pond soil quality. A field study using a commercial probiotic product (2×10^9 CFU/g) containing the abovementioned strain 768 was conducted during intensive farming of white shrimp *Litopenaeus vannamei* to effect environment quality in a commercial pond. The trial was carried out for 57 days with a dosage of 600 g/ha applied every 5 days. It was confirmed that the ponds with the bacterial supplement reached better environmental conditions and enhanced shrimp parameters. Average daily growth of shrimps with the probiotic treatment was improved up to 36 %.

During the performed experiments (Sklyarov et al. 2004), we proved effect of probiotic on the fish eggs and the larvae of the endogenous nutrition (Tables 2 and 3). Obviously, positive effects in these stages could not be affiliated with the alimentary tract but with the surface of the body and the water quality only. The obtained results mean that use of probiotics in aquaculture is important not only for nutrition as the target in the host organisms but also for the rehabilitation of water in whole (Verschuere et al. 2000).

Table 2 Effect of the probiotic *B. subtilis* on the carp fertilized spawn (Sklyarov et al. 2004)

	Larvae yield, % (number of surviving)		
Spawn, g	Probiotic	Blank	*d*, %
500	86	71	+15
500	74	58	+16
450	97	73	+24
450	90	74	+16
350	18	12	+6

Thus, there are no doubts that probiotic bacteria can carry out their functions to suppress pathogens and improve the treated environment both within and, as well, outside of the host organism in the aquatic ecosystems. Obviously, this trend may represent a significant biotechnological approach for treatment of the terrestrial environments too.

2.4 Bacterial Sanitation and Utilization of Poultry Litter and Manure (Our Proprietary Experimental Data)

We studied the possibility to use probiotic bacilli for sanitation and utilization of the poultry farm litter and manure in special experiments. In the first stage, we checked the distribution of enteric bacteria in the different layers of the bedding. Next, we tested the possibility of suppression of enteric bacteria in the model bedding samples which have been artificially infected with *E. coli* and *Salmonella enteritidis*. Finally, we compared utilizing activities of different commercial biopreparations and probiotic strain.

The total number of microorganisms in the exhausted bedding was presented with billions of colony-forming units (CFU) per 1 g (Table 4), which generally is normal for the bedding contaminated and enriched with litter. Coliform bacteria (*E. coli*) were counted by the CFU number on the Chromocult Coliform Agar, and the *Salmonella* CFU were counted on the chromogenic nutrient Rambach Agar by their indicative color, while the spore-forming acid-producing bacteria ("*B. subtilis*-like bacilli") CFU were counted on the Dextrose Casein-Peptone Agar in accordance with changing of the pH indicator color. To be sure that the last enumeration includes the spore-forming bacteria only, the inoculum was injected directly into the melted hot medium. Table 4 presents the distribution of the enumerated groups in three different layers of the exhausted bedding. Only two studied samples (Table 4, Nos. 3 and 9) contained *E. coli*-type colonies; all other enteric bacteria were presented with the *Salmonella* type. In the most cases, the enteric bacteria were discovered in the lowest layer only. In contrast, acidifying spore-forming bacteria were absent or were scared in the lower layers: as a rule,

Table 3 Effect of the probiotic *B. subtilis* on the crucian fertilized spawn and larvae (Sklyarov et al. 2004)

		Result: number of surviving fishes, %			
		Probiotic suspension, ml per 1,000 fishes			
Fish form	Stage to treat with the probiotic suspension	0 ml (blank)	5 ml	10 ml	15 ml
Spawn	1.5 h after beginning of incubation	27	65	75	77
Larvae	Change to exogenous feed	27	59	74	75
Larvae	10 days after change to exogenous feed	27	36	40	45

Table 4 Numbers and distribution of bacteria in the samples of the poultry bedding

Nos.	Chicken group	Bedding layer	Total number, 10^9 CFU/g	Number of coliform bacteria, 10^6 CFU/g	Number of acidifying spore-forming bacilli, 10^6 CFU/g
1	1	Upper	**10.6**	<0.1	**10.0**
2		Middle	2.5	0.2	0.7
3		Lowest	3.2	**40.0**	<0.1
4	2	Upper	3.0	<0.1	<0.1
5		Middle	5.5	<0.1	3.2
6		Lowest	4.3	**16.0**	0.5
7	3	Upper	**11.0**	<0.1	0.5
8		Middle	0.9	<0.1	0.7
9		Lowest	10.8	**34.0**	<0.1
10	4	Upper	1.4	<0.1	2.6
11		Middle	0.6	<0.1	<0.1
12		Lowest	1.8	**43.0**	2.7
13	5	Upper	**11.5**	<0.1	**10.0**
14		Middle	1.1	<0.1	0.4
15		Lowest	4.7	**20.0**	0.1
16	6	Upper	**8.0**	<0.1	2.0
17		Middle	4.8	<0.1	**10.0**
18		Lowest	2.2	**50.0**	5.0

The maximum numbers are in bold

Table 5 Ratios of bacterial number in the upper layer to bacterial number in the lower layer of the poultry bedding

		Ratio of the CFU number in the upper layer to the CFU number in the lower layer	
Group	Cage	Coliform bacteria	Acid-forming bacilli
2	1	0	350
2	2	2.5	0.25
2	3	0	175
3	1	0	2,000
3	2	0	10
3	3	0	200

The data presented are for enteric bacteria and acidifying bacilli

they were located in the top layer. Such distribution may be due to a large access of air to the upper layer of litter and drawback in the bottom. Accordingly, the greater the number of acidifying bacilli, the lesser the number of enteric bacteria (Table 5).

To avoid unevenness in the bacteria distribution and irregularity in the bedding composition, we studied the forthcoming suppressing effect of the probiotic acidifying bacilli on the model samples of unused bedding, which had been infected with *E. coli* O115 or *S. enteritidis*. The probiotic bacilli were presented with the strains *B. subtilis* and *B. licheniformis* from Provimi Corp., Moscow, and *B. polymyxa* B-514 from All-Russian Collection of Microorganisms (VKM). Results of the number relation of the enteric bacteria and the mentioned bacilli in 4 days under the modeled conditions – with nutrients and under air – are presented in the diagrams (Fig. 1). There are no doubts that probiotic bacteria can provide sanitation of litter (in our case – exhausted poultry bedding) by suppressing hazardous enteric bacteria. The specific bacterial agent has to be chosen for the treatment according to the composition of the litter and to the autochthonous pathogens.

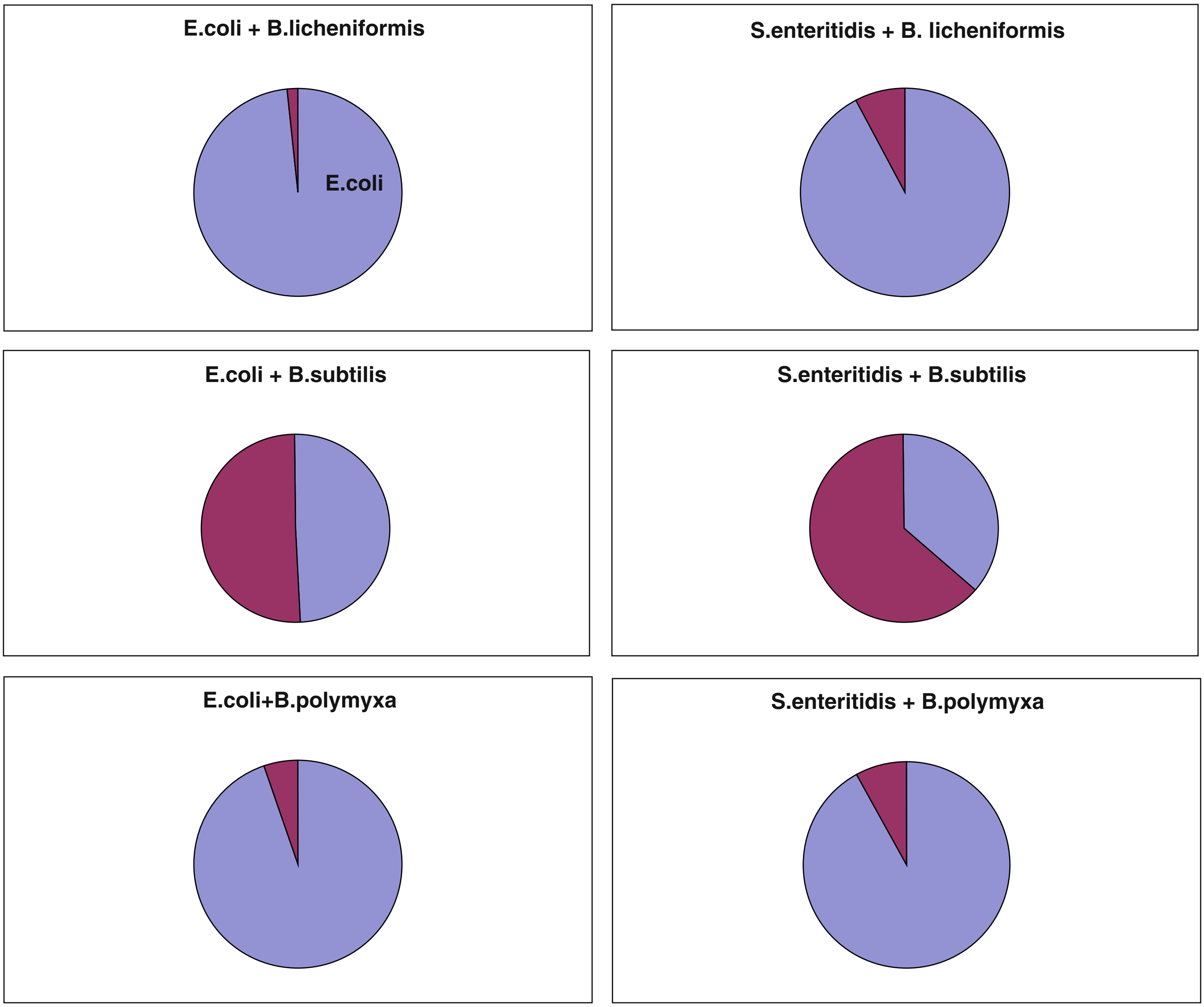

Fig. 1 Ratios of bacilli (*red*) and enteric bacteria (*blue*) in the infected samples in 4 days of their combined growth in model experiments. The data compared the numbers of the viable cells

Sanitation is one side of the whole remediation process, and the other one is utilization of the wastes. The ideal degradation of organic pollutants is a complete oxidation of the matter to carbon dioxide. In this case, we can evaluate the degradation level by the weight loss. Accordingly, we studied a possible role of the probiotic strains in utilization of the model (unused) bedding. The results showed that active utilization of the bedding by probiotic bacteria needed more than 3 weeks at room temperature and exceeded 10 % in 1 month (Table 6).

Exhausted bedding is just a kind of great output of poultry and cattle wastes where the most problematic pollutants are affiliated with animal excrements. There are numerous commercial biopreparations oriented to improve soils and to restore the contaminated and polluted sites. Some of these preparations (e.g., commercial one "Baikal EM1" or "EM – Effective Microorganisms," see Table 6) suggested to present all kinds of probiotic properties (Higa and Parr 1994; Yamada and Xu 2001; Szymanski and Patterson 2003). We proved the effectiveness of different commercial biopreparations in comparison with the probiotic strain *Bacillus sp.* (by the courtesy of A. Repina). It was shown that in a week some commercial preparations and the probiotic strain *Bacillus sp.* increased the rate of degradation of the poultry manure about twice comparatively to natural process (Table 7). However, the non-fresh samples of cattle dung were too complicated for most of the preparations, and just the probiotic strain and "Biokompostin" exceeded the level in a week (Table 8). There are no doubts that different strains (preparations) will produce different effects depending on the composition of the manure and its autochthonous microbiota. Meanwhile, the presented data are an evidence

Table 6 Decrease of the bedding weight affected by its inoculation with bacterial cultures

	Change of the bedding weight, % from the initial weight			
Exposure interval, days	*Bacillus sp.* (*mucilaginosus*)	*Bacillus subtilis*	*Bacillus licheniformis*	Preparation "Baikal EM1"
0	0	0	0	0
10	−3.5	+1.5	−2.0	+0.5
21	+1.0	0	−1.0	−3.0
27	−3.0	−9.5	−4.5	−8.0
33	−13.5	−16.0	−11.0	−14.0

Table 7 Degradation of the poultry manure by *Bacillus sp*. and by commercial preparations

		Decrease in weight in 6 days			
Degrading agent (commercial titles of preparations are in capital letters)	Poultry manure: initial weight, g	g	%	g/day	%/day
– (blank)	15.0	2.3	15.33	0.38	2.56
Bacillus sp.	15.0	4.0	26.67	0.67	**4.44**
BIOFORCE SEPTIC	15.0	2.2	14.67	0.37	2.44
ROEBIC	15.0	4.2	28.00	0.70	**4.67**
SANEX	15.0	2.7	18.00	0.45	3.00
SCHASTLIVYI DACHNIK, SEPTIC SYSTEM MAINTAINER DWT-360	15.0	4.6	30.67	0.77	**5.11**
SCHASTLIVYI DACHNIK, BIOKOMPOSIN BK	15.0	4.8	32.00	0.80	**5.33**

The maximum numbers are in bold

Table 8 Degradation of the cattle manure by *Bacillus sp*. and by commercial preparations

Degrading agent (commercial titles of preparations are in capital letters)	Cattle manure: initial weight, g	Exposition, days	Decrease in weight, g	Decrease in weight, g/day	Decrease in weight, %/day
– (blank)	18.0	13	2.4	0.185	**1.0**
Bacillus sp.	15.0	13	3.5	0.269	**1.8**
BIODESANT	28.0	8	0.0	0.0	0.0
ROEBIC	21.0	8	1.0	0.125	0.6
SANEX	18.0	8	1.0	0.125	0.7
SCHASTLIVYI DACHNIK, SEPTIC SYSTEM MAINTAINER DWT-360	21.0	8	0.0	0.0	0.0
SCHASTLIVYI DACHNIK, BIOKOMPOSIN BK	19.0	8	2.0	0.250	**1.3**

The maximum numbers are in bold

that probiotic bacteria can be used not only for purposes of improvement of health in the digestive tract of different living creatures, but they could provide a health of the treated environment in whole.

3 Discussion and Suggestions

It was mentioned above that selection and choice of probiotic species and strains shall be an individual work for each treated object accordingly to its nature. Introduction into the bowels suggests that these bacteria are affected with acid of gastric juice. By this reason, a special attention was dedicated to spore-forming bacteria which can be introduced into the host organism as spores, i.e., without loss because the spores are not injured with the gastric acid.

Some of these spore-forming strains have additional advantages: they inhibit hazardous bacteria because they produce specific antagonistic compounds. There are East countries that in their cuisine traditionally preserved their food using sour-producing fermentation. The milk-soured food includes not only the fermented milk beverages but sour-fermented vegetables too. For example, soybeans fermented with the spore-forming bacilli are a popular food in China as "Dajiang," in Japan as "Natto," and in Korea as "Doenjang." In scientific literature, this fermenting industrial group of bacteria was published usually under the name *Bacillus subtilis natto*. Recently the medical benefits of the Natto have become widely recognized in Japan, resulting in its increased popularity. Some of the Natto's beneficial effects are preventions of heart attacks, strokes, cancer, osteoporosis, obesity, and intestinal diseases caused by pathogens. The bacterium *B. subtilis natto* produces various enzymes, vitamins, amino acids, and other nutrients during the fermentation; nattokinase and pyrazine prevent or resolve blood clots (Fujita et al. 1993). The *B. subtilis* probiotics produce digestive enzymes (proteolytic, amylolytic, lipolytic, pectinolytic) and biologically active compounds (lysine, histidine, valine, threonine, glutamate, tyrosine, ornithine, alanine). As well, the probiotics based on these bacilli inhibited wide spectra of hazardous bacteria and fungi (including *Salmonella*, *Escherichia*, and other enteric bacteria).

Bacilli in the commercial probiotics can be presented mostly by spores. Survival of the spores at high temperature and under unfavorable conditions and long-term storage provide their commercial preference. The abovementioned examples of the bacillary probiotics in medical and animal breeding practice showed that the listed properties are helpful. Our proprietary experimental data witnessed that this group of probiotics can be applied, as well, for sanitation of environment.

References

American Heritage Dictionaries: The American Heritage Medical Dictionary. Houghton Mifflin Company, Boston (2007)

Balcázar, J.L., de Blas, I., Ruiz-Zarzuela, I., Cunningham, D., Vendrell, D., Múzquiz, J.L.: The role of probiotics in aquaculture. Vet. Microbiol. **114**(3–4), 173–186 (2006)

Berdichevsky, B.: Autobakteria. Stress and a Person (An Anthology of One Observation) (in Russian), p. 410. Meditsinskaya Kniga, Moscow (2001)

Bogadelnikov, I.V., Vyaltseva, Y.V.: Microbiota – an invisible organ of the human organism (in Russian). Zdorovie Rebenka **8**(36), 113–122 (2011)

Bravoa, J.A., Forsythe, P., Chew, M.V., Escaravage, E., Savignac, H.M., Dinan, T.G., Bienenstock, J., Cryan, J.F.: Ingestion of Lactobacillus strain regulates emotional behavior and central GABA receptor expression in a mouse via the vagus nerve. Proc. Natl. Acad. Sci. U.S.A. **108**(38), 16050–16055 (2011)

Core, J.: Probiotics protect poultry from pathogens. Agricultural Research Magazine **52** (1) (2004). http://www.ars.usda.gov/is/ar/archive/jan04/biotic0104.pdf

De Knecht, J. (Chairman): Pharmaceuticals in the environment. Results of an European Environment Agency Workshop. EEA Technical Report No. 1. EEA, Copenhagen, 29p. (2010)

Dolgov, V.S.: Hygiene of Cleaning and Utilization of Manure (in Russian), p. 175. Rosselkhozizdat, Moscow (1984)

Farzanfar, A.: The use of probiotics in shrimp aquaculture. FEMS Immunol. Med. Microbiol. **48**(2), 149–158 (2006)

Fujita, M., Nomura, K., Hong, K., Ito, Y., Asada, A., Nishimuro, S.: Purification and characterization of a strong fibrinolytic enzyme (nattokinase) in the vegetable cheese natto, a popular soybean fermented food in Japan. Biochem. Biophys. Res. Commun. **197**(3), 1340–1347 (1993)

Gehan, Z.M., Anwer, W., Amer, H.M., El-Sabagh, I.M., Rezk, A., Badawy, E.M.: In vitro efficacy comparisons of disinfectants used in the commercial poultry farms. Int. J. Poultry Sci. **8**(3), 237–241 (2009)

Grifftih, D.R.W.: Microbiology and the role of probiotics in Ecuadorian shrimp hatcheries. In: Lavens, P., Jaspers, E., Roelants, I. (eds.) Abstracts of Larvi'95, Fish and Shellfish Larviculture Symposium, p. 478. European Aquaculture Society (Special Publication No. 24), Ghent (1995)

Higa, T., Parr, J.: Beneficial and Effective Microorganisms for a Sustainable Agriculture and Environment, p. 7. International Nature Farming Research Center, Atami (1994)

Ilyin, S.N.: Resource-saving technology of swine manure recycling with biogas manufacture (in Russian). Thesis. Cand. Tech. Sci. (PhD), Ulan-Ude (2005)

Ivanova, E.V.: Biological features of bifidum bacteria and their interaction with microsymbionts of human intestinal microflora (in Russian). Thesis Cand. Med. Sci. (PhD), Orenburg (2010)

Jonas, W.B.: Mosby's Dictionary of Complementary and Alternative Medicine. Elsevier, San Francisco (2005)

Kisil, N.: Ways of manure recycling (in Russian). Poultry Farming **8**, 48–50 (2007)

Kovalev, N.G., Glazkov, I.K.: Engineering of Manure Utilization Systems in Complexes (in Russian), p. 160. Agropromizdat, Moscow (1989)

Kümmerer, K.: Antibiotics in the environment. In: Pharmaceuticals in the Environment. Sources, Fate, Effects and Risks, pp. 75–93, 334p. Springer, Berlin/Heidelberg (2008)

Li, W., Ni, Y.: Use of probiotics to suppress mal-odors of poultry manure. J. Crop. Prod. **3**(1(15)), 215–221 (2000)

Lysenko, V.: Waste recycling in poultry farming – a way to the complex decision of bird influenza problem (in Russian). Ptitsefabrika **6**, 32–35 (2007)

Mayer, E., Gössl, E.M., Santos, G.A., Mohnl, M.: Bioremediation with probiotics in shrimp farming. Environm. Engineer. Manag. J. **11**(3 Suppl), S18 (2012)

Metchnikoff, E.: Etudes Sur La Nature Humaine: Essai de Philosophie Optimiste, 418p. Kessinger Publishing, United States (in French) (2010)

Million, M., Lagier, J.C., Yahav, D., Paul, M.: Gut bacterial microbiota and obesity. Clin. Microbiol. Infect. **19**(4), 305–313 (2013)

Mironov, V.F., Minzanova, S.T., Mindubaev, A.Z., Skvortsov, E.V., Mironova, L.G., Konovalov, A.I., Ryzhikov, D.V.: Method to increase biogas production. Patent RU 2351552 (in Russian) (2006)

Murphy, E.F., Cotter, P.D., Hogan, A., O'Sullivan, O., Joyce, A., Fouhy, F., Clarke, S.F., Marques, T.M., O'Toole, P.W., Stanton, C., Quigley, E.M., Daly, C., Ross, P.R., O'Doherty, R.M., Shanahan, F.: Divergent metabolic outcomes arising from targeted manipulation of the gut microbiota in diet-induced obesity. Gut **62** (2), 220–226 (2013)

Parker, R.B.: Probiotics, the other half of the antibiotic story. Anim. Nutr. Health **29**, 4–8 (1974)

Pikasova, O.V.: A new approach to the molecular diagnostics of bifidum bacteria (in Russian). Thesis. Cand. Biol. Sci. (PhD), Moscow, MSU (2009)

Podgornov, P.A., Ufimtseva, N.F., Anaprienko, T.R.: Method to convert poultry manure and swine dung to organic fertilizer. Patent RU 2409537 (in Russian) (2009)

Sklyarov, V.Y., Mikryakov, V.R., Kulakov, G.V., Kudryashova, E.B., Vainshtein, M.B.: Prospects of preparation application of "Subtilis" probiotics in piscatology for cultivation of fish spawn, embryos, and larvae on the examples of crucian Carassius carassius and carp Cyprinus carpio (order Cypriniformes, family Cyprinidae) (in Russian). Voprosy Rybolovstva **5**(3 (19)), 514–521 (2004)

Szymanski, N., Patterson, R.A.: Effective Microorganisms (EM) and wastewater systems in future directions for on-site systems: best management practice. In: Patterson, R.A., Jones, M.J. (eds.) Proceedings of On-site '03 Conference, pp. 347–354. Lanfax Laboratories, Armidale (2003)

Tlaskalová-Hogenová, H., Štěpánková, R., Kozáková, H., Hudcovic, T., Vannucci, L., Tučková, L., Rossmann, P., Hrnčíř, T., Kverka, M., Zákostelská, Z., Klimešová, K., Přibylová, J., Bártová, J., Sanchez, D., Fundová, P., Borovská, D., Šrůtková, D., Zídek, Z., Schwarzer, M., Drastich, P., Funda, D.P.: Gut microbiota (commensal bacteria) and mucosal barrier in pathogenesis of inflammatory, autoimmune diseases and cancer: contribution of germ-free and gnotobiotic models of human diseases. Cell. Mol. Immunol. **8**, 110–120 (2011)

Verschuere, L., Rombaut, G., Sorgeloos, P., Verstraete, W.: Probiotic bacteria as biological control agents in aquaculture. Microbiol. Mol. Biol. Rev. **64**(4), 655–671 (2000)

Yamada, K., Xu, H.: Properties and applications of an organic fertilizer inoculated with effective microorganisms. J. Crop Prod. **3**(1), 255–268 (2001)

Yasuda, K., Taga, N.: A mass culture method for Artemia salina using bacteria as food. Mer (Bull. Soc. Franco-Jap. Oceanogr.) **18**, 53–62 (1980)

Zenikov, V.I.: Method to prepare compost. Patent RU 2462439 (in Russian) (2006)