Вісник Дніпропетровського університету. Біологія. Медицина
Vìsnik Dnìpropetrovs'kogo unìversitetu. Serìâ Biologìâ, medicina

Visnyk of Dnipropetrovsk University. Biology, medicine.
2013. 4(1)

ISSN 2310-4155

www.medicine.dp.ua

УДК 616.31

Антагоністична активність пробіотиків із бацил до ізолятів бактерій ротової порожнини пацієнтів із пародонтитом

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Значна поширеність запальних захворювань тканин пародонта свідчить про необхідність проведення профілактичних і лікувальних заходів. Наші попередні дослідження показали високу резистентність штамів, виділених із ротової порожнини пацієнтів із пародонтитом, до антибіотиків, що свідчить про необхідність пошуку альтернативних методів лікування цього захворювання. Останніми роками велика увага приділяється вивченню можливості використання пробіотиків із метою корекції мікрофлори ротової порожнини. У статті описано сучасні дані про потенційні переваги та основні властивості спорових пробіотиків роду *Bacillus*, що показують перспективність і актуальність їх використання у стоматологічній практиці. Наведено результати досліджень *in vitro* антагоністичних властивостей бацилярних біопрепаратів («Біоспорин», «Субалін», «Нормофлора», «Ентерожерміна») на клінічні штами мікроорганізмів, виділених із ротової порожнини пацієнтів із запальними захворюваннями тканин пародонта. Найбільшою антагоністичною активністю володіє препарат «Біоспорин». Це може бути пов'язано з наявністю двох пробіотичних штамів у його складі. Тому актуальним залишається проведення подальших досліджень із метою корекції мікробіоценозу ротової порожнини пацієнтів із пародонтитом за допомогою пробіотиків.

Ключові слова: мікрофлора; пародонти; пробіотики роду *Bacillus*; мікробний антагонізм

Antagonistic activity of *Bacillus* probiotics against bacteria isolates of oral cavity of patients with periodontitis

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It is admitted that the normal human microflora plays an important role in supporting homeostasis, forming immune mechanisms and metabolism. Nowadays, there is a constant growth of different diseases due to microbiological imbalance in a human organism. Preparations containing "good bacteria" have been used for therapeutic purposes since ancient times. The mechanism of probiotics influence comprises their ability to compete for adhesion sites with the pathogens, to exhibit antagonistic activity and stimulate the immune system of a host. Most of probiotics commonly used are the spores of *Bacillus*. Initially the main focus of their use was the prevention of gastrointestinal disorders. So, the use of probiotics in dental practice is a poorly studied area. In recent years, probiotics have been investigated to provide the oral health. Therefore the study of using probiotics for correction of the oral microflora in people with inflammatory diseases of the periodontal tissues is promising. Our previous studies have shown changes in microbial community of an oral cavity in patients with periodontitis. In particular, the reducing number of obligate microorganisms and increasing number of pathogens was demonstrated. The paper describes the current data on the potential benefits and basic properties of the *Bacillus* spore probiotics, which demonstrate the viability and relevance in dental practice. The study tested antagonistic activity of commercial *Bacillus* probiotics "Biosporin" ("Biopharma", Ukraine), "Subalinum" ("Biopharma", Ukraine), "Normaflore" ("Sanofi-Aventis Zrt.", Hungary) and "Enterogermina" ("Sanofi-Synthelabo SpA", Italy) against clinical strains of microorganisms isolated from the oral cavity of patients with periodontitis. Thus, further studies on the role of spore probiotics in correction of the oral cavity microflora as a part of complex treatment of periodontitis should be carried out.

Keywords: microflora; periodontitis; *Bacillus* probiotics; microbial antagonism

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Introduction

All local microbe ecosystems by close interaction between themselves and the organism of a human create the unified symbiotic system which sustainable function due to the complicated and diversified mechanisms of regulation (Simonova and Ponomareva, 2008). However, the dynamic balance between the normal and pathogenic microflora in the oral cavity may change under influence of local and / or general factors what may cause some diseases. One of the most common diseases affected the oral cavity are caries and inflammation of periodontal tissue.

Application of the antiseptics and antibacterial drugs for treatment of periodontitis quite often results in the complication of the microbial imbalance of oral cavity. Another point of concern is resistance of microorganism to the antibiotics. Our recent studies demonstrated the high resistance of strains isolated from the periodontal pocket of the individuals affected by periodontitis to antibiotics what testifies to the fact that it is necessary to develop alternative methods for correction of microflora in the oral cavity of the human suffering this disease (Rivis, 2012). Gasula and Cutting (2002) by genetic-engineering chimeric gene fts H-lacZ which is expressed only in the vegetative cells established that the spore *B. subtilis* is able to sprout in the stomach of a mice and proved its ability to have probiotic effect.

Employment of the bacillary probiotics for the correction of the microbial community is considered very instrumental for the following reasons: antagonistic effect on the pathogenic and conditionally pathogenic microorganisms caused by dipicolinic acid of spores and the antibiotics, bacteriocins and ferments which are synthesized by vegetative forms; stimulation of immunocompetent cells, activation of production of interferons (Hong et al., 2004; Skrypnik and Maslova, 2009).

The recent investigations have already proved the efficiency of application in dentistry of probiotic strains of *Lactobacillus* for the correction of the microflora of the oral cavity (Marsh, 2000; Reddy et al., 2011). In particular, Krasse et al. by application of *L. reuteri* in the oral cavity noted reduction of bleeding in the gums of the patients suffering gingivitis and inhibition of its further development (Vaseva, 2010; Narang et al., 2011; Agarwal et al., 2011). Koll-Klais and others discovered that the resident lactobacilli flora is able to inhibit the growth of *Porphyromonas gingivalis* and *Prevotella intermedia* correspondingly by 82 and 65% (Narang et al., 2011; Agarwal et al., 2011; Mohanty et al., 2012). Probiotic strains included into the periodontal bandage in the optimal concentration 10^8 CFU/ml are able to oppress the amount of periodontal pathogenes such as *Bacteroides sp.*, *Actynomices sp.*, *S. intermedius* and *C. albicans* (Agarwal et al., 2011; Narang et al., 2011; Mohanty et al., 2012). The above authors established that the period of remission following the treatment of periodontitis by application of bandages contained *L. casei* lasts 10–12 months. It was observed that the patients who consumed the chewing gum containing probiotics had improved their periodontal status (Mohanty et al., 2012). Elahi et al., testing the character of colonization of *L. acidophilus* and *L. fermentum*, showed rapid dicrease of *C. albicans* in the oral cavity of a mouse after taking of probiotic strains (Narang et al., 2011). The application of *Bacillus* probiotics is considered very prospective in the dentistry. In particular, the alternative method was tested *in vitro* for the prevention of purulent complications occurred after extraction of a tooth by application of *Bacillus* based biopreparations (Rusyn et al., 2012). So far, it is very important to proceed the investigations focused on correction of the microflora of the oral cavity of the people who suffer periodontitis by application of *Bacillus* probiotics.

The purpose of the article is to demonstrate the antagonistic effect of the probiotic products of the genus *Bacillus* ("Biosporin", "Subalinum", "Normoflore", "Enterogermina") as to the clinical strains of the microorganisms isolated from the oral cavity of the people suffering of inflammation of periodontal tissue.

Materials and methods

The subject of investigation are probiotic preparations produced of the microorganisms of the genus *Bacillus*, namely "Biosporin", "Subalinum", "Normaflore", "Enterogermina". "Subalinum" dry (producer "Biopharma", Ukraine) is the microbic mass of live antagonistic active culture *Bacillus subtilis* B-5020, freeze dried by adding sucrose-gelatin medium. "Biosporin" (producer "Biopharma", Ukraine) contains the dry strains of *Bacillus*: *B. subtilis* B-5007 and *B. licheniformis* B-5514. "Entergermina" (producer "Sanofi-Synthelabo S.p.A.", Italy) is the suspension for per oral application which contains spores of polyresistant strains *Bacillus clausii*. "Normaflore" (producer "Sanofi-aventis Zrt.", Hungary) is the suspension for per oral application which contains spores of polyresistant strains *Bacillus clausii*.

The subject of investigation are the cultures of clinical strains of microorganisms isolated from the periodontal pocket of the people with periodontitis, namely *Neisseria sp.*, *Staphylococcus aureus*, *Candida albicans*, *Enterobacter aerogenes* № 1, *Enterobacter aerogenes* № 2, *Escherichia coli*, *Streptococcus mutans*. Intermicrobe interrelations were investigated by application of the method of deferred antagonism (perpendicular streak) as to their ability to oppress the vital activity of various quantity of test-cultures and values of the zones of deferred growth (Ryzhkova et al., 2009). The suspension of cultures of probiotic microorganisms in the concentration equal to one dose of preparation was sown along the diameter of the dried Petri dish with the Gause's medium № 1. The crops were incubated 24 hours in the thermostat under +37 ± 1 °C and 24 hours at room temperature. After that by perpendicular streak 1–2 mm back from the line of growth the suspensions of one day cultures of clinical test strains of the microorganisms were sown. The tests were conducted 3 times. The results of investigations were approved after 18 hours of incubation with reference to the zones of inhibition of growth of test-strains by measuring these zones by a ruler in mm. The simultaneous sowing by streak on the dish with the same medium without investigated probiotics was used for the control of growth of test-cultures. The test strains were considered insensitive when the zone of deferred growth was 0–4 mm, a bit sensitive with the zone of deferred growth 5–10 mm and highly sensitive with the zone of deferred growth 10 mm. In every case for every test-strain it

was calculated the average arithmetic value of deferred growth and its error ($M \pm m$).

Results

By investigation of the antagonistic activity of probiotics it was established that bacteries being the background of the biopreparations, have demonstrated different level of opression effect to various strains of test-cultures. As you see at the table probiotic "Biosporin" demonstrated the highest anatagonistic effect. The highly sensitive to the effect of this preparation were such bacterial strains as *Neisseria sp.*, *S. aureus*, *C. albicans*, *E. coli*, what is testified by signficant zone (s) of deferred growth of these cultures (Table).

"Biosporin" was the only one among the studied probiotics that inhibited the growth of *C. albicans* (competitive inhibition zone was 10 mm). Very low antagonistic effect of this preparation was marked on the clinical strains of *Enterobacter*. Perhaps high antagonistic activity of the "Biosporin" provided by the presence of two *Bacillus* strains (Fig.). The preparation "Subalinum" supresses the growth of clinical strains of genus *Neisseria* and *Staphylococcus*. Its sizes of inhibition growth were 20.3 and 16.7 mm respectively. It turned out that the rest of the strains were either not sensitive or low sensitive to that probiotics.

Table

Antagonistic activity of probiotics based strains genus *Bacillus*

№	Test strain	Zone of inhibition ($M \pm m$, mm)			
		Subalinum *B. subtilis B-5020*	Biosporin *B. subtilis B-5007, B. licheniformis B-5514*	Normaflore *B. clausii*	Enterogermina *B. clausii*
1	*Neisseria sp.*	20,30 ± 0,33	21,00 ± 1,00	2,33 ± 0,33	1,33 ± 0,67
2	*Staphylococcus aureus*	16,70 ± 0,33	15,70 ± 0,90	1,67 ± 0,33	1,67 ± 0,33
3	*Candida albicans*	3,33 ± 0,67	10,00 ± 0,10	2,00 ± 0,10	2,33 ± 0,33
4	*Enterobacter cloacae № 1*	1,33 ± 0,33	1,00 ± 0,10	0,33 ± 0,33	0
5	*Enterobacter cloacae № 2*	1,67 ± 0,33	2,67 ± 0,33	0,67 ± 0,67	1,33 ± 0,33
6	*Escherichia coli*	4,00 ± 0,58	13,00 ± 1,00	3,00 ± 1,00	1,67 ± 0,33
7	*Streptococcus mutans*	0	0	0	0

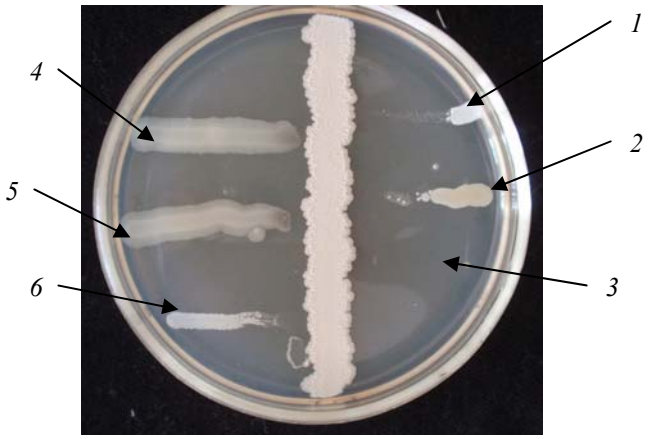

Fig. Bacteriostatic effect of probiotic "Biosporin" on strains of microorganisms isolated from the oral cavity of patients with periodonttits: *1 – Neisseria sp.*, *2 – S. aureus*, *3 – C. albicans*, *4 – E. cloacae* № 1, *5 – E. cloacae* № 2, *6 – E. coli*

Probiotics "Normaflore" and "Entergermina" demonstared low anatogostic effect to all the studied clinical strains. The sizes of inhibition zones vary from 0 to 3 mm.

All investigated biologics demostrated very low anatogonistic effect to the clinical strains of genus *Enterobacter*. The investigation of antogonistic effect of the spore (bacillary) probiotics as to the clinical strains of *Streptococcus* of the oral cavity (in particular *S. mutans*) showed no growth oppression of the above culture.

Discussion

Nowadays widespread use of antibiotics provokes the development and proliferation of the antibiotic resistant strains. The aftermath of that tendency is a microbial imbalance in human organs and systems, and impact on the whole human body. For instance, imbalance of the oral cavity microflora may entail the changes in stomach microflora. The application of probiotics is considered to be the most prospective and effective way for dysbiosis treatment. The majority of the probiotics are designated for treatment and prevention of the microbial imbalance of alimentary canals, while separate probiotics are designated for sanitation of an oral cavity and urogenital system.

The most common probiotic strains belong to the genera Lactobacillus and Bifidobacterium. Currently used probiotic microorganisms in oral treatment are: *L. acidophilus*, *L. rhamnosus*, *L. casei*, *B. bifidum*, *B. longum* and others (Jain and Sharma, 2012; Vishnu, 2012). Some authors consider that there is the interconnection between daily consumption of food and development of periodontitis (Narang et al., 2011; Mohanty et al., 2012). Milk, milk products and yoghurt are the most popular carriers of probiotics. Milk contains calcium, calcium lactate and other organic and inorganic compounds with known anti-cariogenic properties.

Thus, they prevent the colonization of oral cavity by pathogens (Jain and Sharma, 2012).

The results obtained *in vitro* testify that some industrial strains of spore-forming bacteria used in probiotic preparations demonstrate high antagonistic effect to the clinical strains of pathogenic microorganisms isolated from the periodontal pocket. Thereby, continuation of the research of probiotics of the spore-forming bacteria is promising for the rising capabilities for the microbiological balance correction in the oral cavity of people with periodontitis.

Conclusion

The antagonistic effect of the modern probiotic preparations based on the strains of the spore-forming microorganisms is under research. It is established that probiotic preparations "Biosporin" and "Subalinum" are characterized by pronounced antagonistic effect to clinical strains of pathogenic and conditionally pathogenic microorganisms such as *Neisseria sp.* and *S. aureus* . The antagonistic effect to the strains of genus *Enterobacter* and *Streptococcus* isolated from the oral cavity is less pronounced. Probiotic "Biosporin" inhibits its growth of *C. albicans* and *E. coli*. Due to the our research the modern biologics may be recommended as one of the remedies for the microflora correction of oral cavity.

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Надійшла до редколегії 17.03.2013