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Antagonism of lactic acid bacteria against pathogenic agents not related to gastrointestinal tract

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ABSTRACT
Oar researchhas provedthat lactic acid bacteria possess the wide spectrum of antagonistic activity. They inhibit the growth of not only agents of intestinal infections but also agents ofdiseases not related to gastrointestinal tract.
Keywords- lactic acid bacteria, antagonistic activity in vitro, infections located outside the gastrointestinal tract

I. INTRODUCTION

Considerable quantity of works is devoted to antagonistic activity of lactic acid bacteria. The researches mainly concern the struggle with pathogenic gut organisms of a human being and animals [1-4].

Meanwhile there are cases of effective treatment of some pathological processes not related with the gastrointestinal tract, when using biopreparations from living germ culture - representatives of normoflora [5, 6]. So, Yugoslavian scientists have paid attention to the established acceleration of the healing process of wounds and abrasions in patients taking enterally food substances or preparations on the basis of living germ cultures - representatives of *Lactobacillus* or *Bacillus.* French scientists have published data about successful treatment of patients with pyelonephritis with the help of the preparation used for treatment of gastrointestinal disturbance [7].Armenian scientists have suggested that it should be possible to use products of metabolism of lactic acid bacteria to treat septic wounds [8]. It is reported that 1,4% of lactic acid bacteria isolated when fermenting fish food possess antilisterious activity [9]. In connection with the above-stated the researches on revealing of antagonistic activity against pathogen agents not connected with gastrointestinal tract are of great interest.

II. MATERIAL AND METHODS

50 strains of lactic acid bacteria isolated from healthy people and animals were used in the study: *Lactobacillus plantarum*1, 1n, l0d, 53n, 16n, 7k, 25m, 14d, 311, 17d_1*Lactobacillus fermentum*19n, 50, 15, 16, 21, $17_{5\text{-}2}$, 27, 17, 7n; *Lactobacillus brevis*65n, 2d, 27_2, 16_6; *Lactobacillus cellobiosus*20, 7n, 28, 58n, 9, 26c; *Lactobacillus casei*139, 173a, 7, 261, 27_1;*Lactobacillus acidophilus* 15; *Lactobacillus curvatus*18d; *Streptococcus lactis*43n, 33n, 6nc, 41n; *Streptococcusfaecium*14в; *Streptococcus faecalis*.$10_{4\text{-}1}$, 48n; *Streptococcus salivarius*20n, 3d, 17_2; *Streptococcus cremoris*21n, lm; *Streptococcus thermophilus*3d_5, 31.

Strains were cultivated on MRS medium and on combined nutrient medium [10].

Antagonistic activity was determined on the basis of diffusion method in agar [11] with respect to growth suppression zones of the following test-cultures: *Staphylocaccusaureus, Bacillus subtilis, Vibriu scholerae, Bacillus anthracis, Bacieriumcarotavarum, Bacillus mycoides, Pasteurellamultocida, Mycabacierium* B5, *Pseudomonas aeruginosa, Brucellamelitensis*16m, *Brucellaabortus*54, *Brucellasuis*1330, *Brucellaavis* 066, *Brucellaneatamae, Candida albicans, Aspergillusniger.*

In order to test the antibiotic-sensitiveness of bacteria standard disks impregnated with appropriate antibiotics were used.

Tests were repeated not less than three times.

III. RESULTS AND DISCUSSION

The most sensitive to lactic acid bacteria action were *S.aureus, B.subtilis, B.anthracis, B.carotovorum.* At that the quantity of antagonists among the lactobacteria cultivated on combined medium was accordingly 90,3; 87,1; 77,4%, and on MRS medium - 71; 67,7; 87,1; 76,7% accordingly.

The most stable to lactobacteria influence was *B.mycoides*the growth of which was suppressed by 45% of lactobacteria strains cultivated on combined medium and 58%) - on MRS medium.

Active antagonists against *B.carotovarum*were strains of *L. plantarum*1, *L.cellobiosus*20 and *L.plantarum*53n(diameter of the growth suppression zone is 22mm), *L.fermentum*15 (diameter - 24mm), cultivated on combined medium and *S. cremoris*21n (28mm) - on MRS medium.

The growth of *B. mycoides*was actively suppressed by strains of *L. plantarum*1 (30mm) and *L.brevis*65n (27mm) cultivated on combined medium; against *S.aureus*— *L.brevis*65n (32mm), *L. plantarum*1, *L.plantarum*l0d (28mm accordingly) when cultivating in combined medium, and *S.cremoris*21n - on MRS medium; *B.subtilis* - strains of *L.cellobiosus*7n (18mm), *S.thermophilus*31 (20mm) and *S.lactis*6nc (17mm), cultivated in combined medium.

Antagonistic activity against *V. cholerae*was revealed in 74% of lactobacteria strains cultivated in combined medium, at that none of them showed the activity when cultivating in MRS medium. The high activity with respect to the given culture was registered in *L.plantarum*1 (18mm), *S.faecium*14в (17mm), *L.brevis*65n, *L. fermentum*15, *S. cremoris*21n and *L. plantarum*16n (16mm accordingly), the rest strains gave suppression growth zones of the test-culture from 9 till 13 mm.

64%o of the tested lactobacteria strains suppress the growth of *P.aeruginosa*test-culture,a main pathogen of hospital infection of Surgical Department at the Urology Research Center of the Ministry of Health of Kazakhstan.

We have formed the association from lactic acid bacteria (*L. casei*139, *L. fermentum*127_n, *L. casei*173a, *L. brevis*Б-3) and propionic acid bacteria. The association possesses high antagonistic activity against *C.albicans,S. aureus*and *P. aeruginosa*clinical strain. The association is stable to antibiotics used for treatment of inflammatory diseases: cefoperazonome (30mkg/ml), cefamandole (30mkg/ml), seftacidine (30 mkg/ml), norbaxine (l0 mkg/ml), ofloxacine (5mkg/ml), cifrane (30mkg/ml).

Testing of the associations *in vivo* was carried out in the Dzharbusynov Research Center of Urology on patients with benign hyperplasia of prostate of 1-2 degree complicated with the inflammation of inferior urinary tracts after the catheterization. Before treatment urine of those patients was infected by such* pathogens like *S.aureus, P.aeruginosa, P. vulgaris* in titer more than 10000 microbiol cells in 1 ml. Together with complex antibacterial therapy 50-100 ml of liquid culture of the association of lactic acid and propionic acid bacteria were introduced in the urinary bladder of 15 patients in 2001 and 30 patients in 2002 (group 1) with the durability of 30-40 minutes within 7-8 days. 15 and 20 patients accordingly (group 2) were treated without the use of eubiotic.

The positive effect - absence of seeding of pathogenic microbes - was achieved in 73,3-80,3% of patients (group 1) and in 20,6-22,0%) of patients (group 2). So the use of eubiotic in case of "catheter-associated" infection in patients with benign hyperplasia of prostate allows to increase the effectiveness of the standard antibacterial treatment by 2,5-3,7 times.

Besides we have tested the effectiveness of the use of the association of lactic acid and propionic acid bacteria in treatment of patients with chronic calculous pyelonephritis.

As the result of bacteriological analyses of urine in 27 patients from urolithiasis department the following was determined: *E. coli* in 25% of patients, *P. vulgaris, P.rettgeri* - in 24%, *P.aeruginosa* -in 18%, *Citrobacter sp.* - in 8%, *Enterococcus sp.* - in 11%, *Staphylococcus epidermidis, Staphylococcus haemolyticus* - in 7%, fungi of *Candida* type - in 7%.

In the course of treatment 15 patients took eubiotic as oral dosing 20 minutes before the meal for 10 days together with antibacterial therapy. In the control group (12 patients) antibacterial therapy with antibiotics was only used.

After 10 days of therapy 87% of the patients in the test group needed urine sanation. In 13%) of the patients had seeding of *P.aeruginosa*up to 10^4 microbial cells/ml, but the culture resistance against the used antibiotics reduced. In the control group in 65% patients the titre of the seeded microorganisms remained from $10^4$to 10^6 microbial cells/ml.

The study of that association was carried out with the participation of 15patients withurolithiasis complicated by chronic pyelonephritis withstaphylococcal, proteus, candidosisdysbacteriosis of bowels. The quantity of those microorganisms came to 10^7 COE/g. Probiotic was taken enterally as 7day-courses of treatment (15 minutes before the meal). Daily dose was 50 ml of living bacterial cells. No antibiotics were taken during the treatment. Complete recovery was after the third treatment course. At that the level of normal symbiotic flora increased. Hemolytic strains of enterobacteria and staphylococcus, as well as Proteus and fungi of *Candida* type were not registered.

So, the received results testify the high antagonistic activity *in vivo* of the selected association of lactic acid and propionic acid bacteria. It gives the foundation to recommend the association for wide use in the complex therapy of infection diseases of urinary tract [12].

For the prophylaxis and treatment of tuberculosis we have selected the strains of lactic acid bacteria possessing bactericidal activity against *Micbacterium*B5. The most active antagonists against the mentioned test-culture were *L. cellobiosus*7n (growth zone - 19 min), *S.faecalis*48n, *L.brevis*65n and*L.plantarum*1 (17mm), *L.fermentum*$17_{5\text{-}2}$, *L.curvatus*18d, *L.casei*261 (15mm). Antagonistic activity of lactobacteria against the given test-organism also depended on the culture medium. When growing in milk the antagonism was revealed in less degree.

The selected strains of lactobacteria are stable against a number of antibiotics used for treatment of tuberculosis. And these data prove the possibility to use them in complex therapy of the mentioned disease.

Comparative study of lactobacteria antagonistic activity against five reference strains of brucella has revealed six active cultures: *L.brevis*65n (28mm), *L.salivarius*8d (25mm), *L.fermentum*17 (27mm), 7n (25mm), $17_{5\text{-}2}$ (20mm), and 27(24mm). Their antagonistic activity against brucella of all kinds was pronounced and varied only a little.

Chemotherapeutical activity of *L.salivarius*8d strain, active *in vitro* in dilution as 1:10000, was tested *in vivo* on 40 outbred white mice infected with subcutaneous introduction of *B. abortus*54. The mice in comparative group were injected with gentamicin. At that the mice were divided into 8 equal groups where the following periods were different: periods of treatment with gentamicin injected intramuscularly; periods of using lactic acid bacteria (4% of fodder mass) and periods of cutting with necessary bacteriological studies of 8 internals. The animals in the control group were not treated after the infection.

The studies showed that infection indices and intensity of internals contamination in comparison with the control group were for sure lower in the first and second groups in which lactic acid bacteria were added to food within 5 days before the mice were infected with brucella. In the third group in which lactic acid bacteria were added to food within 20 days after the infection and in group 7 in which the mice were treated with gentamicin for 10 days in 20 days after the contamination.

The test results testify that antagonistic activity of *L.salivarius*8d *in vivo* against brucella is highly competitive with gentamicine in action. This fact proves the possibility to use that strain of lactic acid bacteria for treatment and prophylaxis of brucellosis [13].

We have carried out the test of eubiotic developed by us –«Plantafermin» (FS RK42-53 5-2003) - in treatment of gynecological diseases.

Use of antibiotics and sulfanamides for treatment of those diseases especially in case of recurrents of chronic diseases is ineffective and can create complications as a result of impairment of physiological status of macroorganism, reduction of a number of antagonists of vaginal autoflora, because, as a rule, exacerbation iscaused by activation of autoinfection rather than reinfection. In this connection we studied the influence of the new eubiotic«Plantafermin» on the dynamics of inflammatory gynecological diseases, therapeutic effectiveness of this preparation in treatment of 135 women at the age of 16-48 years with inflammatory diseases of genital tract. It was intravaginal introduction of the preparation on cotton-gauze tampon (bolsters) once a day for 7 days. The exposition time was 2-3 hours. The control group consisted of 20 women who took standard drug treatment.

In the course of treatment microscopy of smears and bacteriological study of-pathologic vaginal discharge were carried out on the basis of standard methods.

61 of the examined women had colpitis, 9 - endocervicitis and 2 - adnexitis which was presented mainly as recurrent of chronic form. 8 women with adnexitis had abnormal menstruation cycle. In 27 women the inflammation affected uterine appendages and vagina and in 9 women - vagina and cervical canal. The control group consists of women with mixed pathology.

Bacteriological study of vaginal discharge revealed in patients with inflammatory gynecological diseases evident disbiotic disturbances of vaginal microbiocenose. In 90-95% patients there was great deficiency of lactic-acid flora.

At that the vagina was contaminated with conditionally-pathogenicmicroorganisms. Colpitis was associated mainly with fungi of *Candida* type (80%),pathogenic enterococcus (75%), and hemolyzing strains of colibacillus (60%). In case of adnexitis and mixed pathology pathogenic colibacillus and fungi of*Candida* type were seeded in most patients. Pathogenic staphylococcus and enterococcus were seeded in 20% of patients with adnexitis and in 40% - withmixed pathology.

In case of end ocervicitis conditionally-pathogenic microorganisms were presented by hemoly zing enterococcus, often in association with colibacillus. They were seeded in 57 % of examined women.

After the course of treatment the quantity of leucocytes in vaginal discharge reduced. The discharge was moderate, painful sensation and hyperemia were not registered. Gram-positive bacilli prevailed in the vaginal discharge. Coccal flora was presented mainly by pathogenic streptococci. In the patients with colpitis and endocervicitis the colibacillus and yeast were seeded in single instances. Colibacillus was seeded in 13,3% of patients with adnexitis, the yeast was seeded in 33%. The yeast in small quantity appeared in 15% of patients with mixed pathology.

When using eubiotic«Plantafermin» by the patients with inflammatory gynecological diseases, positive clinical dynamics appeared after 3-5 procedures, vaginal flora became normal. The patients noted good tolerance of the preparation and absence of side effects.

In the control group where the traditional therapy was used, the therapeutic effect appeared later (in some cases the disease state was observed in 14-20 days), and the frequency of side effects came to 20%. Significant improvement in microbiocenose was not registered. All the patients from the control group had great deficiency of lactobacilli. Frequency of seeding of conditionally-pathogenic microorganisms decreased. But that index is less pronounced in

comparison with the patients taking «Plantafermin». The researches revealed inflammatory diseases ofgenital tract in women of childbearing age. At that in 67% of cases the patients belonged to the age group not older than 25. Associate character of etiological factor and great percent of patients with yeast colpitis demanding prolonged treatment are alarming symptoms. Use of traditional drug therapy promotes quick elimination of pathologic factor including conditionally-pathogenic microorganisms. However in this case the deficiency of symbiotic microflora, the leading factor of colonizational resistance of the organism is preserved and in some cases is even redoubled. Absence of this barrier leads to repeated contamination of-the genital tract or to the development of endoinfections caused by conditionally-pathogenic microorganisms and, especially, by yeast.

Sufficient reduction of lactic acid bacteria in the vaginal discharge in case of intensive development of pathogenic flora puts a question on using of bacterial preparations. The attempt to use a new eubiotic «Plantafermin» in correction therapy has demonstrated its effectiveness in case of treatment of inflammatory process in genital tract and flora normalization [14].

So lactic acid bacteria and their associations possess a wide spectrum of antimicrobial action. This testifies about the possibility of their wider use in treatment not only enteric infections but also diseases not related to gastrointestinal tract.

REFERENCES:

[1.] D.E. Corrieretal., Competitive exclusion of Salmonella enteritidisin Leghorn Chicks, *Avian Diseases*,*38*, 1994, 297-303.

[2.] L. Afanaciev, V. Gushul,G. Matrova et al. Use ofLactovit-K probiotic for prophylaxis and treatment of calves with gastrointestinal disturbance, *Epizootology and prophylaxis of contagious diseases in agricultural animals*, 1997,12.

[3.] N.N., Gavrilova, I.A. Ratnikova, L.M.Lukasheva, T.B. Zhangeldin "Polilactovit" preparation for prophylaxis and treatment of gastrointestinal disturbances in agricultural animals, patent № 6164, 1998, 2.

[4.] G.F.Bovkun, V.F.Semenikhina, N.D.Tkatchev, Therapeutic action of bifinorm in case of microecological disturbances if internals in calves,*Veterinary medicine*,*3*, 1999, 42-43.

[5.] I. Blaznik, I. M. Kumel. et al,Sdravljenjekronicnegranulomotoznebolezni z acidofilnemmlecom, *Zdrav. Vestn.*, *45*, 77-79.

[6.] E. Tihole,Fisioloskipomenbacteriemije z jejunalnomicroflora,*Ibid*.,*51*, 1982, 3-5.

[7.] V.V. Smirnov, S.R.Reznicet al., Discussion matters on use of bacterial preparations for correction of microflora of warm-blooded animals,*Microbiology journal*, *54*, 1992, 83-91.

[8.] K.M.Dekhiunyan, A.V. Avakyan,K.M. Ambartsunyan et al. Experimentalgrounds on the use of metabolic products of lactic-acid bacteria for septic wounds treatment. Postoperative complications: articles - Erevan, 1986; 40-42.

[9.] A.Ostergaard, K.R.Embare et al. Characterization of anti-listerial lactic acid bacteria isolated from Thai fermented fish products. *Food Microbial* 1998; 15;2;P.223-233.

[10.] K.Bayakisheva ,I.A.Ratnikova , N.N.Gavrilova , Study of antiinterferon activity of microorganisms. *The Izvestiya* Academy of Sciences Republic of Kazakhstan Med. and Biol. 2001; 3-4; P.23-25.

[11.] N.S.Egorov *Foundations of the doctrine on antibiotics* (M.: Science, 1986).

[12.] L.S.Bectasova, I.A.Ratnikova*Useeubiotics in the therapy of infection diseases of urinary tract.*,collected articles of 3-Annual Conference young scientist-medical. Almaty, 2001.

[13.] N.N. Gavrilova, I.A. Ratnikova , T.A. GrushinaAntagonistic activity of lactic acid bacteria against pathogenslocaled outside the gastrointestinal tract, *Antibiotics and chemotherapy*, *48;2*, 2003, 13-15.

[14.] B.B.Kostenko, L.B.Mishailova, N.N.Gavrilova Use eubiotics plantaferminin the therapy of infection diseases of gynaecology,*Medical.* Almaty,2000, 53-54.