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BIOLOGICAL ACTIVITY OF PROBIOTICS

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Introduction

One way of prophylaxis and treatment of some animal diseases is improvement of parameters of a metabolism and microbial ecology of an organism by application of biological medicines on the basis of lactate and Bifidobacteria. Universal treatment-and-prophylactic properties of such drugs are caused by high concentration in them of the bacteria playing an essential role in normalization proteinaceous, lipidic and mineral metabolism of an organism due to production of a plenty of various ferments, including extra-cellular and cell wise bound proteinase, polysaccharides, glycoproteins and other bioactive compounds.

According to stated above, the purpose of research was to estimate the bioactivity of bifido- and lactate bacteria, including the production of extra-cellular protein, and also the research of influence of probiotic bacteria on protein metabolism characteristics and forming of macroorganism normal flora.

Methods

The objects of research were: bacteria Lactobacillus sp., educed from commercial drug 'Lactobacterinum siccum' produced by "Immunopreparat", Ufa, Russia; bifidobacteria: cultures Bifidobacterium bifidum № 1, B. bifidium LVA-3, B. bifidium 791 B. adolescentis HO-13, B. adolescentis MC-42, B. longum B379M kindly provided by scientists of Gabrichevsky research institute. Cultures B. adolescentis 91-BIM and B. adolescentis 94-BIM are obtained in laboratory of microorganism biology and are deposited as B. adolescentis BIM B-91 and B. adolescentis BIM B-87 in Scientific collection of typical and production-valuable non-pathogenic microorganisms stored in Microbiology institute by National Byelorussian academy of science.

Cultivation of lactate and bifidobacteria was carried out in a thermostat by 37 C on mediums of various structure (MRS, MR.S-4, Blaurock, thioglycolic, casein-yeast (CY), productive medium with a beef broth) depending on experiment goal. The amount of viable bacteria cells in 1 ml of suspension (number of colony-forming cell - CFC) was estimated by method of limit delusion by cultivating on agar-containing (0.2% of agar) nutrient mediums.

The actual acidity of mediums (pH) was measured potentiometrically. The proteolytic activity of lactate and bifidobacteria was measured with Anson's modified method by different pH level (2.5, 7.0, 9.0) by 37C using natrium caseate as a substrate. The bile-resistance was estimated by submerge bifidobacteria cultivation method on CY medium containing 1, 20 and 40 vol.% of bile.

Experiments to estimate the impact of medications Bifidobacter and Bifilac upon the protein metabolism was held in specially formed groups of piglets – 1 control group and 2 experimental (10 piglets in each). The piglets in control group were raised in conditions of standard technology. The animals of experiment groups within the first 6 days were given probiotics in dose 4 ml/kg. After reaching 2-weeks age, the course of injections was repeated in same dose. At the beginning and at the end of each experiment stage the blood was taken to estimate the whole protein and protein fractions concentration; the cultivation of bowels content on selected nutrient mediums according to standard methods was made, and also the scrapes of rectum tunica mucosa were researched for estimating the quality and quantity content of microflora in pet's organism.

The content of whole protein in animal's blood serum was measured by biuretic method, the concentration of albumin – with chromatography method (biochromatograph POINTE-180 PLUS, USA). The reagents of standard sets produced by POINTE SCIENTIFIC, USA, were used. The fractions of α-,β-,γ–globulins were estimated by electrophoretic method using the electrophoresis system CORMAY, USA.

To measure the concentration of bifido- and lactate bacteria in animals' bowels the method of accumulating isolates cultures and method of continuous cultivation (the content of bowels was re-slurried in sterile distilled water in proportion 1:99 with following planting of 5-12th cultivation on selected nutrient mediums) were used. To measure the amount of lactate bacteria the cultivation on dense MRS-4 medium was held, for bifidobacteria the semi-fluid thioglycolic medium was taken. The cultivation of bacteria was held in thermostat within 72 hours by 37±1 C temperature. The calculation of bacteria colonies was held after 24, 48 and 72 hours.

Results and discussion

Initially, we have made a screening of researched bacterium cultures according to following characteristics: production of extra-cellular proteolytic enzymes, growth activity and biomass accumulation. It is an established fact, that by cultivation in CY medium, the bifidobacterium produces the extra-cellular proteinases, active in various scope of pH level, at

that the level of extra-cellular proteinase activity of cultures B. bifidium № 1, № 791, LVA-3, B. adolescentis HO-13 MC-42, 91-BIM is considerably lower than of B. adolescentis 94-BIM (the culture was obtained by method of auto-selection by the level of proteolytic activity). So, in stationary growth phase (24 h) for B. bifidum № 1 and LVA-3 it's typical to produce the proteolytic enzymes active in neutral and alcaline conditions of protein hydrolyze reaction (the total level of proteolytic activity E ≈220 units/ml), and for B. bifidum № 791, correspondingly, in acid and alkaline conditions (E≈412 units/ml). Simultaneously for the cultures B. adolescentis HO-13 (E≈278 units/ml), MC-42 (E≈538 units/ml), 91-BIM (E≈392 units/ml) and B. longum B379M (E≈548 units/ml) it's typical to produce the proteinase, active in wide scope of pH level. The maximal level of proteolytical activity is remarked by B. adolescentis 94-BIM (E≈1096 units/ml). It is proved that the character of high level of proteolytic enzymes production is correlating with the higher growth activity ($5.3*10^9$-2.4-10^{10} CFC/ml) and biomass accumulation (0.84-0.98 mg/ml) of cultures B. adolescentis MC-42, 91-BIM, 94-BIM, HO-13 and B. longum B379M compared to B. bifidium №1, LVA-3 and 791 (growth – $6.2*10^7$-$3.2*10^9$ CFC/ml, biomass 0.37-0.67 mg/ml). As a result, according to features of growth and enzymes activity we have chosen cultures B. adolescentis 91-BIM and 94-BIM.

The following phase of our research was the study of bile-resistance of bifidobacteria on example of B. adolescentis 91-BIM. It is established, that at presence of bile in concentration of 20% and 40% a small increase of biomass accumulation along decrease of growth activity and acid forming is noticed by B. adolescentis 91-BIM. The intensity of biomass accumulation in dynamic of bifidobacteria population development depends on bile concentration in cultivation medium. Thus, to adaptate bacteria to bile and ferment products stimulation, the selection of cultures B.adolescentis 91-BIM and B.Adolescentis 94-BIM by features of growth activity and extra- cellular proteinase forming in CY medium. It is established that intensive biomass increase for B. adolescentis 94-BIM in bile-containing medium with natrium casemate is noticed only on 3-4^{th} day of cultivation, bacterium titre after 24 hours reached in control group $8.1*10^9$ CFC/ml, in experimental group - $9.6*10^9$ CFC/ml. Total level of proteolytical activity of the culture increased within the time of adaptation 1.33 times. B. adolescentis 91-BIM culture compared to B. adolescentis 94-BIM shows faster speed of biomass growth in bile-containing medium and natrium caseate – the intensive growth was noticed already on the second day of cultivation, the bacterium titre after 24 h was: in control group $7.4*10^7$ CFC/ml, in experimental – 1.5-10^{10} CFC/ml. By stimulating the production of proteinase by introducing natrium casemate into nutrient

medium, the total level of proteolytical activity of B. adolescentis 91-BIM increased 1.9 times compared to control level. Culture Lacotbacillus sp. was also noticed to provide fast growth and biomass accumulation in CY medium with bile and natrium casemate: the intensive biomass increase was noticed within the 1st day of cultivation, bacterium titre after 24 h was: 1.4*10 CFC/ml in control, 5.6*10 CFC/ml in experiment. Within adaptation time the total level of proteolytic activity of lactate bacterium raised 2.28 times compared to control.

Further on we analyzed the growth activity, acid-forming, extra-cellular proteinase forming of bifido- and lactate bacteria on productive nutrient medium with beef broth. It was estimated that chosen lactate and bifido bacteria have a higher growth and acid-forming activity, higher proteolytical enzymes production, when being cultivated on productive medium with beef broth.

We estimated the potential of bifido- and lactate bacteria to produce extra-cellular proteolytic enzymes on productive medium with beef broth containing additionally 1% of bile and 0.5 % of natrium caseate. Summarizing the obtained data, we established that as a result of adaptating to productive medium with beef broth, bile and natrium caseate; we obtained the increasing of cell titre and proteolytic enzymes production B. adolescentis 91-BIM and Lactobacillus sp. multiplied by 1.65 and 1.82 correspondingly. Thus, as a result of research of set of features: higher growth activity, acid-forming ability, biomass accumulation intensity, protein substrates and bile acids level, the cultures B. adolescentis 91-BIM and Lactobacillus sp. were chosen, and also a new medicine based on B. adolescentis 91-BIM enriched with culture Lactobacillus sp. was created and named Bifilac.

The next phase of our research was to estimate the biological impact of probiotics Bifidobacter and Bifilac based of bifido- and lactate bacterium with ability for intensive production of proteolytic enzymes, on raising of efficiency of exogenous protein metabolism, and also possible interconnection between protein metabolism normalization, changes in quality and quantity content of gastrointestinal tract microflora and raising of macroorganism resistance. It's shown that applying probiotics made for activization of protein metabolism in animal' organism – the level of whole protein in blood serum increased for 14.9-15.9%, the protein fractions were re-distributed for raise of globulins content (α-globulins – for 17.3-22.1%, β-globulins – for 14.8-16.9%, γ-globulins – 44.4-56.5%) and decrease of albumins concentration (for 9.3-10.6%). The results of morphology study for cultures contained in medicines showed that for lactate bacterium in evolution cycle the forming of regular rod-shaped forms. In lactate bacteria populations the rod shapes with round ends are prevailing and are placed in chains or stand-alone. The results of microbiological research of animals'

bowels content show that injecting symbiotic microflora makes for suppression of conventional-pathogenic bacteria development, allows making micro-biocenose correction of gastrointestinal tract for lactate- and bifidobacteria prevailing. The cells of lactate and bifidobacteria after first isolation out of bowels content have the particular morphology. The cultural features of isolates are noticeably distinguished from museum bifidobacterium cultures.

Thus, applying probiotics Bifilac and Bifidobacter on basis of physiologically active cells of lactate and bifidobacterium having higher level of proteolytical enzymes production made for intensification of protein metabolism and animals' bowels microbiocenose normalization.

Within the framework of State program of fundamental researches "Biotechnology" in the Republic of Belarus we have held the approbation of the new probiotics Bifilac and Bifidobacter to increase the resistance, growth and development activity, activate metabolism of animals. The economical profit of 10160$ is obtained. The high medical and prophylactic efficiency of probiotics (80±5%, $P<0.01$) by gastrointestinal disturbance of animals was proved. Introducing of Bifilac and Bifidobacter medications in agricultural enterprises might create the necessary prerequisites for more efficient treatment and prophylactic of diseases and rising of being intact and productivity of agricultural animals.