Antagonistic activity of strains of lactic acid bacteria isolated from Carpathian cheese

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Abstract

Introduction

Over the recent years, the scientists have been focussing more on antibacterial activity of lactic acid bacteria (LAB) due to their important role in provision of microbiological quality of food products (Vasylyuk et al., 2014; Choi et al., 2018), prolongation of their storage period (Balciunas et al., 2013; Favaro et al., 2015), and also inhibition of pathogenic microflora and formation of microbiocenose of the gastrointestinal tracts of humans and animals (Chen et al., 2018). Antibacterial activity of LAB is determined by their adhesion to the mucous membrane of the intestine and correspondingly the decrease in the adhesion of pathogenic bacteria, aggregation and co-aggregation, and also production of antimicrobial substances such as organic acids, hydrogen peroxide and bacteriocins (Rahme et al., 2017).

Mechanisms of manifestation of antagonistic effect vary. In some cases antimicrobial effect of LAB occurs due to the influence of the main product of metabolism – lactic acid which reduces pH of environment and functions as a bactericidal factor (Castillo et al., 2015). In other cases antagonistic action is caused by neutral products, sometimes pigments produced by the cell into the environment. In oxygen, LAB can also produce hydrogen peroxide which inhibits a number of bacteria, for example Staphylococcus aureus, Pseudomonas and others. Antimicrobial effect of hydrogen peroxide takes place as a result of denaturation of some enzymes, increase in penetrability of the membranes and decomposition of DNA under the impact of free radicals. Furthermore, hydrogen peroxide activates the lactoperoxidase system, and at the same time there forms hypothiocyanite – inhibitor of a broad range of Gram-positive and Gram-negative bacteria (Chornogor et al., 2006). Another known mechanism of antibacterial activity of LAB is the ability to produce lysozyme which destroys the wall of bacterial cells, creating a non-specific antibacterial barrier. Some LAB-producing substances are characterized by high antagonistic activity even at low concentrations in the environment. This category includes antibiotic substances (lactacyd, lactobrevin, nisin, lactobacillin, etc.) (Kamruzzaman et al., 2013; Deep & Kundu, 2015). Among all LAB metabolites, bacteriocins deserve special attention due to their high potential for inhibiting activity towards pathogenic bacteria (Wang & Liu, 2016; Ahn et al., 2017; Zhang et al., 2018). Bacteriocins are low-molecular peptides or genetically-coded proteins which are synthesized on ribosomes and produced extracellularly (Cavicchioli et al., 2017; Yukalo & Storozh, 2018). After binding with the surficial receptors or penetrating into target cells, bacteriocins can function in them through formation of pores, degradation of cellular DNA and inhibition of the system of peptidoglycan.
doglycan synthesis. Bacteriocins may be used as biopreservatives added directly to the food product, or bacterio-cyanogenic strains of LAB which produce these peptides in situ may be added (Deegan et al., 2006).

Search for new LAB strains which can exert biological activity, particularly, antibacterial activity, is an important task of microbiology and biotechnology (Ajay et al., 2015). Valuable sources for isolating promising strains of lactic acid bacteria are natural ecospheres which have been formed over a long period of time. For example, traditional raw-milk dairy products from various geographical regions. Production of such products has an important place in the cultural heritage of each nation. A traditional Ukrainian food product is brined bryndza cheese (Shyvka et al., 2015b) which is made from sheep milk in non-industrial conditions. Over the process of preparation of bryndza out of raw milk, the bacterial cultures are not being added, thus over ripening the natural lactic acid microflora preserves. The current requirements to industrial LAB strains promising for inclusion in bacterial preparations include their safety, technological and probiotic properties (Zhang et al., 2016). Strains of bacterial preparations should be identified according to phenol- and geno-typical traits, have genetic ID, be stable, correspond to technological requirements and have a broad spectrum of antagonistic properties against pathogenic and conditionally pathogenic microflora (Cavicchioli et al., 2015).

Today, since pathogenic microorganisms develop resistance to broad-spectrum antibiotics, probiotic preparations are becoming more and more common. Because of the fact that probiotics based on lactic acid bacteria as the main inhabitants of the gastro-intestinal tract have low antagonistic activity, scientists have focused their studies on seeking a culture with higher antagonistic activity (Moore et al., 2013). Antagonistic activity towards pathogenic and conditionally pathogenic strains can be different and vary for strains, underlying the main difference between the types of probiotics (Krivota & Grebenshelkova, 2018).

Lactic acid bacteria can be used instead of chemical preservatives for inhibition of growth of such pathogenic and conditionally pathogenic microorganisms as Listeria monocytogenes, Salmonella typhimurium, Staphylococcus aureus, Escherichia coli (Castillo et al., 2015; Langa et al., 2016). Currently there is a need for research on new probiotics, which is explained by particularly selection of strains – disease pathogens unsusceptible to probiotic microorganisms (Abeer, 2018).

A promising orientation of improving probiotics is development of complex preparations which include different types of bacterial cultures which would complement each other’s specificity of activity and effect on pathogenic and conditionally pathogenic microorganisms (Fijan, 2016). Therefore, study of antibacterial properties of strains of lactic acid bacteria isolated from natural ecospheres between which interrelations were determined in a relevant and timely task.

Thus, the objective of our study was antagonistic activity towards pathogenic and conditionally pathogenic microorganisms of LAB strains isolated from traditional Carpathian bryndza. As reference strains we used species of Gram-positive bacteria commonly present in milk and dairy products: S. aureus (Cuzzola et al., 2020; Pachuta et al., 2020), L. monocytogenes (Ricchi et al., 2019) and Gram-negative E. coli (Ribeiro et al., 2019), S. typhimurium (Amrinder et al., 2020). Antibacterial activity of lactic acid bacteria towards Gram-positive bacteria is studied more actively than Gram-negative bacteria (Heredia-Castro et al., 2015).

Materials and methods

Experimental studies of technological parameters and antagonistic activity of LAB strains were performed at the Institute of Biology and Biotechnology of the Rzeszów University (Republic of Poland). In the studies we used lactic acid bacteria isolated from bryndza made of raw sheep milk in non-industrial conditions of the Carpathian Region of Ukraine. The data on the origin of cheese are given in Table 1.

Cultures of LAB were identified using classic microbiological and modern molecular-genetic methods (RAPD-PCR, RFLP-PCR, 16S rRNA gene sequencing) (Shyvka et al., 2015a, 2015b). From three samples of cheese, made of milk of different breeds of sheep and selected from different regions with different climatic conditions, we identified the following LAB species: Lactococcus lactis, L. garvieae, Enterococcus faecium and E. durans (Shyvka et al., 2018). Therefore, we consider the

| Kind of cheese | Designations of cheese samples hereinafter | Location of cheese selection | Sheep breed |
|----------------|------------------------------------------|-----------------------------|-------------|
| Bryndza        | A Putyla, Chemiivski Oblast              | Ukrainian Carpathian        |
|                | (highland)                               |                             |             |
| Buzd*          | B Putyla, Chemiivski Oblast              | Ukrainian Carpathian        |
|                | (highland)                               |                             |             |
| Buzd*          | C Duna farm, Koteleva Village, Chemiivski | Bukovina type of Karakul     |
|                | Oblast (pre-mountain area)               | breed                       |             |

Note: * – Buzd sheep cheese according to the technology is Bryndza before salting.

Phylogenetical analysis was performed by determining forward nucleotide sequence of 16S rRNA gene with following comparison of nucleotide identity with the sequences deposited in the International Gene Bank Data Base.

As the main criteria of evaluation of LAB cultures’ suitability for inclusion in the fermentation starter preparation, we used the following technological parameters – acid-forming activity of milk fermentation, resistance to high concentrations of NaCl and temperature optimums for cultivation of lactic acid bacteria strains. Acid-forming activity was assessed according to the decrease in pH of defatted milk, fermented with corresponding bacterial strain. Lactic acid bacteria were incubated in sterile defatted milk without introduction of additional components, which was poured into 5 mL test tubes, and to which 1% inoculate was added, and incubated at the temperature of 30 °C in a thermostat for 3, 6, 9, 24 h. Titratable acidity of milk was determined according to GOST 3624-92 “Milk and dairy products. Titrating methods of determining acidity”. Active acidity was measured using Mutter Toledo MP220 electronic pH-meter.

Salt resistance of the cultures of lactic acid bacteria was determined in 2.0, 4.0 and 6.5% concentrations of NaCl. For this purpose, 0.5 mL of bacteria-inoculated MRS broth was added to the test tube with 5 mL of specially prepared broth for determining the ability of bacteria to grow in different concentrations of NaCl. Test tubes were incubated in the temperature of +30 °C over 7 days. Change in the colour of broth in the test tube from violet to yellow indicated the ability of the bacterium to develop in certain concentrations of NaCl.

The ability of LAB strains to develop in different temperatures was determined at +10, +15 and +45 °C. For this purpose, 0.5 mL of bacteria-inoculated MRS broth was added to test tube with 5 mL of specially prepared broth. After the inoculation, test tubes were incubated for 7 days. Growth of cells in any of these temperatures was determined according to the change in the colour of the broth – violet to yellow.

Antagonistic activity of LAB was determined using the agar diffusion method (agar-well diffusion assay method) (Balouani et al., 2016) and using Multiscan FC microplate reader manufactured by Thermo scientific (USA) at the wavelength of 620 nm (Jeong et al., 2018). Test-cultures were four reference strains of pathogenic and conditionally pathogenic microorganisms: Listeria monocytogenes PCM 2191, Staphylococcus aureus PCM 458, Escherichia coli PCM 2208, Salmonella typhimurium PCM 2182. Strains of test cultures were obtained from the collection of microorganisms at the Institute of Biology and Biotechnology of Rzeszów University (Republic of Poland). Strains of reference microorganisms were cultivated in nutrient broth (BTL sp. z o.o., Poland) in the temperature of 37 °C over 24 h.

Daily cultures of lactic acid bacteria were grown in MRS broth at the temperature of 37 °C. During the study of antagonistic activity of LAB, we used culture broth and supernatant obtained by centrifuging the culture broth at 12,000 rpm for 10 min at the temperature of 4 °C and subsequent filtration through filters of 0.22 µm diameter (Minisart). Agar diffusion

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method was used in dense nutrient medium, comparing the sizes of growth inhibition zones of the test cultures. For this purpose, to Petri dishes with 25 mL of MRS agar (Himedia, India), we inoculated a lawn with corresponding cultures of conditionally pathogenic microorganisms in the amount of 100 µm and concentration of 1×10^6 CFU/cm^3. After keeping corresponding cultures of conditionally pathogenic microorganisms in the growth activity of bacterial cultures in various concentrations of NaCl and 7% (for production of bryndza from raw sheep milk) is one of important characteristics.

E. durans

E. faecium

L. garvieae

L. lactis

Acid-producing activity of LAB strains over fermentation of defatted milk was measured quantitatively. The results of the dynamics of changes in pH and acidity, °Т.

Table 2

Analysis of 16S rDNA sequencing analysis (BLAST) of the LAB strains isolated from Carpathian cheese

| LAB strains        | Strain number | Inventory number in Gen Bank | Percentage of homology, % |
|--------------------|---------------|------------------------------|---------------------------|
| L. lactis IMAU32258| A5            | KF148942.1                   | 90                        |
| L. garvieae KB2626472 | B19          | KM400707.1                   | 99                        |
| Enterococcus faecium IMAU9421 | A8         | KF149058.1                   | 99                        |
| E. faecium L3-23   | C29           | KT229861.1                   | 99                        |
| E. durans FMA8     | C31           | HQ721252.1                   | 99                        |

Acid-producing activity of LAB strains over fermentation of defatted milk was measured quantitatively. The results of the dynamics of changes in pH and acidity, °Т.

Table 3

Acid-forming activity of the LAB strains isolated from Carpathian cheese (x ± SD, n = 3)

| LAB strains          | 3 h       | 6 h       | 9 h       | 24 h      |
|----------------------|-----------|-----------|-----------|-----------|
|                      | active acidity, pH | titrated acidity, °Т | active acidity, pH | titrated acidity, °Т | active acidity, pH | titrated acidity, °Т | active acidity, pH | titrated acidity, °Т |
| L. lactis IMAU32258  | 6.20 ± 0.38* | 39.0 ± 3.8 | 5.10 ± 0.54 | 45.0 ± 0.45 | 5.38 ± 0.51 | 65.0 ± 5.9 | 4.90 ± 0.68 | 94.0 ± 6.5* |
| E. faecium IMAU9421  | 6.90 ± 0.51 | 34.0 ± 3.2 | 6.15 ± 0.59 | 41.0 ± 5.3 | 5.50 ± 0.40 | 56.0 ± 4.1 | 5.01 ± 0.53 | 83.0 ± 4.9 |
| L. garvieae KB2626472 | 6.65 ± 0.42 | 33.0 ± 4.1 | 6.30 ± 0.52 | 42.0 ± 4.6 | 5.86 ± 0.67 | 49.0 ± 5.0 | 5.33 ± 0.24 | 65.0 ± 5.2 |
| E. faecium L3-23    | 6.61 ± 0.35 | 34.0 ± 4.3 | 6.17 ± 0.46 | 42.0 ± 4.9 | 5.67 ± 0.48 | 54.0 ± 5.7 | 4.93 ± 0.44 | 87.0 ± 6.1 |
| E. durans FMA8       | 6.58 ± 0.36 | 34.0 ± 3.3 | 6.15 ± 1.04 | 42.0 ± 4.7 | 5.87 ± 0.82 | 50.0 ± 4.2 | 5.18 ± 0.68 | 74.0 ± 5.4 |

Acid-producing activity of LAB strains over fermentation of defatted milk was measured quantitatively. The results of the dynamics of changes in pH and acidity, °Т.

Table 4

Growth characteristics of the strains at different NaCl concentration and temperature

| LAB strains          | NaCl, % | Temperature, °С |
|----------------------|---------|-----------------|
|                      | 2.0     | 4.0             | 6.5             | 10             | 15             | 45              |
| L. lactis IMAU32258  | +       | +               | +               | +              | +              | +               |
| E. faecium IMAU9421  | +       | +               | +               | +              | +              | +               |
| L. garvieae KB2626472 | +      | +               | +               | +              | +              | +               |
| E. faecium L3-23    | +       | +               | +               | +              | +              | +               |
| E. durans FMA8       | +       | +               | +               | +              | +              | +               |

Acid-producing activity of LAB strains over fermentation of defatted milk was measured quantitatively. The results of the dynamics of changes in pH and acidity, °Т.

Note: * – values significantly different from one another within a column on the results of comparison (P < 0.05) with Bonferroni correction.

Resistance of lactic acid bacteria to high concentrations of NaCl measured 5% (for production of bryndza from pasteurized sheep milk) and 7% (for production of bryndza from raw sheep milk) is one of important characteristics for selecting cultures for production of bryndza. The results of activity of bacterial cultures in various concentrations of NaCl and different temperatures are given in Table 4. Highest resistance to high concentration of NaCl (6.5%) were demonstrated by the cultures of E. faecium, L. garvieae and E. durans. The strain of L. lactis IMAU32258 was characterized by higher sensitivity to heightened concentration of salt, and manifested no growth in the concentration of 6.5%.

As for temperature optima, we determined that strains of E. faecium and E. durans grow at the temperature from 30 to 35 °С, as well as at 45 °С, whereas L. lactis and L. garvieae did not grow at the temperature of 45 °С.

During the study of antagonistic activity of LAB strains isolated from the Carpathian bryndza, we determined that they have different extent of inhibiting effect on the test cultures (Table 5). During the use of the culture broth, the highest antagonistic activity was determined for strain of E. durans, with growth inhibition zone measuring 20 mm and more for all test cultures used in the study. The strain of L. garvieae exerted low-active antagonistic activity towards pathogenic microorganisms, producing growth inhibition zones of 8.4–10.2 mm. Among strains of E. faecium, the highest antagonistic activity was displayed by L3-23 strain. Highly active antagonistic activity was also exhibited by L. lactis. Except L. garvieae, high susceptibility to all the strains of lactic acid bacteria was seen in test culture of S. typhimurium PCM 2182, growth inhibition zone measuring 20.3 ± 1.7 mm to 24.1 ± 1.2 mm.
Lactic acid bacteria are given attention as potential probiotics due to their properties to produce antibiotic substances of various nature, including bacteriocins, mostly, of peptide or protein nature. Therefore, the next stage was the study of antagonistic activity of culture broth supernatant of the studied strains of lactic acid bacteria against conditionally pathogenic microorganisms (Table 6). We determined that growth-inhibiting effect of supernatants of the studied strains of lactic acid bacteria on the test cultures varied. Supernatants of the strains of lactic acid bacteria poorly inhibited growth of *S. aureus*, contrast to the results obtained using culture broth. The difference between the growth inhibition zones produced by culture broth and supernatant equalled 8–12 mm. Obviously the inhibition of growth of *S. aureus* is participated by cells or their components.

Table 5

Antagonistic effect of the LAB strains (culture fluid) isolated from Carpathian cheese against pathogenic bacteria (mm, x ± SD, n = 3)

| LAB strains       | *S. aureus* PCM 458 | *L. monocytogenes* PCM 2191 | *S. typhimurium* PCM 2182 | *E. coli* PCM 2208 |
|-------------------|----------------------|-----------------------------|---------------------------|---------------------|
| *L. lactis* IMAU32258 | 20.2 ± 2.8           | 18.5 ± 1.6                  | 24.1 ± 1.9                | 18.7 ± 1.6          |
| *E. faecium* IMAU9421 | 18.1 ± 1.4           | 14.4 ± 1.3                  | 20.3 ± 1.7                | 23.6 ± 2.4          |
| *L. garvieae* JB2826472 | 8.4 ± 1.1*          | 10.2 ± 0.9*                 | 9.5 ± 1.4*                | 8.4 ± 0.7*          |
| *E. faecium* L3-23  | 19.5 ± 1.6           | 21.4 ± 0.8                  | 25.2 ± 1.2                | 20.1 ± 1.2          |
| *E. durans* FMA8   | 20.6 ± 2.1           | 23.6 ± 1.3                  | 24.0 ± 0.9                | 20.3 ± 1.9          |

Note: * – values significantly different one from another within a column on the results of comparison (P < 0.05) with Bonferroni correction.

Strain *L. garvieae* JB2826472 displayed the highest degree of antagonistic activity towards pathogenic and conditionally pathogenic microorganisms (6.2–7.4, P < 0.05). At the same time, *E. faecium* IMAU9421 exerted moderate antagonistic impact on all the test cultures, whereas activity of *E. faecium* L3-23 was higher. In all the variants of the experiments, a high level of antagonistic activity was exerted by *E. durans*, particularly towards *L. monocytogenes* PCM 2191 (growth inhibition zone of 22.6 ± 1.8 mm) and *S. typhimurium* PCM 2182 (growth inhibition zone measuring 23.8 ± 1.2 mm), which may suggest using this strain for developing a probiotic of metabolite type as a constituent in bacterial preparations.

Table 6

Antagonistic effect of the LAB strains (supernatant) isolated from Carpathian cheese against pathogenic bacteria (mm, x ± SD, n = 3)

| LAB strains       | *S. aureus* PCM 458 | *L. monocytogenes* PCM 2191 | *S. typhimurium* PCM 2182 | *E. coli* PCM 2208 |
|-------------------|----------------------|-----------------------------|---------------------------|---------------------|
| *L. lactis* IMAU32258 | 8.4 ± 1.3           | 17.1 ± 1.2                  | 18.9 ± 0.6                | 17.3 ± 2.1          |
| *L. garvieae* JB2826472 | 6.3 ± 0.9           | 6.2 ± 0.4*                  | 7.4 ± 1.3*                | 7.1 ± 1.3*          |
| *E. faecium* IMAU9421 | 6.1 ± 0.6           | 14.3 ± 2.1                  | 15.7 ± 1.7                | 16.5 ± 1.5          |
| *E. faecium* L3-23  | 10.2 ± 1.7           | 18.5 ± 1.7                  | 20.1 ± 2.5                | 18.4 ± 2.7          |
| *E. durans* FMA8   | 12.5 ± 2.9           | 22.6 ± 1.8                  | 23.8 ± 1.2                | 20.4 ± 1.4          |

Note: see Table 5.

The extent of inhibition of growth of test-cultures in the dynamics is determined according to the change in optical density of their culture broth after addition to supernatant of lactic acid bacteria and incubation for 24 h in the temperature of 37 °C (Fig. 1).

![Fig. 1. Antagonistic activities of the supernatant LAB at 37 °C for 24 h:](image)

*Fig. 1. Antagonistic activities of the supernatant LAB at 37 °C for 24 h: a – Staphylococcus aureus PCM 458, b – Listeria monocytogenes PCM 2191, c – Salmonella typhimurium PCM 2182, d – Escherichia coli PCM 2208; control – growth of pathogenic bacteria without the supernatant from LAB; x ± SD, n = 3*
We performed cultivation in aerobic conditions, therefore we cannot differentiate the factors due to which the antagonistic activity was displayed — i.e. bacteriocins, hydrogen peroxide, organic acids or enzymes.

We determined that *E. durans* FMA8 strain was characterized by high antagonistic activity towards *L. monocytogenes* and *S. typhimurium*, optical densities of which after 24 h of cultivation accounted for 0.195 and 0.178 respectively (Fig. 1b, c), i.e. practically at the initial level. High extent of antagonism was exerted by *E. durans* FMA8 strain against *E. coli* (Fig. 1b). Strain *L. garvieae* JB2826472 took lowest-degree antagonistic impact on pathogenic and conditionally pathogenic microorganisms in the conditions of using agar diffusion technique and change in optical density. Other surveyed strains also demonstrated higher antagonistic activity.

In the analysis of the graphs which reflect the antibacterial activities of LAB strains (Fig. 2), we should note that the most active strain was *E. durans* FMA8 — growth of *L. monocytogenes* equaled only 9.3% compared with 83.1% in the control (without addition of supernatant of LAB), *S. typhimurium* — 5.8% compared with 54.0% in the control, and *E. coli* — 10.0% compared with 78.0% in the control. Supernatants of strains *L. lactis* IMAU32258, *E. faecium* IMAU9421 and *E. faecium* L3-23 were also quite active in inhibiting growth of all the test cultures, except *S. aureus*, though strains *E. faecium* L3-23 and *E. durans* FMA8 decreased the intensity of its growth by over 30%.

**Discussion**

While selecting LAB strains in accordance with their properties, it is important to take into consideration the extent and velocity of acid production of bacterial microorganisms, temperature optima and resistance to high concentrations of salt, because this directly influences the speed of obtaining end product and interaction with other constituents of bacterial preparation, taste of the product, its physical qualities and maintenance (Thierry et al., 2015). Species *E. faecium* exerted high acid producing ability, which is consistent with the results (Amaral et al., 2016). Less expressed acid-producing ability was determined for *E. durans* and *L. garvieae*. Low acid-producing ability of *L. garvieae* of dairy origin was reported in the study (Fortina et al., 2007). Several surveys confirm high technological practicability of *E. faecium* and *E. durans* (Mcauley et al., 2015) and good combination with both mesophilous and thermophilous cultures (Sanantinopoulou et al., 2002). Regarding salt-tolerance, according to the results of the study (Amaral et al., 2017), the salt concentrations of 5% and 7% were limiting factors for *E. durans* SRP29, which does not correlate with our results and indicates strain differences, at the same time our results confirm broad temperature range for growth of enterococci.

High antibacterial activity is one of the main requirements to probiotic strains (Yerlikaya & Akbulut, 2019). Among the surveyed lactic acid bacteria, the lowest antagonistic activities towards both Gram-positive and Gram-negative test-cultures were exerted by strain *L. garvieae* JB2826472. These data are completely consistent with the data obtained using the agar diffusion method, though they do not coincide with reports about high antagonistic activity of this species against *S. aureus* (Abdelfatah & Mahboub, 2018). At the same time, the authors emphasize that this high antibacterial activity manifested according to the results of their studies is due to synthesis of bacteriocins, because the cultivation was performed in anaerobic conditions, having supernatant neutralized before the introduction. Several hypotheses are proposed to explain the differences between the expressions of antibacterial activity of culture broth and supernatant towards *S. aureus*, one of which is adsorption of bacteriocin-like compounds by the filter. Our data about low antibacterial activity of supernatant of LAB against *S. aureus* correlate with other reports (Ammor et al., 2006; Rasovic et al., 2017). It should be noted that the differences between antibacterial activities of culture broth and supernatant of the other test-cultures were much less manifested. Notable antibacterial impact was taken by *E. durans* FMA8 on *L. monocytogenes* — one of the most dangerous pathogens in food products, which causes high mortality rates (Swaminathan & Gerner-Smidt, 2007). Presence of *L. monocytogenes* in cheeses is disturbing, contamination by *L. monocytogenes* was reported for example in cheese-making factories in Brazil (Baranelchi et al., 2014), and cheeses are consumed without undergoing processing. Therefore, supernatants of strains *E. faecium* L3-23 and *E. durans* FMA8 exerted...
well expressed antagonistic activity towards strains of *S. typhimurium* PCM 2182 and *E. coli* PCM 2208 and *L. monocytogenes* PCM 2191 and low activity against *S. aureus*, entirely correlating with the results obtained using method of diffusion of supernatant in agar. The ability of enterococci to exert antibacterial activity due to synthesis of bacteriocins is reported in a number of studies (Alhunay, 2014; Cavicchioli et al., 2015).

Strain *L. garvieae* JB2826472 had the lowest antagonistic impact on pathogenic and conditionally pathogenic microorganisms. Unlike *L. garvieae* JB2826472, strains *L. lactis* IAM132258 and *E. faecium* IAM9421 exhibited moderate antagonistic activity.

We determined no significant differences between antagonistic activities of the studied strains against conditionally and conditionally pathogenic bacteria, which is consistent with a number of studies (de Almeida et al., 2015; Reuben et al., 2019). However, Añas et al. (2008) reported that LAB exert a higher level of antagonistic activity towards Gram-positive than Gram-negative bacteria. Lactic acid bacteria can produce bacteriocins which have an antibacterial impact on such Gram-negative bacteria as *E. coli*, *S. typhimurium*, *Helicobacter pylori* NCIPID 230, and also *Campylobacter jejuni* (Todorov et al., 2010; Reis et al., 2012).

According to the results of the study on the representatives of *Enterococcus* genus, *E. faecium* and *E. durans* may have other probiotic properties (Amaral et al., 2016). Combination use of *E. faecium* and *E. durans* would allow creation of a bacterial composition for production of dairy products with probiotic properties. Enterococci are part of the normal microflora of the human intestine, and are currently being considered as probiotics (Goh & Philip, 2015), and also cultures for enrichment of taste and aroma properties of dairy products, first of all cheeses (Yerlikaya & Fortina, 2007). We isolated *L. garvieae* from raw milk and cheeses as well (Fernández et al., 2010), though it was isolated from milk and cheeses as well (Fernández et al., 2010), the genetic similarity between the specimens from different sources (fishes and dairy products) being insignificant (Foschino et al., 2008). It is worth noting that no relationship was determined between consumption of products from raw milk and *L. garvieae*-caused infections (Fernández et al., 2010).

We isolated *L. garvieae* from all the samples of Carpathian byrhnza, though there is little literature data on its presence in dairy products. Species *L. garvieae* is known for producing a number of bacteriocins (Tosaldhuvong et al., 2012; Maldonado et al., 2012; Ovchinnikov et al., 2016) and it is currently considered from the perspective of possible control of pathogenic microflora (Ablibeltah & Mahboub, 2018).

**Conclusions**

We identified strains of lactic acid bacteria isolated from Carpathian byrhnza. According to nucleotide sequences, we determined taxonomic position with homology of 99% for 4 isolates of LAB. Taxa belonged to 4 species which causes diseases in fishes (Vendrell et al., 2006), though it was isolated from raw milk and cheeses as well (Fernández et al., 2010), the genetic similarity between the specimens from different sources (fishes and dairy products) being insignificant (Foschino et al., 2008). It is worth noting that no relationship was determined between consumption of products from raw milk and *L. garvieae*-caused infections (Fernández et al., 2010). We isolated *L. garvieae* from all the samples of Carpathian byrhnza, though there is little literature data on its presence in dairy products. Species *L. garvieae* is known for producing a number of bacteriocins (Tosaldhuvong et al., 2012; Maldonado et al., 2012; Ovchinnikov et al., 2016) and it is currently considered from the perspective of possible control of pathogenic microflora (Ablibeltah & Mahboub, 2018).

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