Characterization Of Exopolysaccharide From Lactobacillus Casei K7/3

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Abstract: An exopolysaccharide (EPS) in the amount of 20 mg/L and 1200 mg/L was obtained from the culture supernatant of Lactobacillus casei K7/3 grown in MRS broth and in a broth based on the whey of cottage cheese, respectivily. In the IR spectrum of EPS, intense absorption bands were found, generally characteristic of a class of carbohydrates. The molecular weight of the EPS obtained from L. casei K7/3 was 5600 Da, the polydispersity index was 1.9. The EPS from L. casei K7/3 consisted from a dextrose, mannose and galactose in molar ratio of 2.7:1.5:1, respectively. Minimal Inhibition Concentration (MIC) values of an EPS against Listeria monocytogenes ATCC 1911 revealed between 10 mg/mL and 80 mg/mL. An in vitro study of the antioxidant activity of L. casei K7/3 EPS in the diphenylpicrylhydrazine (DPPH) system showed that radical scavenging activity (RSA) has being increased depending on the concentration of the investigated EPS. The maximum %RSA was detected at 4 mg/ml being 26%.

Keywords:lactobacilli, exopolysaccharide, monosaccharide composition, antimicrobial activity, antioxidant.

1. INTRODUCTION

Lactic acid bacteria (LAB) has an important role in many food fermentation processes. Some species of the genus Lactobacillus (Lb.) are included in this group. The lactic acid fermentation has long been known and applied by humans for making different food stuffs. For many centuries, LAB have been an effective form of natural preservation. In addition, they strongly determine the flavor, texture and frequently, the nutritional value of food and feed products. However, the application of well-studied starter cultures has been established for decades (Lee, B.H., 1996., Tserovska, L. et al., 2002).

The growth of LAB is often accompanied by the production of polysaccharides, which are found outside the cell wall. These exopolysaccharides (EPS) may be found as capsule attached to the bacteria or they may be released to the environment as slime or both (Sutherland IW., 1977). Polysaccharides important to bacteria for adhesion, infection and protection may as well have commercial value. Some polysaccharides are known to have gelling properties that is; agar and gel rite (Lin, C.C. et al., 1984; Davis, B.D. et al., 1980).

Exopolysaccharides of lactic acid bacteria also positively affect the taste, smell and preservation of the final product (S.Badel et al. 2011; Ruas-Madiedo, P. et al., 2005). Many of the exopolysaccharide-producing strains of lactobacilli belong to the genera *Streptococcus, Lactobacillus, Lactococcus, Leuconostoc, and Pediococcus* (Berg D.J.C. et al., 1995).

EPSs of lactobacilli have several medical applications, including immunostimulation through application as polysaccharide vaccines; they possess both antitumor and antiviral activity as well as act to lower blood cholesterol levels (Cheng-Chih Tsai, et al. 2014). EPSs are high-molecular weight polymers composed of sugar residues that are secreted by microorganisms into their surrounding environments. EPSs are comprised of various

monosaccharides, mostly glucose and galactose, but also including rhamnose, fructose, mannose, galactosamine, and other sugars (Tallon R. et al. 2003).

A key problem in the industrial production of EPSs by lactobacilli is the high cost of culture medium. For industrial production, the culture medium must contain all the *Lactobacillus*-specific nutrients (Seesuriyachan P., 2011). EPSs produced by lactic acid bacteria are influenced by many factors, e.g. medium components, pH, temperature, and fermentation time course. Many studies showed that the best carbon sources for EPS production of lactic acid bacteria are lactose, glucose, and sucrose. Furthermore, nitrogen sources also an important medium component determining the EPS yield (Zhang Y., et al., 2011; Fukuda K. et al., 2010; Pham P. et al., 2000; Yuksekdag Z. et al., 2008). Due to complex nutritional requirements of *Lactobacillus* strains several exopolysaccharides selection medium has been developed which include whey, whey based media, modified Exopolysaccharide selection medium (mESM), chemically defined and semi-defined medium and de Mann Rogosa Sharpe medium (mMRS) (D.J.C. van den Berg et al., 1993; V.M.Marshall et al., 1995; R. van Kranenburg et al., 1997; S. A. Kimmel, 1998; E.W.J. van Niel et al., 1999; Degeest B. et al. 2001). Therefore, it is particularly necessary to reinforce the study on the improvement of yield of Lactobacilli EPS by isolation new strains or optimization the EPS production conditions, which is significant for the EPS commercial exploitation.

The purpose of the work is investigate the EPS production by *L.casei* K7/3 and evaluation its antibacterial and antioxidant activity.

2. MATERIALS AND METHODS

Bacterial strain and starter culture preparation: The strain of *L.casei* K7/3 was isolated from domestic sauerkraut according to the generally accepted method, identified by morphological, physiological, biochemical properties and deposited in the collection of microorganisms at the Institute of Microbiology of the Academy of Sciences of the Republic of Uzbekistan.

Production and Isolation of EPS from L.casei K7/3: Production and isolation of polysaccharide from *L.casei* K7/3 culture supernatant was carried out according to the method described by Cerning, J. et al. (Cerning J et al., 1999). Culture *L.casei K7/3* restored from the lyophilized state 2-3 transfers in MRS-broth and incubated at 37 °C for 48 hours. An inoculum in a volume of 20 ml (2%, w / v) was added to 1 L of MRS-broth and grew in shaker-incubator at 150 rpm. A medium based on the whey of cottage cheese (tvorog) proposed by K.D. Karapetyan et al. was used as an alternative to the MRS-medium. The composition of the medium: 0.6% sodium citrate, 0.018% manganese sulfate, 0.5% sodium acetate, 0.5% yeast extract, 1.0 % peptone, 2.0% carbon source, 0.5-1.5% ammonium sulfate, 0.2% magnesium sulfate (K.D. Karapetyan et al. 2008). Under aerobic conditions were incubated at 37°C for 48 hours. After culture incubation, TCA was added to a final concentration of 4% (w/v) and stirred for 30 minutes at room temperature. Cells and precipitated proteins were removed by centrifugation at 7,000 x g for 30 minutes at 4 °C. Chilled ethanol was added to the supernatant in an equal volume and kept at 4 °C for 48 hours. Precipitated EPS were collected by centrifugation at 7000 x g for 30 minutes at 4 °C. The precipitate was dissolved in distilled water and dialyzed at 4 °C for 48 hours, then dried by lyophilization. Total carbohydrates in lyophilic – dried exopolysaccharides of lactobacilli were determined by the phenol sulfuric acid method (Dubois M. et al., 1956). The quantitative determination of proteins in the composition of crude exopolysaccharide was carried out by the method described by A. Yermakov et al. (A. Yermakov et al., 1982).

Infrared spectroscopic (IR) analysis of crude exopolysaccharide from L.casei K7/3: IR-spectra of exopolysaccharide from *L.casei* K7/3, registered Fourier transform infrared spectrometer Vector-22 (Bruker, Germany) in the frequency range 400-4000 cm^{-1}. 2 mg of

exopolysaccharide was mixed with 200 mg of potassium bromide (KBr) (1: 100 ratios), then the mixture was pressed into a mold with a diameter of 16 mm and conducted IR spectroscopy for detection of functional groups characteristic of polysaccharides (Yeanly W. et al., 2016).

Monosaccharide and molecular weight (Mw) determination for the EPS from L.casei K7/3: The monosaccharides composition determination of *L.casei* K7/3 EPS was carried out by gas chromatography (GC) (V.V. Arasimovich, 1984). The Mw of the polysaccharide was determined by size exclusion chromatography (SEC) on an Agilent 1260 Infinity size exclusion chromatograph (Agilent Technologies, USA).

Antimicrobial Activities of EPS from L.casei K7/3: The antibacterial activity of EPS was investigated by agar well diffusion assay as described by Chen et al. (2014) with a slight modification (Chen et al., 2014). Overnight incubation culture of the *Listeria monocytogenes* was diluted to 10^6 to 10^7 CFU/mL and then spread on 10 mL of BHI-agar in a Petri dish. With a stainless cylinder (of diameter 6.0±0.1 mm) were done wells on agar. Then, 100 μL aqueous solutions of EPS in concentrations of 80, 60, 40, 30 mg/mL were added to the wells. The plates were incubated at 37^{o}C for 24 hours and the diameter of the clear zones around the wells was measured.

Antioxidant activity analysis: Antioxidant activity was judged by the binding of the oxide radicals of diphenylpicrylhydrazine (DPPH) (Wang K. et al., 2015b). 2.0 ml of an alcohol solution of DPPH (0.4 mM) was added per 1 ml of an aqueous solution of an exopolysaccharide from *L.casei* K7/3. The mixture was thoroughly mixed and incubated at room temperature in a dark place for 30 minutes. The absorption coefficient of the mixture was measured at 517 nm on a Cary 60 UV-Vis spectrophotometer (Agilent Technologies, USA). Antiradical activity was calculated by the formula: $\%RSA=\frac{(Ao-A1)}{Ao}x100$, where - A_1 is the absorption coefficient of the sample solution; A_o- the absorption coefficient of the DPPH solution without a sample. An aqueous solution of ascorbic acid was used as a control.

Statistical analysis: All experiments were carried out in triplicate. In statistical processing determined the arithmetic mean, standard deviation, confidence intervals, the significance of differences was determined using Student's criterion. Differences were considered significant at a significance level of $p\leq0.05$. The results were analyzed using standard packages of licensed programs MS Excel 2003 and STATISTICA 6.0 (Larkin M.A., et al., 2007).

3. RESULTS

*Microorganism***:** Cells of *L.casei* K7/3 culture are Gram-positive, catalase negative short bacilli, located singly and in pairs, 0.6 μm x 1.2-3.6 μm in size. The culture actively ferments cellobiose, salicin, mannose, mannitol, melibiose, ribose, maltose, trehalose, sucrose, fructose, lactose and galactose, neutralizes the lithmus, grows with 6.5% NaCl and 0.4% bile.

Isolation of polysaccharide from L.casei K7/3 culture supernatant: When growing *L.casei* K7/3 culture in MRS-broth yield of lyophilic - dried crude EPS was 20 mg/L. at the cultivation in medium based on the whey of cottage cheese (tvorog) proposed by K.D. Karapetyan et al. was 1200 mg/L, that is 6 fold more than in MRS-broth. Lyophilized EPS was an amorphous cream-colored powder, it was well dissolved in water, had a smooth fibrous structure. Crude EPS contained 65.59 ± 0.7% of the total polysaccharides and 7.63% of the protein (nitrogen content 1.22%).

Infrared spectroscopic (IR) analysis of the crude exopolysaccharide from L.casei K7/3: The IR spectrum of investigated EPS was studied in the region between 400 cm^{-1} and 4000 cm^{-1} and showed numerous peaks from 3434 cm^{-1} to 534 cm^{-1}. In the IR spectrum of EPS of *L.casei K7/3*, intense absorption bands were found that was generally characteristic of the carbohydrate class (Figure 1).

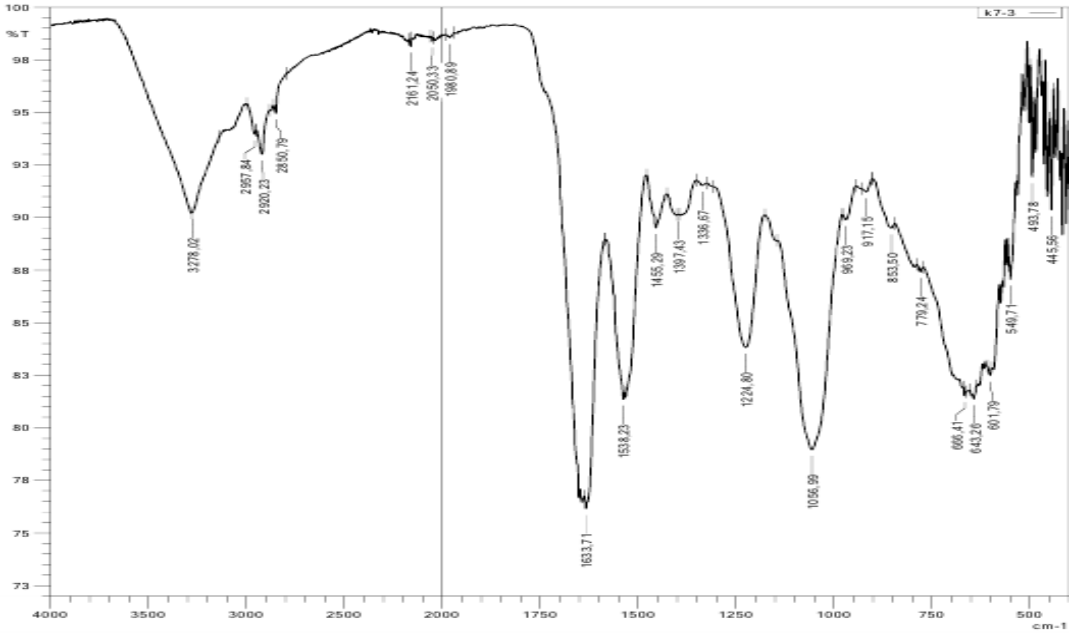

Figure 1. IR spectrum of raw EPS from *L.casei* K7/3

Monosaccharide composition and molecular weight of an exopolysaccharide from L.casei K7/3: GC analysis of the monosaccharide composition of EPS from *L.casei* K7/3 showed that this EPS consists of dextrose, mannose and galactose in a molar ratio of 2.7:1.5:1, respectively (Figure 2). The molecular weight, EPS of *L.casei* K7/3 was 5600 Da, the polydispersity index was 1.9.

Antimicrobial Activity of EPS from L.casei K7/3: Antibacterial activity of EPS from *L.casei* K7/3 is shown in Figure 1. This EPS showed high antimicrobial activity against *Listeria monocytogenes* ATCC 1911 in all studied concentrations. The diameter of zones of growth suppression at 80 mg/mL is 25 mm, at 60 mg/mL is 22 mm, at 40 mg/mL is 22 mm and at 30 mg/mL is 20 mm (Figure 3).

Antioxidant activity of EPS from L.casei K7/3: The antioxidant activity of EPS from *L.casei K7/3* was judged by the activity of binding of free oxide radicals of DPPH.

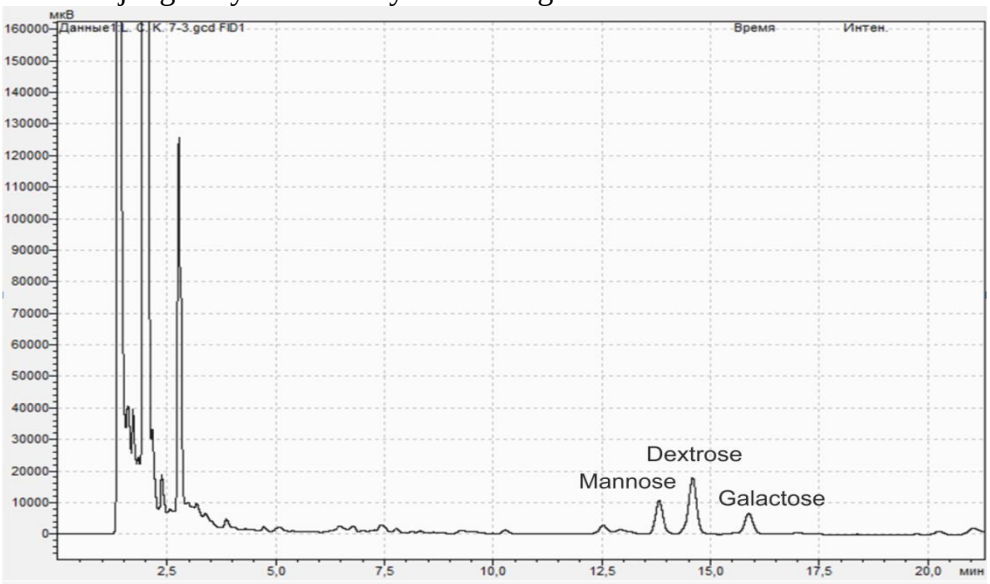

Figure 2. GC chromatogram of the monosaccharide composition of the polysaccharide from *L.casei* K7/3

DPPH free radicals are stable radicals with an unpaired valence electron of one atom of the nitrogen bridge, which significantly decrease under the influence of protons of the radical acceptor (Kodali V.R. et al, 2008). In our work, the radical scavenging activity of EPS of *L.casei K7/3* was determined using a colorimetric method at concentrations of 2, 3, and 4 mg/ml, ascorbic acid served as a positive control. The radical scavenging activity of EPS from *L.casei K7/3* increases, depending on the concentration of the investigated EPS and % RSA were at an EPS concentration of 2 mg/ml 12%; at 3 mg/ml 12% and at 4 mg/ml 26%.

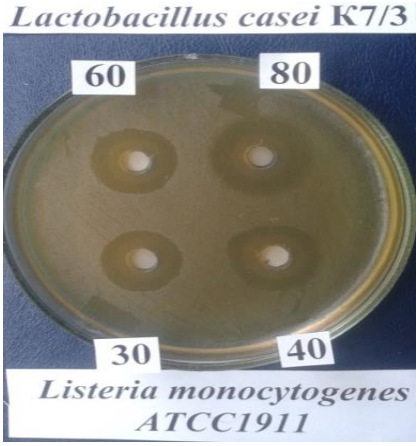

Figure 3. Antibacterial activity of EPS from *L.casei* K7/3

4. DISCUSSION

Isolation of polysaccharide from L.casei K7/3 culture supernatant: In the work of Xin Wang et al. (2017) analysis of the raw EPS of *Lactobacillus plantarum* KX041 showed that the content of total sugars in this EPS is 60.59 ± 0.92%. Also, by the Sevage method were determined the protein content equal to 12.88 ± 0.64%. After removal of free EPS proteins by the Sevage method, residual proteins that are covalently linked to the polysaccharide molecule were detected (Xin Wang et al, 2017). Proteins contained in exopolysaccharides, play an important role in modifying the physicochemical, thermal properties, and bioactivity of polysaccharides (F.C. de Oliveira et al. 2016).

The yield of heteropolysaccharides is from 0.05 to 0.60 g/l (Broadbent J.R., et al 2003) on the contrary, homopolysaccharides are synthesized in large quantities up to a few grams/liter (Ruas-Madiedo P. et al., 2009).

Whey comprises 80 to 90% of the volume of milk entering the cheese making process and contains about 50% of the solids present in the original whole milk, including 20% of the protein and most of the lactose, minerals, and water-soluble vitamins (Marshall K.R. et al, 1982). Greater polysaccharide production by lactobacilli strains recorded in Whey has been explained by Sutherland (1972). It was reported that when cells were grown slowly, synthesis of cell wall polymer was also slow, making more isoprenoid phosphate available for EPS synthesis (Sutherland I., 1972).

1. *Infrared spectroscopy (IR) analysis of exopolysaccharides:* Fourier transform-infrared spectroscopy has been an effective method in monitoring structural changes in biopolymers (Wilson R. H. et al, 1998). Widely located peak at 3278.02 cm^{-1} indicated the presence of O–H stretching in hydrogen bonds, which was indicative of strong inter- and intra-molecular interactions of the EPS chains (Shengjie Li et al., 2014). A weak peak at 2920.21 cm^{-1} indicates the presence of aliphatic CH_2 groups, which are contained in proteins and other organic substances. The peak at 1633.71 cm^{-1} represents the extended vibrations of the C = O group (Sutherland I.W., 1977). Short peaks in the region below 1538.23 cm^{-1} indicate the presence of sulfated groups and that this substance is a polysaccharide (Sutherland I.W.,

1972). Also, the peak at 1224.80 cm^{-1} belongs to CO bonds in ether or alcohol groups (Tallon R. et al., 2003).The peaks in the range of 1056.99 - 920.27 cm^{-1} means vibrations of C-O and C-O-C glycosidic bonds in the carbohydrates (Tserovska L. et al., 2002). The presence of carboxyl groups in the IR spectrum of the polysaccharide can serve as a binding site for bivalent cations (Van den Berg D.J.C. et al., 1993).

Monosaccharide composition and molecular weight of exopolysaccharides: According to the literature, EPS from various strains of *Lactobacillus* are heteropolysaccharides and mainly consist of glucose, galactose, mannose, arabinose, and rarely xylose or fructose. When analyzing the monosaccharide composition of L-EPS from *Lactobacillus casei* WXD030 strain was mainly composed of glucose, glucosamine, mannose, in an approximate molar ratio of 1.4:1.1:1 (Lei Xiua, et al, 2018).

The molecular mass of exopolysaccharides of lactobacilli ranges from 40 to 6000 kDa (Ruas-Madiedo P. et al., 2002). The molecular weight of EPS obtained from *L. plantarum* YW32 is determined to be 1.03 x 10^5 Da. The polydispersity index was 1.255, which means the presence of a homogeneous EPS material in the test sample (Ji Wang et al, 2015).

It has been reported by Mende et al (2016) that the low molecular weight polymer correlates with capsular polysaccharides, whereas the high molecular weight is found free in the extracellular matrix (Mende, Rohm and Jaros 2016).

Many authors claim that molecular weight has a great importance in the manifestation of the biological activity of EPS. EPS with a high molecular weight possess antitumor activity than EPS with a low molecular weight (Peng Y. et al, 2010).

Antimicrobial Activity of EPS: A few studies showed that EPS from microorganisms had strong antimicrobial activity against several pathogens in vitro and have proposed several putative antibacterial mechanisms of EPS, such as impairing cell division, disrupting the cell wall and cytoplasmatic membrane, and decomposing DNA (He F. et al, 2010).

Agar diffusion assay showed that EPS from *Lactobacillus plantarum* R315 (L-EPS) exhibited antibacterial activities against tested pathogens such as *Cronobacter sakazakii, Escherichia coli, Listeria monocytogenes, Staphylococcus aureus, Candida albicans, Bacillus cereus, Salmonella typhimurium* and *Shigella sonnei* at 300 mg/mL (Shengjie Li et al, 2014).

In the work of Abdelkarim Mahdhi (2017) The EPS of the strain of lactobacilli had a molecular weight of 36 kDa and polydispersity index estimated to be 1.2. The tested EPS LB had an antibacterial activity, with a Minimal Inhibition Concentration (MIC) values ranging between 1 mg/ml and 10 mg/ml, displayed an antibiofilm effect concentration dependent on Gram positive and negative strains (Abdelkarim Mahdhi et al, 2017).

Antioxidant activity of EPS: In the work of Zouaoui Benattouche et al. the purified EPS from *S. thermophilus* had a DPPH radical-scavenging activity, with an IC50 value of 225 µg/ml. which was much higher than that of the standard antioxidant ascorbic acid (48 µg /mL) (Zouaoui Benattouche et al, 2018). Due to the presence in the raw EPS of other antioxidant substances, such as proteins, peptides and trace elements, crude EPS has greater antioxidant activity than purified. Moreover, they act as synergists and interact with the binding of free radicals (Wei Li et al, 2014).

EPS bioactivity may depend on many factors, such as chemical composition, molecular weight, structure, configuration, extraction and purification conditions (Chen, H. et al, 2008). Xu R, et al. showed that the high antioxidant activity of EPS from *Bifidobacterium animalis* PH is due to its low molecular weight (Xu R. et al, 2011).

5. CONCLUSIONS

In this study, *L.casei* K7/3 strain studied could produce EPS under laboratory conditions, and a medium on basis of the whey of cottage cheese (sour milk tvorog) proposed by K.D. Karapetyan et al. was more effectively compared with MRS-broth. EPS had a low molecular weight and consists of dextrose, mannose and galactose in a molar ratio of 2.7:1.5:1,

respectively. The results showed that EPS from *L.casei* K7/3 strain had strong antibacterial ability against the tested pathogen strain of *Listeria monocytogenes*. Although EPS from *L.casei* K7/3 showed novel biological activities, further studies are needed to evaluate their structure and the components responsible for antimicrobial activity and mode of actions of EPS.

6. REFERENCES

[1] Abdelkarim Mahdhi, Nadia Leban, Ibtissem Chakroun, Mohamed Aymen Chaouch, Jawhar Hafsa, Kais Fdhila, Kacem Mahdouani, Hatem Majdoub (2017): Extracellular polysaccharide derived from potential probiotic strain with antioxidant and antibacterial activities as a prebiotic agent to control pathogenic bacterial biofilm formation. Microbial Pathogenesis, Volume 109, P. 214-220.

[2] Arasimovich V.V. (1984): Biochemical methods of fruit analysis. Chisinau.

[3] Badel S., Bernardi T., Michaud P. (2011): New perspectives for Lactobacilli exopolysaccharides. *Biotechnology Advances*, vol.29, no. 1, P. 54–66.

[4] Berg D.J.C., Robijn G.W., Janssen A.C (1995): Applied and Environmental microbiology, 61(8), P. 2840-2844.

[5] Botelho P. S., Maciel M. I., Bueno L. A., Maria de F´atima F. M., Marques D. N. and S. Silva T. M. (2014): Characterization of a new exopolysaccharide obtained from of fermented kefir grains in soymilk. *Carbohydrate polymers*, vol. 107, P. 1–6.

[6] Bramhachari P. V., Kavi Kishor P. B., Ramadevi R., Kumar R., Rama Rao B., Dubey S. K. (2007): Isolation and characterization of mocous exopolysaccharide (EPS) produced by Vibrio furnissii strain VB0S3. *Journal of Microbiology and Biotechnology*, vol. 17, no. 1, P. 44–51.

[7] Broadbent JR, McMahon DJ, Welker DL, Oberg CJ, Moineau S (2003): Biochemistry, genetics, and applications of exopolysaccharide production in *Streptococcus thermophilus*: a review. J Dairy Sci, 86: P. 407–423.

[8] Cerning, J. and Marshall VME (1999): Exopolysaccharides produced by the dairy lactic acid bacteria. Recent Results and Developments in Microbiol. 3: P.195 – 209.

[9] Chen, H., Zhang, M., Qu, Z., & Xie, B (2008): Antioxidant activities of different fractions of polysaccharide conjugates from green tea (camellia sinensis). Food Chemistry, 106 (2), P. 559-563.

[10] Chen, T., Wu, Q., Li, S., Xiong, S., Jiang, S., Tan, Q., Zhang, Z., Zhu, D., and Wei, H (2014): Microbiological quality and characteristics of probiotic products in China. *J. Sci. Food Agric.*; 94: P.131–138.

[11] Cheng-Chih Tsai, Pei-Pei Lin, You-Miin Hsieh, Zi-yi Zhang, Hui-Ching Wu, Chun-Chih Huang (2014): Cholesterol-Lowering Potentials of Lactic Acid Bacteria Based on Bile-Salt Hydrolase Activity and Effect of Potent Strains on Cholesterol Metabolism In Vitro and In Vivo. Scientific World Journal Volume 2014, P. 1-10. http://dx.doi.org/10.1155/2014/690752

[12] Davis, B.D., Dulbecco, R., Eisen, H.N. and Ginsberg, H.S (1980): Microbiology 3rd ed. Harper and Row Publishers Inc. New York. P. 67-68.

[13] de Oliveira F.C., J.S. dos Reis Coimbra, E.B. de Oliveira, A.D. Giraldo Zuniga, E.E.Garcia Rojas (2016): Food protein-polysaccharide conjugates obtained via the maillard reaction: a review, Crit. Rev. Food Sci. Nutr. 56 (7), P. 1108–1125.

[14] Degeest B., Vaningelgem F. and L. De Vuyst (2001): Microbial physiology, fermentation kinetics and process engineering of heteropolysaccharides production by lactic acid bacteria. Int. Dairy J. 11: P. 747–758.

[15] Doleyres Y, Schaub L, Lacroix C (2005): Comparison of functionality of exopolysaccharides produced in situ or added as bioingredients on yoghurt properties. J DairySci. 88: P. 4146–4156.
[16] Dubois M, Gilles K, Hamilton J., Rebers P, Smith F (1956): Colorimetric methods for determination of sugars and related substances. Annals of chemistry; 28: P. 350-356.
[17] Fukuda K., Shi T., Nagami K., Leo F., Nakamura T., Yasuda K., Senda A., Motoshima H., Murashima T (2010): Effects of carbohydrate source on physicochemical properties of the exopolysaccharide produced by *Lactobacillus fermentum* TDS030603 in a chemically defined medium. Carbonhydrate polymer 9, P. 1040-1045.
[18] Ge, Y., Duan, Y., Fang, G., Zhang, Y., & Wang, S (2009): Polysaccharides from fruit calyx of Physalis alkekengi var. francheti: Isolation, purification, structural features and antioxidant activities. Carbohydrate Polymers, 77(2), P. 188–193.
[19] He, F., Yang, Y., Yang, G., and Yu, L (2010): Studies on antibacterial activity and antibacterial mechanism of a novel polysaccharide from *Streptomyces virginia* H03. *Food Contr.* 2010; 21: P. 1257–1262.
[20] Ji Wang, Xiao Zhao, Yawei Yang, Aimei Zhao, Zhennai Yanga (2015): Characterization and bioactivities of an exopolysaccharide produced by *Lactobacillus plantarum* YW32. International Journal of Biological Macromolecules 74. P. 119–126.
[21] Karapetyan K.D., Hakobyan A.S., Tkchruni F.N., Balabekyan C.R. (2008): Influence of cultural conditions on the properties of Lactic acid bacteria. Scientific notes Yerevan State University, 3. P. 125-130.
[22] Kimmel S. A., Roberts R. F., Ziegler G. R. (1998): Optimization of exopolysaccharides production by Lactobacillus delbruecki ssp bulgaricus RR grown in a semi - defined medium. Appl. Environ. Microbiol. 64: P. 659-664.
[23] Kodali, V.R., Sen, R (2008): Antioxidant and free radical scavenging activities of an exopolysaccharides from a probiotic bacterium // Biotechnology journal. 3(2), P. 245-251.
[24] Larkin M.A., Blackshields G., Brown N.P., Chenna R., Mc Gettigan P.A., Mc William H., Valentin F., Wallace I.M., Wilm A., Lopez R., Thompson J.D., Gibson T.J., Higgins D.G. Clustal W (2007): Clustal X. Version 2.0. Bioinformatics. Vol. 23. P. 2947-2948.
[25] Lee, B.H (1996): Bacteria-based Processes and Products. In: Fundamentals of Food Biotechnology, Lee, B.H. (Ed.) John Wiley and Sons, New York, P. 219-290.
[26] Lei Xiua, Haochi Zhang, Zhongpeng Hua, Yanchen Liang, Shuai Guo, Ming Yang, Ruiping Dub and Xiao Wanga (2018): Immunostimulatory activity of exopolysaccharides from probiotic Lactobacillus casei WXD030 strain as a novel adjuvant in vitro and in vivo. Food and agricultural immunology, VOL. 29, NO. 1, P. 1086–1105.
[27] Lin, C.C. and. Casida, L.E (1984): Gelrite as a gelling agent in media for the growth of thermophilic organisms. Appl. Environ. Microbiology. 47: P. 427-429.
[28] Marshall V.M., Cowie E.N., Moreton R.S. (1995): Analysis and production of two exopolysaccharides from Lactococcus lactis ssp. cremoris LC 330. J. Dairy Res. 62: P. 621-628.
[29] Marshall, K. R. (1982): Industrial isolation of milk proteins: Whey proteins. Pages 339–373 in Developments in Dairy Chemistry. Vol. 1. P. F. Fox, ed. Applied Science Publishers, London.
[30] Mende S, Rohm H, Jaros D., 2016: Influence of exopolysaccharides on the structure, texture, stability and sensory properties of yoghurt and related products. *Int Dairy J*; 52: P. 57–71.
[31] Peng, Y.; Zhang, L.; Zeng, F.; Kennedy, J.F (2005): Structure and antitumor activities of the water-soluble polysaccharides from *Ganoderma tsugae mycelium*. Carbohydr. Polym. 59, P. 385–392.

[32] Pham P. L., Dupont I., Roy D., Lapointe G., and Cerning J (2000): Production of exopolysaccharide by *Lactobacillus rhamnosus* R and Analysis of Its Enzymatic Degradation during Prolonged Fermentation, Applied and Environment Microbiology 66 (6), P. 2302-2310.

[33] Ruas-Madiedo P., Hugenholtz J., and Zoon P. (2002): An overview of the functionality of exopolysaccharides produced by lactic acid bacteria. *International Dairy Journal*, vol. 12, P. 163–171.

[34] Ruas-Madiedo, P., and C. G. de los Reyes-Gavilon (2005): Methods for the screening, isolation, and characterization of exopolysaccharides produced by lactic acid bacteria// J. Dairy Sci. 88: P. 843–856.

[35] Ruas-Madiedo, P., Salazar, N., & De los Reyes-Gavilon, C. G (2009): Biosynthesis and chemical composition of exopolysaccharides produced by lactic acid bacteria. In Bacterial Polysaccharide. In M. Ullrich (Ed.), Current Innovations and Future Trends, Norwich, UK: Caister Academic Press. P. 279–310.

[36] Seesuriyachan P., Kuntiyal A., Hanmoungjai P. and Techapun C. (2011): Exopolysaccharide production by *Lactobacillus confusus* TISTR 1498 using coconut water as an alternative carbon source: the effect of peptone, yeast extract and beef extract, Songklanakarin Journal of Science and Technology **33** (4), P. 379-387.

[37] Shengjie Li, Renhui Huang, Nagendra P. Shah, Xueying Tao, Yonghua Xiong, Hua Wei, (2014): Antioxidant and antibacterial activities of exopolysaccharides from *Bifidobacterium bifidum* WBIN03 and *Lactobacillus plantarum* R315. Journal of Dairy Science, Volume 97, Issue 12, P. 7334–7343

[38] Sutherland I.W. (1977): Bacterial exopolysaccharides: their nature and production. In: Sutherland I.W. (ed) Surface carbohydrates of the procaryotic cell. Academic Press, London New York San Francisco, P. 27–96.

[39] Sutherland I.W. (ed.) (1972): Surface carbohydrates of the prokaryotic cell. Academic Press Inc. New York.

[40] Tallon R., Bressollier P., Urdaci C. M. (2003): Isolation and characterization of two exopolysaccharides produced by *Lactobacillus plantarum* EP56, Research in Microbiology 154. P. 705-712.

[41] Tserovska L., Stefanova and T. Yordanuva (2002): Identification of lactic acid bacteria isolated from Katyk, goat's milk and cheese. J. Cult. Collec., 3: P. 48-52.

[42] Van den Berg D.J.C., Smith A., Pot B., A.M. Ledeboer, K. Kerstens, J.M.A. Verbakel and C.T. Verrips (1993): Isolation, screening and identification of lactic acid bacteria from traditional food fermentation processes and culture collections. Food Biotechnol. 7: P. 189-205.

[43] Van Dyk J. S., Kee N. L. A., Frost C. L. and B. I. Pletschke (2012): Extracellular polysaccharide production in Bacillus licheniformis SVD1 and its immunomodulatory effect. *BioResources*, vol. 7, no. 4, P. 4976–4993.

[44] Van Kranenburg R., Marugg J.D., Swam II van, Willem N.J., de Vos W.M. (1997): Molecular characterization of the plasmid-en-coded eps gene cluster essential for exopolysaccharide biosynthesis in Lactococcus lactis. Mol Microbiol. 24: P. 387-397

[45] Van Niel E.W.J. and B. Hahn-Hägerdal (1999): Nutrient requirements of Lactococci in defined growth media. Appl Microbiol Biotechnol. 52: P. 617-627.

[46] Wang K, Li W, Rui X, Li T, Chen X, Jiang M, Dong M. (2015b): Chemical modification, characterization and bioactivity of a released exopolysaccharide (r-EPS1) from *Lactobacillus plantarum* 70810. Glycoconj J. 32 (1-2): P. 17-27.

[47] Wang K., Li W., X. Rui, X. Chen, M. Jiang, M. Dong (2014): Characterization of a novel exopolysaccharide with antitumor activity from *Lactobacillus plantarum* 70810, Int. J. Biol. Macromol. 63. P. 133–139.

[48] Wei Li, Juan Ji, Xiaong Chen, Mei Jiang, Xin Rui, Mingsheng Dong (2014): Structural elucidation and antioxidant activities of exopolysaccharides from Lactobacillus helveticus MB2-1// Carbohydrate Polymers. 102, P. 351-359.
[49] Wilson R. H., Goodfellow, B. J., Belton, P. S. (1998): Fourier transform infrared spectroscopy for the study of food biohydrocoloids. Food Hydrocolloids, 2, P. 169–178.
[50] Xin Wang, Chunge Shao, Lian Liu, Xing Guo, Yuanmei Xu, Xin Lü (2017): Optimization, partial characterization and antioxidant activity of an exopolysaccharide from *Lactobacillus plantarum* KX041. International Journal of Biological Macromolecules 103. P. 1173–1184
[51] Xu R, Shen Q, Ding X, Gao W, Li P (2011): Chemical characterization and antioxidant activity of an exopolysaccharide fraction isolated from *Bifidobacterium animalis* RH. Eur. Food Res. Technol. 232: P. 231-240.
[52] Yadav V., Prappulla S. G., Jha A., Poonia A. (2011): A novel exopolysaccharide from probiotic *Lactobacillus fermentum* cfr 2195: Production, purification and characterization. *Biotechnol. Bioinf. Bioeng.*, vol. 1, P. 415–421.
[53] Yeanly Wuena Pinaria, Nyoman Semadi Antara, G.P. Ganda Putra, Nengah Sujaya. (2016): Characterization of exopolysaccharide Produced by *Lactobacillus casei* AL15 Isolated from sap of *Arenga pinnata* // Journal of Natural Sciences Research. Vol. 6, No. 22.
[54] Yermakov A.I., Arasimovich V.V (1982): In the book: Methods of biochemical research of plants M. p.430. [Russian]
[55] Yuksekdag Z. N., Aslim B (2008): Influence of different carbon sources on exopolysaccharide production by *Lactobacillus delbrueckii subsp. bulgaricus* (B3, G12) and *Streptococcus thermophilus* (W22). Brazilian archives of Biology and Technology 51 (3), P. 581-585.
[56] Zhang Y., Li S., Zhang C., Luo Y., Zhang H., Yang Z. (2011): Growth and exopolysaccharide production by *Lactobacillus fermentum* F6 in skim milk. African Journal of Biotechnology 10 (11), P. 2080-2091.
[57] Zouaoui Benattouche, Djillali Bouhadi, Ghalem Bachir Raho (2018): Antioxidant and Antibacterial Activities of Exopolysaccharides Produced by Lactic Acid Bacteria Isolated from Yogurt// International Journal of Food Studies. Volume 7. P. 30-37.