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CHARACTERISTICS OF EXOPOLYSACCHARIDE *LACTOBACILLUS CASEI* CO_1 AND ITS ANTIOXIDANT ACTIVITY

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РЕЗЮМЕ

В работе приведены результаты исследований по изучению способности синтезировать экзополисахариды (ЭПС) местным штаммом *Lactobacillus casei* CO_1, выделенного из эпифитной микрофлоры цветов сирени. В ИК-спектре ЭПС обнаружены интенсивные полосы поглощения в целом характерные для класса углеводов. Установлен моносахаридный состав и определена молекулярная масса экзополисахарида. Выявлена %АРА ЭПС в системе ДФПГ (дифенилпикрилгидразин).
Ключевые слова: лактобактерии, экзополисахарид, моносахаридный состав, антиоксидант.

ABSTRACT

In this work presents the results of studies on the ability to synthesize exopolysaccharides (EPS) by a local strain of *Lactobacillus casei* CO_1, isolated from the epiphytic microflora of lilac flowers. In the IR spectrum of EPS, intense absorption bands were found, generally characteristic of the carbohydrate class. The monosaccharide composition was established and the molecular weight of the exopolysaccharide was determined. Revealed % RSA of EPS in the system of DPPH (diphenylpycrylhydrazil).
Keywords: lactobacilli, exopolysaccharide, monosaccharide composition, antioxidant.

INTRODUCTION

Among lactic acid bacteria, special attention is paid to bacteria of the genus *Lactobacillus*, whose representatives are widely distributed in nature. Various researchers have shown that lactobacilli have great potential for the synthesis of exopolysaccharides [1].
Some LAB (lactic acid bacteria) form polysaccharides, which are released from the cell as components of the cell wall (peptidoglycans). The latter are either firmly attached to the surface of the microbial cell in the form of a capsule (capsular polysaccharide, CPS) or released into the environment as exopolysaccharides (EPS) [2].
Cultures *L.casei, L.lactis, L..sakei, L.rhamnosus,St.thermophilus* refer toheteropolysaccharide-forming bacteria [3]. The structure of heteropolysaccharides can contain several copies of oligosaccharides, which contain from three to eight residues.Two or more different monosaccharides are usually present in each repeating unit and show different types of bonds. It was found that EPS from LAB have significant antioxidant and antitumor activity, and recently they have attracted much attention. L. Zhang et al. [4] found that EPS from *Lactobacillus plantarum* C88 is effective in removing reactive oxygen species (ROS) *in vitro*. EPS from *L.acidophilus* 606 can inhibit the proliferation of HT-29 colon cancer cells, directly affecting cell morphology [5].
The aim of the work is to identify the synthesis of exopolysaccharide by *Lactobacillus casei* CO_1 culture, to study the physicochemical properties and antioxidant activity of the polysaccharide obtained.

Materials and methods.
The *Lactobacillus casei* CO_1 culture was isolated from the epiphytic microflora of lilac flowers, identified by the classical method and deposited in the collection of the Institute of Microbiology of the Academy of Sciences of the Republic of Uzbekistan.

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The isolation of the polysaccharide from the culture fluid *L. casei* CO_1 was performed according to the method described in Cerning, J. et al. [6]. The culture of *L. casei* CO_1 was recovered from the lyophilized state by 2-3 subcultures in MRS-broth and incubation at 37 ° C for 48 hours.The inoculum in a volume of 20 ml (2%, w/v) was added to the MRS-broth medium and incubated at 37°C for 48 hours under aerobic conditions. After incubation, cultures were added with TCA 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 7,000xg for 30 minutes at 4°C. Equal volume of the cooled ethanol was added to the supernatant and kept at 4°C for 48 hours. The precipitated EPS was collected by centrifugation at 7,000 x g for 30 minutes at 4°C. The precipitate was dissolved in distilled water and dialyzed at 4 ° C for 48 hours and then dried by lyophilization. The total amount of carbohydrates in the lyophilized exopolysaccharides of lactobacilli was determined by the phenol-sulphuric method [7]. Quantitative determination of proteins in the composition of the raw exopolysaccharide was carried out by the method described by Yermakov A.I. and others [8].

Infrared spectroscopic (IR) analysis of the crude exopolysaccharide. The IR spectra of exopolysaccharide of *L. casei* CO_1 were recorded on a Fourier transform by a Vector-22 IR spectrophotometer (Bruker, Germany) in the frequency range 400–4000 cm^{-1}. Two mg of exopolysaccharide was mixed with 200 mg of potassium bromide (KBr) (1: 100 ratio), then the mixture was pressed into a form with a diameter of 16 mm and IR spectroscopy was performed to detect functional groups characteristic of polysaccharides [9].

Analysis of monosaccharide composition of exopolysaccharide from *L. casei* CO_1. The installation of the monosaccharide composition obtained by EPS using gas chromatography [10].
To establish the monosaccharide composition, the polysaccharide was hydrolyzed with 1N concentrated sulphuric acid at a temperature of 100°C for 6 hours. Then the hydrolyzate was neutralized with barium carbonate, deionized with KJ-2(H^+) and evaporated o 1 ml on a rotary evaporator. The identification and quantitative composition of the monosaccharides was determined on a GC Plus2010 gas chromotograph (Shimadzu, Japan) under the following conditions: temperature of injector 250°C, total flow 60 ml/min, flow through the column 0,89 ml/min, carrier gas - nitrogen, column - Rxi-624SI MS, column length – 3 m, inner diameter ID – 0,25 mm, the column temperature is 230°C, the temperature of the detector is 250°C, the form of derivatives is acetates of aldonitriles.

Molecular mass characteristics were determined on an Agilent 1260 Infinity SEC chromatograph (Agilent Technologies, USA).

Antioxidant activity. Antioxidant activity was judged by the binding of 1,1-diphenyl-2-pycryl-hydrazile oxide radicals (DPPH) [11]. To 1 ml of an aqueous solution of exopolysaccharide from *L. casei* CO_1 was added 2.0 ml of an alcohol solution of DPPH (0.4 mM). 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). Radical scavenging activity was calculated by the formula: DPPH radicals scavenging activity (%) $=\frac{(Ao-Ai)}{Ao}$x100, where - A1 - the absorption coefficient of the solution with a certain sample content; Ao is the absorption coefficient of the DPPH solution without a sample. An aqueous solution of ascorbic acid was used as a control.

Results and discussion

Isolation of the polysaccharide from the culture fluid *L. casei* CO_1. The output of freeze-dried crude EPS *L. casei* CO_1 was 400 mg/L. The lyophilized EPS of creamy beige color, well dissolved in water, had a smooth fibrous structure. Crude EPS contained 7.11% protein (nitrogen content 1.13%).
Exopolysaccharide biosynthesis is a complex process involving a large number of enzymes and regulatory proteins. In mesophilic LAB strains, like *Lactococcus*, the genes encoding proteins involved in the synthesis of EPS are located in plasmids, and in thermophilic streptococci and lactobacilli in chromosomes [12].Typically, the yield of heteropolysaccharides is from 0.05 to 0.60 g/l [13], on the contrary, homopolysaccharides are synthesized in large quantities to almost a few grams/liter [14].
MRS broth is the most suitable medium for the growth and synthesis of LAB biopolymers. But for industrial purposes and from an economic point of view, the use of waste from other industries as a basis for the nutrient medium is appropriate [15]. There are many discussions about the formation of EPS under the influence of various conditions.It is generally recognized that the cultivation conditions or several other factors (pH, temperature, incubation time and composition of the growing medium) have a significant impact on the yield and EPS composition.The results of studies by some authors showthat the lactobacilli strain, depending on the carbon source of the nutrient medium, can produce EPS with different rheological properties [16]. B. Adebayo-Tayo et al (2008) notes that serum is the best medium for EPS production in which *L. casei* LCN1 synthesized 198.69 mg/l of polymer [17].

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Infrared spectroscopic (IR) analysis of the crude exopolysaccharide*L. casei CO$_1$*. In the IR spectrum of EPS, intense absorption bands were found, generally characteristic of a class of carbohydrates (**figure 1**).

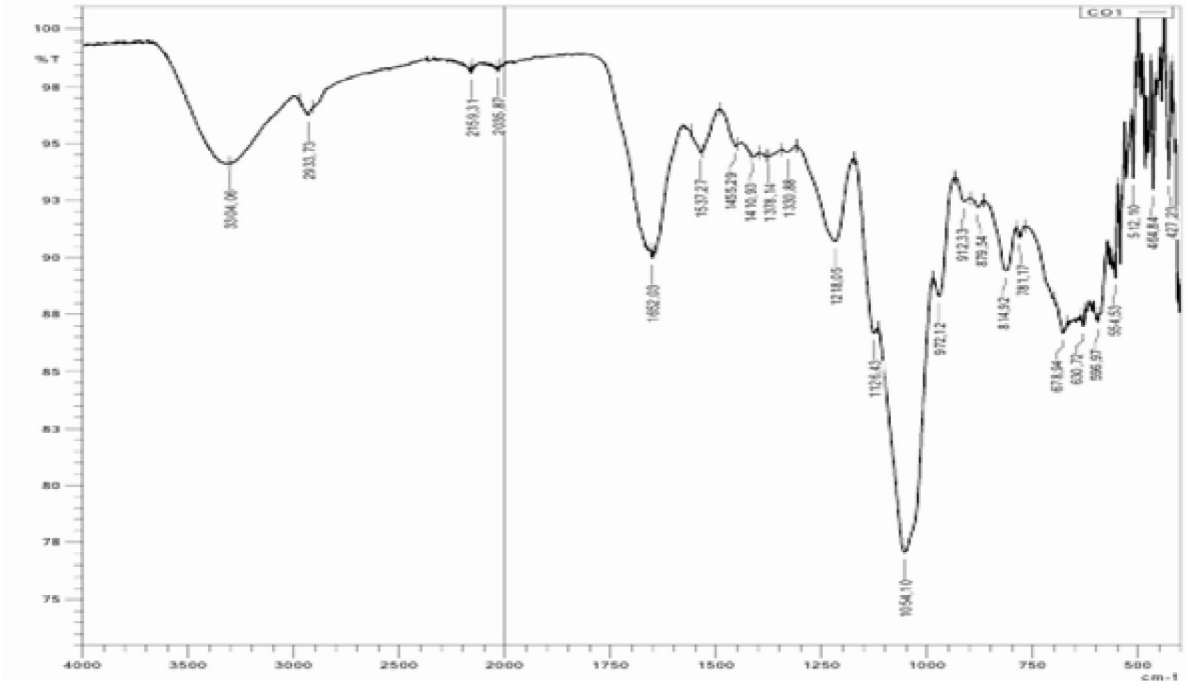

Figure 1. IR spectrum of EPS from *L. casei CO$_1$*.

The widely located peak at 3304.00 cm^{-1} belongs to the hydroxyl group [18]. A weak peak at 2933.73 cm^{-1} indicates the presence of aliphatic CH_2, which are found in proteins and other organic substances. Peak at 1662.03 cm^{-1} resembles a mannose or galactose ring. A symmetrically extended absorption peak at 1378.14 cm^{-1} is formed from the –COO – group. Peaks in the range of 1218.05-1054.16 cm^{-1} indicate C-O and C-O-C glycosidic link vibrations, demonstrating the presence of carbohydrates.The sharp peak at 1054.16 cm^{-1} indicates the presence of a polysaccharide [19].

Monosaccharide composition of exopolysaccharide from *L. casei* CO$_1$.
GC analysis of the monosaccharide composition of EPS from *L. casei* CO$_1$ showed that this EPS consists of mannose, glucose and rhamnose in an approximate ratio of 11.3:1.7:1, respectively. The polysaccharide isolated from *L. casei* CGII grown in a medium containing 20 g/l as a carbon source, consisted of glucose (76%) and rhamnose (21%), and traces of mannose and galactose were also detected [20]. HPLC analysis of the monosaccharide composition of EPS obtained from *Lactobacillus sakei* CY1 showed that glucose and galactose predominate in its composition [21]. Verges et al. reported that the EPS of *Lactobacillus sakei* consists mainly of glucose and rhamnose in a ratio of 3:2 [22]. The purified exopolysaccharide produced by *Lactobacillus plantarum YW32* had a molecular weight of 1.03×10^5 Da and consisted of mannose, fructose, galactose and glucose in an approximate mass fraction of 8.2:1:4.1:4.2, respectively [23].
The molecular weight of the EPS obtained from *L.casei* CO$_1$ was 7.1x10^4 Da, the polydispersity index was 1.9 (**figure 2**).

M_w/M_n=1,9 71000 6300

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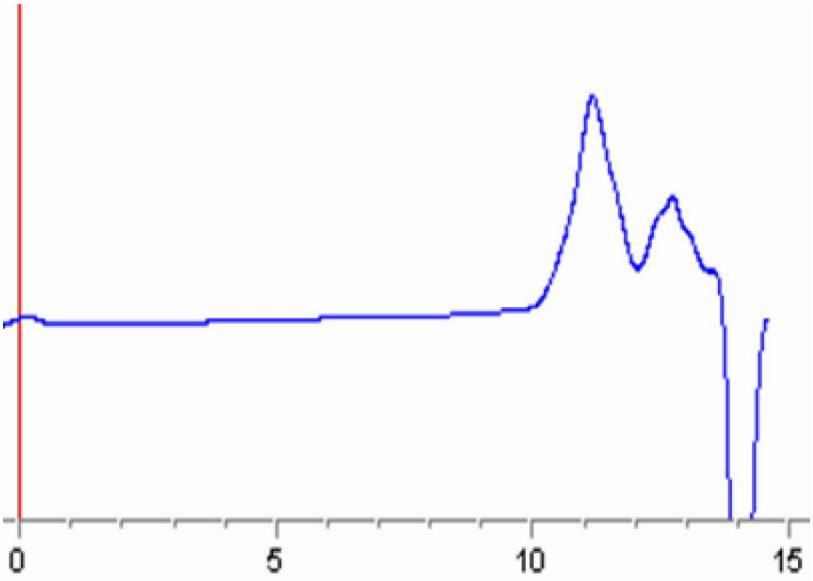

Figure 2. Chromatogram of molecular weight of EPS from *L. casei* CO_1

The molecular weight of EPS obtained from *L. plantarum* YW32 is determined to be $1.03x10^5$Da.The polydispersity index was equal to 1.255, which means the presence of a homogeneous material EPS in the sample under study [23]. Many authors argue that the molecular weight is of great importance in the manifestation of the biological activity of EPS. It was shown that high antioxidant activity of EPS from *Bifidobacterium animalis RH* is due to its low molecular weight [24]. EPS with a high molecular weight have antitumor activity than EPS with a low molecular weight [25].

Antioxidant activity of EPS from *L. casei* CO_1. Antioxidant activity may be due to a variety of reactions and mechanisms. In our work, we studied the antioxidant activity of EPS from *L. casei* CO_1 in order to bind the diphenylpycrylhydrazil oxide radicals (DPPH) *in vitro* in comparison with ascorbic acid. As can be seen from figure 3, the radical scavenging activity of EPS from *L. casei* CO_1 increases depending on the concentration of the investigated EPS: % of the radical scavenging activity (RSA) was at a concentration of EPS 2 mg/ml 17.7%; at 3 mg / ml 24% and at 4 mg / ml 26%.

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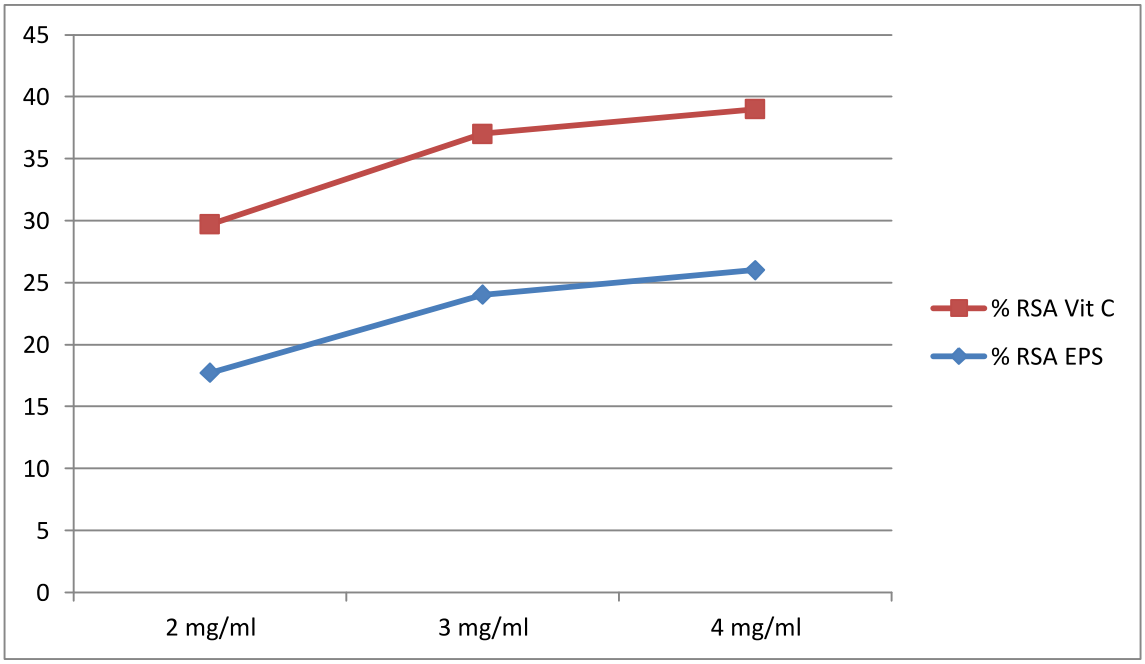

Figure 3. Antioxidant activity of EPS and ascorbic acid.

Excessive production of free radicals leads to oxidative damage to biomolecules (lipids, proteins, DNA), resulting in many chronic diseases, such as atherosclerosis, cancer, diabetes, rheumatoid arthritis, post-ischemic perfusion injury, myocardial infarction, cardiovascular diseases, chronic inflammation of organs, stroke and septic shock, aging and other diseases [26]. In Zouaoui Benattouche et al. notes shows that when studying the activity of EPS from S. *thermophilus*, the highest antioxidant activity of 55.83% was observed with the concentration of PES 1000 g/ml [27]. Although the EPS from *L. plantarum* YW32 comparatively low results were obtained than with ascorbic acid, but at a dose of 5 mg/ml, EPS shows promising antioxidant activity with 30% absorption of DPPH radicals [23].
The bioactivity of EPS may depend on many factors, such as chemical composition, molecular weight, structure, configuration, extraction and purification conditions.The molecular weight of EPS plays an important role in antioxidant activity [28].

CONCLUSIONS

EPS isolated from *L. casei* CO_1 has a molecular weight of $7.1x10^4$ Da and consists of mannose, glucose and ramnose, in a ratio of 11.3: 1.7: 1, respectively.Also, this EPS has antiradical activity and can be used as an alternative to chemical antioxidants in the food and pharmaceutical industry.

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