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RHEOLOGICAL FEATURES OF ISOLATES OF *BRADYRHIZOBIUM* NODULE BACTERIA

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Висвітлено, що властивості бактеріальних екзополісахаридів (ЕПС) дають змогу застосовувати їх у промисловості для іммобілізації мікроорганізмів у гельних препаратах. У промисловості вони можуть слугувати згущувачами, гелеутворювачами та стабілізаторами, оскільки мають здатність посилювати в`язкість розчинів. Встановлено, що вони також сприяють зберіганню бактеріальних клітин у природному середовищі та субстраті біопрепаратів для удобрення сільськогосподарських культур упродовж тривалого періоду. Ізолят LG 2 може утворювати 3,2 г/л ЕПС, що переважає контрольний штам інокулянту Ризоактив Р на 13,4%, а ізолят LG 5 мав здатність синтезувати ЕПС у кількості 2,8 г/л. Кількісний уміст (у %) моносахаридів, їх реологічні властивості оцінювали за площами піків на хроматографах. Визначено реологічні властивості культуральних розчинів отриманих ізолятів бульбочкових бактерій сої LG 2 та LG 5. Отримані ізоляти бульбочкових бактерій LG 2 та LG 5 завдяки інтенсивному синтезу ЕПС та значним реологічним властивостям можуть бути застосовані для створення сучасних препаратів на їх основі.

Ключові слова: *екзополісахариди, Bradyrhizobium japonicum, бобово-ризобіальний симбіоз, соя.*

EPS of nitrogen-fixing rhizobial bacteria are also potential biopolymers for industry, but they are still not widely used. One of the most important issues is the possibility of storage of microbial preparations for a long period. This is possible thanks to the isolation and efficient use of EPS of nodule bacteria. In the process of interaction and during the transmission of signals in legume-rhizobial symbiosis, polysaccharides play a significant role, which effectively interact with leguminous plants and enhance their adaptive mechanisms. They are excreted by many types of soil microorganisms. Bacterial exopolysaccharides (EPS) are widely used in different industries, they can also be used to immobilize microorganisms in gel preparations. In industry, they can serve as thickeners, gelling agents, stabilizers, for their ability to increase the viscosity of solutions [1].

It is known that rhizobia have the ability to form several types of surface polysaccharides, among which there are exopolysaccharides (EPS), lipopolysaccharide (LPS), capsular polysaccharides (CPS), neutral polysaccharides (NPS), gel-forming polysaccharides (GPS) and cellulose fibrils (CF). EPS has a nourishing, protective and reserve function, and also plays an important role in adhesion and recognition [2]. Microbial polysaccharides contain the necessary information about the symbiotic potential of bacteria: specificity, virulence, nitrogen-fixing activity, competitiveness, which is ensured by the carbohydrate-protein correspondence of the microsymbiont and macrosymbiont, and the formation of legume-rhizobial symbiosis most of all depends on the level of lectin-polysaccharide interaction [3]. Bacterial polysaccharides and plant lectins are responsible not only for the formation of symbiosis, but also for its functioning [4, 5].

Exopolysaccharides have a large suppressor effect, which is activated when plants are affected by nodule bacteria [6]. They also pro-

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tect the enzyme nitrogenase of the symbiotic apparatus of leguminous plants [7]. In recent years, microbial exopolysaccharides become one of the main object of research not only because of their importance in the metabolism of microorganisms, but also due to the diversity of the physicochemical structure that determines the properties of these polymers [8]. Microbial EPS can be used as suspending, gelling and emulsifying agents, due to their rheological properties [9, 10].

Exopolysaccharides of nitrogen-fixing rhizobial bacteria are also potential biopolymers for the production of drugs, because in the culture broth it increases its viscosity and provides for their adaptation to environmental conditions both in symbiosis with legumes and in a saprophytic status. The microbial exopolysaccharide can be used as an alternative to peat carrier for preparations. There are biopolymers of bacterial cells, they are synthesized and released into the extracellular environment, this allows us not to use minerals as a vector in the future to produce bacterial preparations [11–14].

The aim of the study was to test rheological abilities of *Bradyrhizobium* isolates, which are active producers of EPS.

MATERIALS AND METHODS

The object of the study was isolates of the nodule bacteria *Bradyrhizobium* sp., which are forming symbiosis with soybean *Glycine max* (L) Merril. Deep cultivation was carried out in 250 ml bottles, under conditions of constant mixing 180 rpm, and the temperature of 28±1°C. Bacteria were grown in a liquid mineral nutrient medium yeast mannitol agar (YMA) of the next composition (g/l): mannitol — 8.0; yeast extract — 2.0; glucose — 2.0; $(NH_4)_2SO_4$ — 0.5; K_2HPO_4 — 0.35; KH_2PO_4 — 0.35; $MgSO_4$ — 0.2; agar-agar — 20.0; pH 7.2 [15].

The isolation of the exopolysaccharides began with the separation of the culture fluid from the biomass, through centrifugation at 8.000 rpm for 10 minutes. The isolated culture broth was then added to isopropyl in a proportion of 5:1. The isolated precipitate was collected and dried in a vacuum drying cabinetuntil complete evaporation of isopropyl [16]

The dynamic viscosity of the cultures was determined using a capillary viscometer VPZh-2 [17].

To measure the optical density, every 8 hours a sample of 1 ml was taken at the same time with the biomass estimate. The resulting liquid culture was centrifuged at 12.000 rpm for 2 minutes until precipitate, washed with water and again centrifuged, repeating this procedure three times. Then, 1 ml of distilled water was added to the resulting precipitate and the optical density was measured on PG INSTRUMENTS T60 UV-Visible spectrophotometer.

The monosaccharide composition of exopolysaccharides was determined in the late logarithmic phase. For this, the bacterial mass was washed with a physiological solution, precipitated by centrifugation at 10.000 rpm for 20 minutes and washed twice. Bacterial cells were dried with acetone and diethyl ether. The supernatant was diluted with saline in the ratio of 1:1 and re-centrifuged at 10.000 rpm for 20 minutes. Exopolysaccharides were isolated from the supernatant by single precipitation with ammonium sulphate. The resulting preparation was dialyzed against a distilled carrier to a full type of SO_4 ions and lyophilized [18].

RESULTS OF THE RESEARCH AND DISCUSSION

From natural ecological niches, we isolated several highly active and competitive isolates of nodule bacteria of soybean. The range of synthesis of EPS by bacteria was in the range of 0.09–3.21 g/l. However, two isolates LG 2 and LG 5 were the most active and the number of synthesized EPS was at the level of the reference strain of Rizoaktiv R and also higher (fig. 1).

Among all eleven isolates of soybean nodule bacteria, two were characterized by a high content of EPS. The LG 2 isolate could syntheze 3.2 g/l exopolysaccharides, which is dominated by the reference strain of Rizoaktiv R by 13.4%. Also, LG 5 isolate was able to synthesize exopolysaccharides in the amount

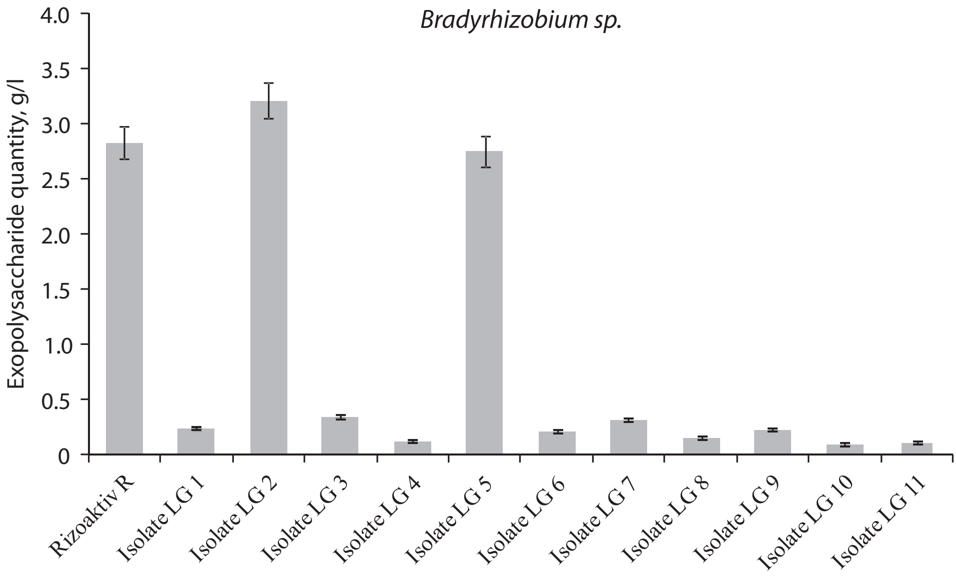

Fig. 1. The synthesis of exopolysaccharides by soybean nodule bacteria *Bradyrhizobium*

of 2.8 g/l. Actually the fact that this number of exopolysaccharides was known at the level of the control variant, these two isolates were chosen for further studies. Well-known fact that nodule bacteria synthesize exopolysaccharides in the amount of 0.5–1.5 g/l [2].

The presence of EPS in modern microbial preparations is important for the storage of high titer and high physiological activity of bacterial cells for a long period. So, based on these data, we can conclude about the effectiveness of the isolates given in our studies. The synthesis of exopolysaccharides by bacterial strains significantly contributes to the improvement of soil fertility, plant growth and development, as well as the formation of an effective symbiosis between the plant and the bacterium.

The practical value of EPS is determined by their rheological properties, the ability to increase the viscosity and plasticity of solutions. Therefore, we checked the rheological properties of two isolates that produced the greatest number of exopolysaccharides [19].

During the cultivation of EPS producers (isolates of soybean nodule bacteria), on the YMA medium showed an intensive growth of biomass in the variant represented by LG 2 isolate. It is known that under certain cultivation conditions the chemical composition of EPS, the balance of monosaccharides can change the practical value of these polymers.

The viscosity of the culture broth of the control variant – strain from Rizoaktiv R inoculant, reached 280 mPa·s. Meanwhile, from the soybean nodule bacteria presented in our studies, LG 2 isolate had the ability to form exopolysaccharides in an amount that exceeded the reference strain by 7.8% (fig. 2). The number of synthesized EPS of LG 5 isolate was within the range of chosen model

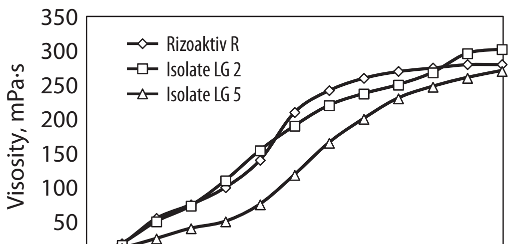

Fig. 2. Viscosity of the bacterial culture broth

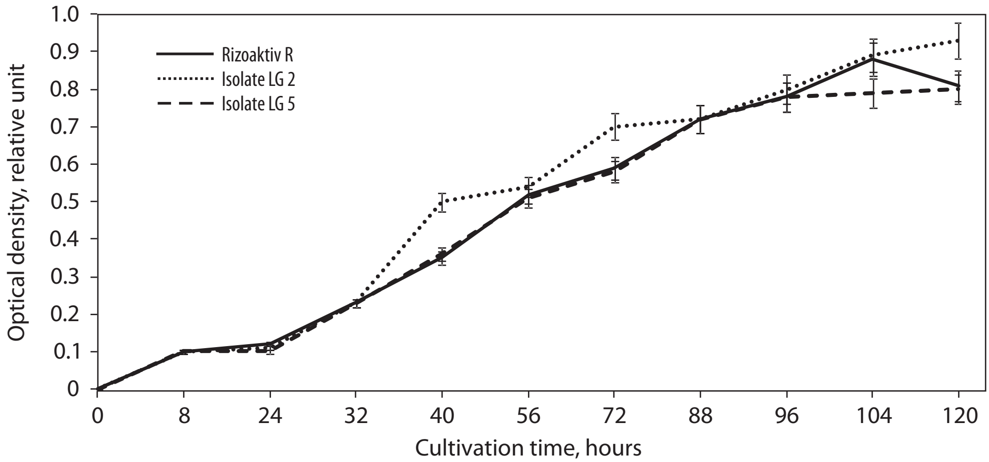

Fig. 3. Optical density of culture broth of *Bradyrhizobium* isolates

The monosaccharide composition of exopolysaccharides synthesized by *Bradyrhizobium japonicum* isolates

Variants of experiment	Monosaccharides, %			
	Glucose	Mannose	Galactose	Rhamnose
Rizoaktiv R (Control)	51,2	38,2	14,3	0,9
Isolate LG 2	47,5	34,5	17,6	0,4
Isolate LG 5	51,4	32,6	15,4	0,6

Note: the amount of monosaccharides is given as a percentage of the total area of the peaks.

strain of Rizoaktiv R. It is important how the rheological property of EPS as the optical density of the culture broth of isolates of nodule bacteria changes, depending on the cultivation time (fig. 3).

The analysis of the research results shows that the optical density increases according to the biomass of the presented experimental variants. Thus, the largest optical density was characterized by LG 2 isolate, it was 0.93±0.06 relative unit.

Consequently, EPS with pronounced rheological features are an effective stabilizing component when creating a gel medium for the cultivation of bacteria *Bradyrhizobium*. An important technological characteristic of microbial preparations is the duration of their storage.

The monosaccharide composition of exopolysaccharides synthesized by isolates was nearly the same The dominant monosaccharides of our studied isolates are glucose, mannose, and galactose (tabl.).

The number of rhamnosis in the EPS of all bacteria represented in this study was in a minor amount, that is, less than 1%. A similar composition of EPS was in *Rhizobium* strains, which were in the studies of Algerian and French scientists [20].

We consider, that it will be interesting to use these isolates of slow-growing *B. japonicum* bacteria in the future as the basis for liquid or gel biologics due to the fact that the qualitative composition of monosaccharides defined in the studies was at the level of the production inoculant Rizoaktiv R.

CONCLUSIONS

Isolates of soybean nodule bacteria *Bradyrhizobium* sp. are characterized by intensive synthesis of EPS and significant rheological properties. The further testing of the effect of these isolates on soybean yield and on plant biometrics will be important. It is necessary not to forget that these bacteria are isolated from the soil-climatic zone of Ukraine, so they will be effective in our country, they can quickly adapt to environment and increase the soil fertility.

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