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SYNTHESIS OF EXOPOLYSACCHARIDES BY BRADYRHIZOBIUM JAPONICUM ISOLATES

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Exopolysaccharides of nitrogen-fixing rhizobial bacteria are potential biopolymers for industry. One of the most important issues is the possibility of storage of microbial drugs for a long period. The isolate LG 2 is capable of forming 3,2 g/l exopolysaccharides, which is dominated by the control strain B. japonicum eko/001 by 13,4%, and the isolate LG 5 synthesized EPS in the amount of 2,8 g/l. Characterized by the rheological properties of culture solutions of isolates (viscosity, optical density). The resulting isolates of nodule bacteria LG 2 and LG 5, owing to the intensive synthesis of EPS and significant rheological properties, can be used to create modern preparations of soybean nodule bacteria. *Key words:* exopolysaccharides, legume-rhizobial symbiosis, soybean Bradyrhizobium japonicum.

Синтез екзополісахаридів ізолятами Bradyrhizobium japonicum. Гуменюк І.І., Грузінський С.Ю., Бровко І.С., Чабанюк Я.В. Екзополісахариди азотфіксувальних ризобіальних бактерій є потенційними біополімерами для промисловості. Одним із найважливіших питань залишається можливість зберігання мікробних препаратів упродовж тривалого періоду. Ізолят LG 2 здатний утворювати 3,2 г/л екзополісахаридів, що переважає контрольний еталонний штам B. Japonicum eko/001 на 13,4%, а ізолят LG 5 мав здатність синтезувати екзополісахариди в кількості 2,8 г/л. Охарактеризували реологічні властивості культуральних розчинів ізолятів (в'язкість, оптичну густину). Отримані ізоляти бульбочкових бактерій LG 2 та LG 5 завдяки інтенсивному синтезу ЕПС та значним реологічним властивостями можуть бути застосовані для створення сучасних препаратів бульбочкових бактерій сої. *Ключові слова:* екзополісахариди, бобово-ризобіальний симбіоз, соя Bradyrhizobium japonicum.

Синтез экзополисахаридов изолятами Bradyrhizobium japonicum. Гуменюк И.И., Грузинский С.Ю., Бровко И.С., Чабанюк Я.В. Экзополисахариды азотфиксирующих ризобиальных бактерий являются потенциальными биополимерамы для промышленности. Одним из важнейших вопросов остается возможность хранения микробных препаратов в течение длительного периода. Изолят LG 2 способен образовывать 3,2 г/л экзополисахаридов, что превышает контрольный штамм B. japonicum eko/001 на 13,4%, а изолят LG 5 синтезировал ЭПС в количестве 2,8 г/л. Охарактеризовали реологические свойства культуральных растворов изолятов (вязкость, оптическую плотность). Полученные изоляты клубеньковых бактерий LG 2 и LG 5 благодаря интенсивному синтезу ЭПС и значительным реологические свойства могут быть применены для создания современных препаратов клубеньковых бактерий сои. *Ключевые слова:* экзополисахариды, бобово-ризобиальный симбиоз, соя Bradyrhizobium japonicum.

Introduction. 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 the food and textile industry, 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]. 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 drugs for a long period. This is possible thanks to the isolation and efficient use of EPS of nodule bacteria.

Literary review. It is known that rhizobia have the ability to form several types of surface polysaccharides, among which 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 – the interaction with the lectins of the host plant [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 micro- and macrosymbiont, and the formation of legume-rhizobial symbiosis (LRS) depends on the level of lectin-polysaccharide interaction. Bacterial polysaccharides and plant lectins are responsible not only for the formation of symbiosis, but also for its functioning [3; 4].

EPS also have a suppressor effect, which is activated when plants are infected by nodule bacteria [5]. They also protect the enzyme nitrogenase of the symbiotic apparatus of leguminous plants [6]. In recent years, microbial EPS have 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 and allows them to be used in industry. Solutions of microbial polysaccharides should have a high viscosity at low concentration, stability in a wide temperature range and pH, resistance to mechanical destruction [7]. Microbial EPS can be used as suspending, gelling and emulsifying agents, due to their rheological properties [8; 9].

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 [4; 10; 11]. Rhizobia release a large number of EPS in the rhizosphere [6; 12]. Therefore, as an alternative to peat carrier, microbial EPS can be used [14; 15]. Since these are polymers of bacterial cells, they are synthesized and released into the extracellular environment, this allows us not to use minerals as a vectorin the future to produce bacterial preparations [6; 12; 15; 16].

The aim of the study was to identify strains of *Bradyrhizobium* – active producers of EPS with a high rheological ability.

Materials and methods. The object of the study was isolates of the nodule bacteria *Bradyrhizobium sp*., which forming symbiosis with soy *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 on a liquid mineral nutrient medium of the next composition (g/l): mannitol – 8; yeast extract – 2; glucose – 2; $(NH_4)_2SO_4$ – 0,5; K_2HPO_4 – 0,35; KH_2PO_4 – 0,35; $MgSO_4$ – 0,2; agar-agar – 20; pH 7.2. The isolation of the exopolysaccharides began with the separation of the culture fluid from the biomass, throughcentrifugation 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 vacuumdryingcabinetuntil complete evaporation of isopropyl [17].

The dynamic viscosity of the cultures was determined using a capillary viscometer VPZh-2 [18].

To measure the optical density, every 8:00, 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 a PG INSTRUMENTS T60 UV-Visible spectrophotometer.

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 best and the number of synthesized EPSs was at the level of the reference strain *B. japonicum* eko/001, as well and above it (Fig. 1).

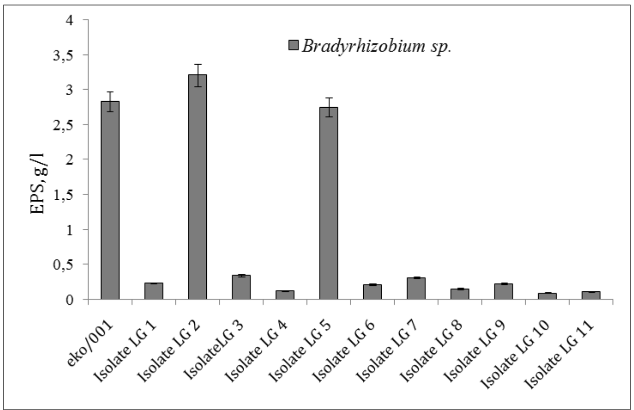

Fig. 1. The synthesis of exopolysaccharides by nodule bacteria of soybean

Among all eleven isolates of soybean nodule bacteria, two were characterized by a high content of EPS. LG2 isolate is capable of synthezing 3,2 g/l exopolysaccharides, which is dominated by the reference reference strain *B. japonicum eko*/001 by 13,4%. Also, LG5 isolate was able to synthesize exopolysaccharides in the amount of 2,8 g/l. Despite the fact that this number of exopolysaccharides was known at the level of the control variant, these two isolates were chosen for further studies. Usually, 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, the improvement of plant growth and development, as well as the formation of an effective symbiosis between the plant and the bacterium [3].

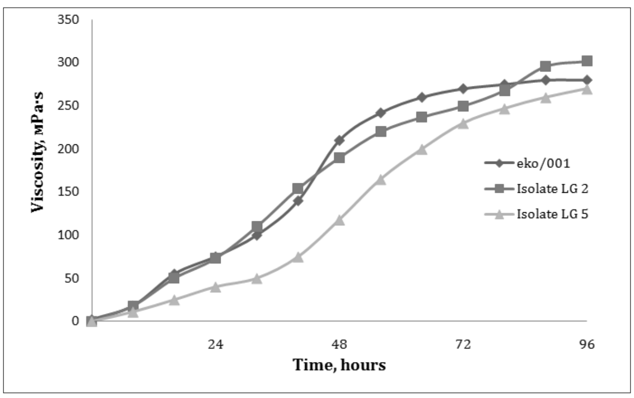

Fig. 2. The culture broth viscosity

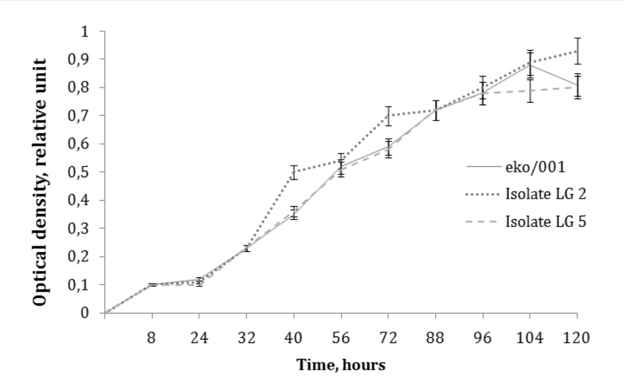

Fig. 3. Optical density of culture broth of isolates Bradyrhizobium

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 ratio of monosaccharides can change the practical value of these polymers.

The viscosity of the culture brothof the control variant – strain *B. japonicum* eko/001, 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 strain *B. japonicum* eko/001. 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.

Conclusions. Isolates of soybean nodule bacteria *Bradyrhizobium sp*. are characterized by intensive synthesis of EPS and significant rheological properties. Taking into account the results, further testing of the effect of these isolates on soybean yield and on plant biometrics will be important. It is necessary to search for isolates adapted to our conditions of existence, through their adaptation and ability to influence environmental indicators.

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