Study of the microstructure and properties of exopolysaccharide (producer of Rhizobium leguminosarum)

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Abstract. As a result of cultivation by the deep periodic method, the bacteria Rhizobium leguminosarum obtained a polysaccharide. The study of its microstructure and properties showed the presence of fibrillar micro-formations and the presence of photochemically active components. Determination of viscosity suggests that the exopolysaccharide has a highly branched structure. Studies have shown that polysaccharide has antioxidant and antimicrobial activity. The study of the effect of polysaccharide on the quantity and quality of gluten of wheat flour showed that its introduction during kneading helps to strengthen gluten and improve its quality. The resulting exopolysaccharide can be used in baking technology.

1. Introduction
Rhizobium - symbiotic legumes and inhabitants of the rhizosphere of various types of plants. These bacteria are involved in the infection of legume nodules that can fix nitrogen. There are two methods of bacterial infection, which are mainly determined by the types of legumes. The attachment of Rhizobium to the roots and root hairs is a very important step in the initiation of symbiosis and occurs both due to secreted proteins and surface polysaccharides. Proximity to root hairs provides bacteria with nutrients that allow them to grow on and around the root [1].

Symbiotic bacteria produce exopolysaccharides, which are secreted by the cell and can act as an important signaling molecule in the initiation of symbiosis with legumes. In addition, exopolysaccharides protect bacteria in their natural environment from stressful conditions, such as salinization, drying, and pollution by heavy metal ions [2]. The rhizobacteria exopolysaccharides are species-specific branched heteropolymers consisting of a series of repeating subunits containing various monosaccharides and groups of a different nature, which impart an acidic character to these molecules. The heterogeneity of the chemical composition of exopolysaccharides produced by various strains of Rhizobiaceae is very high and includes a monosaccharide composition, the size of repeating subunits, the type of glycosidic bonds, the nature of non-carbohydrate residues and the degree of polymerization [3].
Despite the high diversity of bacterial endopolysaccharides and their physicochemical properties, only a few of them are used in industry, for example, xanthan gum from Pseudomonas aeruginosa and Pseudomonas aeruginosa are used as a thickener and stabilizer in the food industry [4]. Bacterial endopolysaccharides are also used as carriers of drugs, bioflocculants, biosorbents. Their ability to bind heavy metals is successfully used in bioremediation processes, especially in the metallurgical and mining industries [5]. It has recently been shown that certain bacterial exopolysaccharides also have antioxidant, antitumor and immunomodulating properties, so they can be used as pharmaceuticals.

In recent years, the production of microbial exopolysaccharides used in the food industry has developed [6]. This is due to the fact that microbial polysaccharides have more diverse functional properties, are easily reproducible and safe; they are obtained regardless of the time of year at the lowest cost [7]. Polysaccharides are used in bread baking. An effective way to improve the quality of bread from low-gluten flour is to use hydrophilic additives of various origin, including microbial polysaccharides, as improvers.

The purpose of the study was to study the microstructure, antioxidant and antimicrobial properties of the bacterial polysaccharide (producer of Rhizobium leguminosarum).

2. Materials and methods
The industrial value of many polysaccharides resides in their capacity to modify the rheological properties of the solutions by gel formation and by modification of the flow characteristics. Polysaccharide solutions are characterized by an abnormal viscosity. With an increased concentration of polysaccharides, the number of bonds between molecules increases and gels are formed. Determination of the kinematic viscosity of a 1.5% solution of exopolysaccharide of a water-soluble fraction showed that it was 1.17 mm² / s, while the viscosity of water was 0.84 mm² / s. The determination of the kinematic viscosity has shown that the exopolysaccharide solution has a low viscosity.

The studies were carried out using a JEOL JSM 6390 scanning electron microscope. A sample was selected from an average sample of a biosynthesis product with a mass of 5 g, which was plated by platinum in a vacuum benchtop JEC-3000FC and placed on a strip coated with carbon dioxide. The resolution of the microscope is 4 Nm at an acceleration voltage of 20 kV (image of the secondary electrons).

To study the ability of an isolated bacterial polysaccharide to display the properties of a fluorophore, the fluorescence imaging method was used. The studies were carried out in a laboratory configuration designed by the biophotonics laboratory, including a DCC 3260C camera, source: Ocean Optics (LLS-455), filters at source output: blue (MF445-45), filters at camera input: blue (FGL495). The radiation at the exit of the fiber is focused by a collimating lens.

The viscosity was measured using a VPZh-2 viscometer and of Pinkevich type. A polysaccharide solution was used to carry out these analyzes. The samples were resuspended in purified water at a temperature of 20 °C and a concentration of 1.5 g L⁻¹. Then the samples were kept for at least 24 hours to ensure complete wetting.

To determine hexoses in terms of glucose, a reaction with phenol in the presence of concentrated sulfuric acid was used. Antioxidant activity was determined spectrophotometrically in an alcohol extract [8] based on percentage of inhibition of DPPH radical (2.2-diphenyl-1-picrylhydrazyl). We determined the optical density of solutions in the interaction DFPG with extractive substances of plants by spectrophotometer “Specord M40” (Carl Zeiss Industrielle Messtechnik GmbH, Germany) at a wavelength of 515 nm.

The study of antimicrobial activity was carried out by a method based on the diffusion of exopolysaccharide into nutrient agar containing test cultures of microorganisms, followed by measurement of the zone of absence of colony growth.

The properties of gluten of wheat flour were investigated using an IDK device.
3. Results and discussion

The surface microstructure of the polysaccharide synthesized by Rhizobium leguminosarum (VKM B-1003) was studied. Figure 1 shows the appearance of the obtained polysaccharide and micrograph of its surface (magnification x40). A photograph of the appearance of the polysaccharide shows that it is an amorphous mass of white.

The surface microstructure of the polysaccharide has fibrillar micro-formations of various lengths and tortuosity. Visually, you can determine that the selected substance is a natural biopolymer.

![Figure 1. Photograph of the appearance of the selected polysaccharide and micrograph of the structure of its surface.](image)

Visualization using fluorescence imaging allows you to distinguish between fluorescence emanating from different fluorochromes and autofluorescence of sample molecules. Visualization is used to study spatial and temporal protein-protein interactions, membrane properties, and identification of biologically active compounds.

Using a blue filter (λ = 440-480 nm) at the input and output of the chamber, the effect of intense exciting light on the processes induced by it of the photochemically active components of the obtained metabolic product was obtained. The photograph (figure 2) clearly distinguishes fluorescent components, which in the presented wavelength range give a green glow.

![Figure 2. Fluorescence imaging of Rhizobium leguminosarum metabolism product samples.](image)
The indicated wavelength range is characterized by the presence of fluorescence in substances of a polysaccharide nature. This allows us to conclude that the metabolic product synthesized by the bacterium Rhizobium leguminosarum is an exopolysaccharide.

The determination of neutral sugars in terms of glucose in the obtained samples of the drug from the metabolic products of the bacteria was carried out by the phenol-sulfur method. Under the action of concentrated sulfuric acid, glucose is dehydrated for 45 minutes until the formation of hydroxymethyl furfural. And then, under the action of phenol on oxymethylfurfural, an aurine dye is formed, having in the visible region of the spectrum an absorption maximum of \( \lambda_{\text{max}} \) 483-485 nm.

The total content of neutral sugars, in terms of glucose, expressed as a percentage, is presented in Table 1.

| Sampling time, h | Glucose content, % |
|-----------------|--------------------|
| 72              | 40.0               |
| 144             | 51.4               |
| 192             | 62.2               |

The results show that in polysaccharide samples obtained by microbial synthesis, the content of neutral sugars in terms of glucose increases with the duration of cultivation of Rhizobium leguminosarum and after 192 hours is above 50%.

The viscosity of polymer solutions is determined not only by molecular weight, but also by the shape of the molecule, depending on the structure of the polymer (linearity, branching), the concentration of the solution and the nature of the interaction of the polymer with the solvent. Therefore, viscometry cannot be used to determine the absolute values of molecular weight, but the simplicity of this method leads to the fact that it is widely used to compare the properties of different fractions of polysaccharides.

The industrial value of many polysaccharides lies in their ability to change the rheological properties of solutions by gel formation and changes in flow characteristics. Polysaccharide solutions are characterized by abnormal viscosity. With an increased concentration of polysaccharides, the number of bonds between molecules increases and gels form. Determination of the kinematic viscosity of a 1.5% solution of exopolysaccharide of a water-soluble fraction showed that it was 1.17 \( \text{mm}^2/\text{s} \), while the viscosity of water was 0.84 \( \text{mm}^2/\text{s} \). Determination of kinematic viscosity showed that the exopolysaccharide solution has a low viscosity.

Therefore, according to the literature, the exopolysaccharide synthesized by the bacterium Rhizobium leguminosarum has a highly branched structure. Perhaps the polysaccharide has an arabinogalactan nature and can find wide application in food technology.

The antioxidant activity of the drug product of the metabolism of Rhizobium leguminosarum was determined. Antioxidant activity accounted for 43.4% of the inhibition of the DPPH radical.

Thus, experimental studies have shown that the resulting exopolysaccharide metabolic product of the bacterium Rhizobium leguminosarum has the ability to inhibit the growth of microorganisms in the test cultures of Staphylococcus aureus and Escherichia coli (Table 2).

| Test culture of microorganisms | The diameter of the zone of lack of growth, mm |
|------------------------------|---------------------------------------------|
| Staphylococcus aureus         | 1.8±0.2                                    |
| Escherichia coli              | 3.0±0.2                                    |

The effect of the polysaccharide Rhizobium leguminosarum on the “strength” of wheat flour was studied. The obtained microbial polysaccharide was introduced in an amount of 1%, 2% and 3% by weight of flour when kneading the dough in an unpaired way, taking into account the recalculation of water for dry substances. The control sample did not contain a microbial polysaccharide. The quantity
and quality of gluten in the test was determined at the end of the fermentation process until the test reaches an acidity of 2.5-3 degrees. The results of a study of the effect of the polysaccharide Rhizobium leguminosarum on the quantity and quality of gluten of premium wheat flour are presented in table 3. The experimental data presented show that with an increase in the polysaccharide dose, there is a tendency to a decrease in the amount of raw gluten in premium wheat flour and the index of IDK. This indicates the strengthening of gluten and improving its quality.

Table 3. Effect of the polysaccharide Rhizobium leguminosarum on the quantity and quality of gluten of premium wheat flour.

| Sample Name | The content of crude gluten, % | Values of IDK, units instrument |
|-------------|--------------------------------|---------------------------------|
| Control     | 28.8±0.2                       | 74±0.5                          |
| 1%          | 27.5±0.3                       | 67±0.5                          |
| 2%          | 27.3±0.2                       | 65±0.5                          |
| 3%          | 27.1±0.1                       | 60±0.5                          |

Strengthening gluten is associated with the presence in the polysaccharide of impurities of substances with antioxidant properties. In addition, the formation of protein-polysaccharide complexes and glycoproteins leads to hardening of the structure of the gluten framework.

4. Conclusion
Following the culture of Rhizobium leguminosarum, a polysaccharide of microbial origin was obtained. Studies on its microstructure and its properties have shown that it contains fibrillar micro-formations of different lengths and tortuosities. The polysaccharide sample was visualized using fluorescence imaging, which confirmed the polysaccharide nature of the biopolymer and the presence of photochemically active components in its composition. In the samples of the polysaccharide obtained by microbial synthesis, the content of neutral sugars in terms of glucose increases with the culture time of Rhizobium leguminosarum and after 192 hours is greater than 50%.

Determination of kinematic viscosity showed that the exopolysaccharide solution has a low viscosity. It is likely that the exopolysaccharide synthesized by the bacterium Rhizobium leguminosarum has a highly branched structure, which allows it to be recommended for use in food technology. Studies have shown that the resulting polysaccharide has antioxidant and antimicrobial activity.

The effect of the Rhizobium leguminosarum polysaccharide on the "strength" of wheat flour has been studied. Determination of the effect of microbial polysaccharide on the quantity and quality of gluten in premium wheat flour has shown that with an increase in the dose of polysaccharide, there is a tendency to decrease the amount of raw gluten in the premium wheat flour and the IDK index and, therefore, to strengthen gluten and improve its quality.

The results obtained allow us to recommend the polysaccharide obtained by microbial synthesis in baking technologies.

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