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Charakterystyka antygenów O, glukanów i egzopolisacharydów bakterii z rodzaju *Ochrobactrum*

(Characterization of O antigens, glucans and exopolysaccharides from *Ochrobactrum*)

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Abstract

The bacteria collectively known as rhizobia are a very diverse group, both phylogenetically and morphologically. They share the unique ability to establish symbiosis with many plant species from the legume family (*Fabaceae*). These microorganisms play a very important role in ecosystems, creating one of the most efficient systems for the biological reduction of atmospheric nitrogen.

Cyclic glucans (OPG), egzopolisaccharides (EPS), lipopolysaccharide (LPS) and capsular polysaccharides (CPS), the surface components of rhizobial cell walls, play an important role in the creating of an effective symbiosis. These polymers are important in the early stages of symbiosis development, providing the proper adhesion of bacteria to root hairs and then allow bacteria and bakteroids adaptation to changing osmotic conditions prevailing inside cells of root nodules. Lipopolysaccharides, which are an integral component of Gram-negative bacteria envelopes, are of particular importance in the creation of symbiotic systems. Apropriate LPS structure ensures the integrity of the outer membrane and stabilizes its structure. Through close contact with the plant cell membrane of the infective thread end, it determines its growth, affects the effectiveness of endocytosis and determine the appropriate organization and symbiosomes maturation. There are numerous publications regarding the surface component structures of bacteria belonging to *Rhizobium*, *Sinorhizobium* (*Ensifer*) and *Mesorhizobium* genus and slightly fewer reports on *Bradyrhizobium*, but lipopolysaccharides from *Ochrobactrum* has never been investigated.

The genus *Ochrobactrum* is classified within the *Brucellaceae* family, which includes also species that are human and animal pathogens. Representatives of this group of bacteria also play an important role in the atmospheric nitrogen fixation. This group includes recently isolated species of *O. cytisi* and *O. lupini*, which are symbionts of *Cytisus scoparius* and *Lupinus honoratus*, respectively. Some strains belonging to *Ochrobactrum* also have the ability to metabolize harmful chemicals, such as atrazine, nicotine, phenol and polycyclic aromatic hydrocarbons, and to biosorb heavy metal ions (Cu, Zn and Cd). Many of these substances are toxic to the environment and to humans and therefore studies on these bacteria are important from the ecological point of view.

The aim of the dissertation was to characterize successively: O-specific LPS chains and glucans as well as describe exopolysaccharides (mainly in terms of their

sorption capacity in relation to heavy metal ions) synthesized by bacteria symbiotically fixing atmospheric nitrogen belonging to the species O*chrobactrum cytisi* and *Ochrobactrum lupini.*

The analyses were carried out on the basis of classical chemical methods, gas chromatography coupled with mass spectrometry and NMR spectroscopy.

As a result of the conducted research it was shown that the O-specific polysaccharide synthesized by *O. cytisi* ESC1^T is composed of subunits consisting of two sugars: α-D-fucose and β-D-galactosamine residues linked by (1→3) bonds. The final structure of the O-specific polysaccharide *Ochrobactrum lupini* LUP21^T has not been determined. Analysis of the high molecular weight fraction of degraded polysaccharide allowed to establish that *O. lupini* LUP21^T produces at least two types of O antigens. The core of the both O-specific polysaccharides are an *N*-acetyl-D-galactosamin homopolymers decorated with D-*altro*-heptulose.

Analysis of the periplasmic glucans of *O. cytisi* and *O. lupini* showed that both strains synthesize cyclic β-(1→ 2)–glucans with an identical degree of polymerization of 17 to 25 glucose residues.

Attempts to describe the chemical structures of *Ochrobactrum cytisi* and *O. lupini* exopolysaccharides showed that both polymers contain mainly mannose, glucose and galactose appearing in similar proportions (6:3:2). It was showed that *O. lupini* LUP21^T better tolerates environment polluted with lead than cadmium ions.Testing the sorption capacity of heavy metal ions (lead and cadmium) by EPS, it was found that the pH of the environment does not have a major impact on the sorption process. By means of infrared spectroscopy, it was shown that metal ions sorption process involves groups containing oxygen atoms, i.e. carboxyl, carbonyl and hydroxyl groups. The first two are usually elements of EPS non-sugar substituents, the last are free hydroxyl groups of sugars.

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ABSTRACT