**Summary of doctoral dissertation**

„Structural studies of the O-polysaccharides isolated from *Franconibacter helveticus*, *Pseudomonas donghuensis* and *Pectobacterium brasiliense*"

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Structural studies of O-polysaccharides isolated from *Pectobacterium* strains can be exceptionally useful in better understanding of bacteria-plant interactions and evaluating the influence of OPS on the pathogenic process. The acquired knowledge on chemical diversity of O-polysaccharides might contribute to developing of rapid and effective identification tests for phytopathogens. In fact, research on such tests has already been conducted. For example, there are PCR tests, which are perpetually improved, or enzyme-linked immunosorbent assays (ELISA). However, due to constantly changing taxonomic division, *Pectobacterium* strains are difficult to identify. Therefore, knowledge concerning surface structures might contribute to a better classification system for these phytopathogens.

Determining chemical structure of the OPSs isolated from endophytes *Franconibacter helveticus* and *Pseudomonas donghuensis* is very important in terms of studying interactions between bacteriophages and bacteria. In future, this knowledge might contribute to preparing effective plant protection products, which in turn could lead to reduction of economic losses caused by phytopathogens.

*P. brasiliense* 5527 and *P. donghuensis* P482 were selected and grown by scientists from the Intercollegiate Faculty of Biotechnology at the University of Gdańsk and the Medical University of Gdańsk. *F. helveticus* 1975 was selected by Stephen Forsythe from Nottingham Trent University (UK) as part of research cooperation.

I began my research with the isolation of lipopolysaccharide. First of all, bacterial cells was subjected to classical phenol-water extraction using Westphal and Jann method and PCP extraction. Then, I removed nucleic acids using ethanol precipitation method and enzymatic digestion. The lipopolysaccharide was separated and then lipid A was cleaved by mild acid hydrolysis. Subsequently, I separated and purified the OPS by size exclusion chromatography (GPC).

Structural studies of the O-polysaccharides were carried out using chemical modifications: sugar analysis, methylation analysis and reaction with optically active butan-2-ol. The obtained derivatives were then analyzed by gas chromatography (GC) and gas

chromatography coupled with mass spectrometry (GC-MS). On the basis of the obtained results, I was able to determine sugar composition in the OPSs, define linkage positions of sugar residues and assign D or L configuration to individual monosaccharides. The bacterial polysaccharides were analyzed using nuclear magnetic resonance spectroscopy technique (NMR). For the tested samples, NMR spectra ($^{1}$H NMR as well as two-dimensional homocorrelation and heterocorrelation spectra such as COSY, TOCSY, ROESY, HSQC, HSQC-TOCSY and HMBC) were recorded. On the basis of the NMR spectra, I was able to confirm the results obtained through the sugar and methylation analysis, as well as determine the anomeric configuration of individual sugar residues and define the sequence of monosaccharides in the OPS. As a result of structural studies including chemical modifications and the NMR spectra, chemical structures of the O-polysaccharides isolated from *Franconibacter helveticus* 1975, *Pseudomonas donghuensis* P482, and *Pectobacterium brasiliense* 5527, were elucidated:

*Franconibacter helveticus* 1975

α-D-Glc*p*
1
↓
2
→3)-α-L-6Tal*p*-(1→ 2)-α-L-6Tal*p*-(1→ 2)-α-L-6Tal*p*-(1→

*Pseudomonas donghuensis* P482

β-Man*p*NAc
1
↓
4
→3)-α-Rha*p*-(1→ 3)-β-Glc*p*-(1→

*Pectobacterium brasiliense* 5527