Summary of doctoral dissertation

„Determination of the chemical structure of the O-antigens of phytopathogenic bacteria of the genera *Dickeya* and *Pectobacterium*"

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The bacteria belonging to the genera *Dickeya* and *Pectobacterium* are phytopathogenic microorganisms. As disease agents of a wide variety of crops (e.g. potatoes, carrots, tomatoes, cucumbers, chicory, cabbage, corn, rice, bananas, sunflower seeds, sugar cane and many others), they contribute to significant losses in their cultivation. Knowledge of the surface structures of these microorganisms can greatly contribute to a better understanding and characterization of the factors responsible for their pathogenicity, as well as those involved in the mechanisms of pathogen-plant interactions. In turn, this knowledge might lead to preparing easier and faster tests for the identification of these microorganisms in the future, as well as to preparing effective methods of combating and preventing infections caused by these phytopathogens.

The main goal of my research was to determine the chemical structures of the O-antigens of twelve bacterial strains belonging to five species of the genera *Dickeya* and *Pectobacterium*. All bacterial strains selected for the research came from the collection of the Intercollegiate Faculty of Biotechnology of the University of Gdańsk and the Medical University of Gdańsk.

The intermediate goals included the isolation and purification of O-antigens, and then subjecting them to structural studies.

In the first part of my research, I isolated and purified the O-antigens. I subjected the dry bacterial cells to phenol-water extraction. Then, I removed nucleic acids and then isolated the lipopolysaccharides. I subjected the obtained LPS to mild hydrolysis. From the saccharide fractions remaining after lipid A separation, I isolated and purified the O-polysaccharides by size exclusion chromatography (SEC).

In the second part of the work, I subjected the analyzed O-antigens to structural studies using chemical methods such as: sugar analysis, methylation analysis and reaction with optically active butan-2-ol. The derivatives obtained as a result of chemical modifications were analyzed using gas-liquid chromatography (GLC) and gas-liquid chromatography coupled with mass spectrometry (GLC-MS). On the basis of the obtained results, I was able to determine the

amount and the type of sugar residues, the size of sugar rings and to define linkage positions of sugar residues and assign the respective sugar residues to the conformational D or L.

The last stage of my research involved the interpretation of NMR spectra. Based on the result, I was able to determine the α or β configuration of anomeric carbon of individual sugar residues, as well as the sequence of individual sugar residues and to characterize the components of the analyzed OPS, which had been unidentified at an earlier stage while using chemical analyses.

Based on the obtained results of all of the analyses performed, both using chemical analyses and spectroscopic methods, I determined the chemical structures of the repetitive units of the O-antigens of *Dickeya dadanti* IFB0016, *Dickeya dianthicola* IFB0485, *Dickeya zeae* IFB0031, four strains of *Dickeya solani* (IFB0099, IFB0123, IFB0158, IFB0223) and five strains of bacteria *Pectobacterium parmentieri* (IFB5308, IFB5408, IFB5427, IFB5432 and IFB5441):

***Dickeya dadanti* IFB0016, *Dickeya dianthicola* IFB0485, *Dickeya zeae* IFB0031 and *Dickeya solani* (IFB0099, IFB0123, IFB0158 i IFB0223):**

→2)-β-D-6dAlt*p*-(1→

***Pectobacterium parmentieri* IFB5308 and IFB5432:**

→3)-β-D-Gal*f*-(1→3)-α-D-Gal*p*-(1→8)-β-Pse4Ac5Ac7Ac-(2→6)-α-D-Glc*p*-(1→6)-β-D-Glc*p*-(1→

***Pectobacterium parmentieri* IFB5408 and IFB5427:**

β-D-Man*p*NAc
1
↓
4
→3)-β-D-Gal*p*NAc-(1→3)-α-D-Gal*p*-(1→4)-β-D-Gal*p*-(1→

***Pectobacterium parmentieri* IFB5441:**

→6)-β-D-Gal*f*-(1→3)-β-D-Gal*p*-(1→4)-β-D-Gal*p*-(→
3
↑
2
β-Pse4Ac5Ac7(S3Hb)