ABSTRACT

CHEMICAL STRUCTURES OF O-POLYSACCHARIDES OF SELECTED STRAINS OF BACTERIA *DICKEYA* AND *PECTOBACTERIUM* GENERA

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Bacteria surrounding plants usually have no noticeable effect on their growth and physiology. A large group are species that bring benefits, e.g. by providing nutrients or stimulating growth. Unfortunately, there are also phytopathogenic bacteria that cause serious diseases, leading to the death of plants. Of particular note are plant pathogens from the *Pectobacteraceae* family (Soft Rot *Pectobacteraceae*; SRP), including pectinolytic bacteria from the genera *Pectobacterium* and *Dickeya*. These microorganisms cause diseases such as blackleg and soft rot on potatoes, as well as many other cultivated plants (e.g. tomatoes, chicory, rice, corn) and ornamental plants (e.g. chrysanthemums, carnations, violets) worldwide, contributing to significant crop losses. Phytopathogens from the genera *Pectobacterium* and *Dickeya* are able to penetrate the host tissue due to the efficient production of plant cell wall degrading enzymes with pectinolytic, cellulolytic, proteolytic and lipolytic properties. Another important virulence factor is lipopolysaccharide (LPS), due to its participation in bacterial adhesion to plant tissue and interaction with host defense systems. The needs of better understanding the bacterial-plant interaction, as well as to characterize the factors responsible for the pathogenicity of SRP, led to the study of the surface structures of these phytopathogens. Knowledge of the structure of polysaccharides may help to explain the mechanisms of the bacterium-plant interaction and complement the classification system of these microorganisms.

The aim of my doctoral thesis was to determine the chemical structure of O-specific polysaccharides (OPS), which are the most exposed fragments of LPS, of selected strains of bacteria of the genera *Dickeya* and *Pectobacterium*, isolated from the aquatic environment.

LPS was isolated from dry bacterial cells using extraction with a mixture of phenol-chloroform-petroleum ether or classical phenol-water extraction. DNA, RNA and proteins were removed from the sample by enzymatic digestion and dialysis. The obtained LPS was subjected to mild hydrolysis. Lipid A was centrifuged and the sugar fraction was purified using size-exclusion chromatography. Chemical analyses such as sugar analysis, methylation analysis and reaction with optically active butan-2-ol, as well as nuclear magnetic resonance spectroscopy

(NMR) were used for structural studies of O-antigens. Derivatives obtained as a result of chemical modifications of OPS were analyzed by gas chromatography and gas chromatography coupled with mass spectrometry. The analysis of obtained chromatograms and MS spectra provided preliminary information about the chemical structure of the OPS repeating unit. The number and type of sugar residues, their substitution sites were determined and monosaccharides were assigned to a D or L configuration series. Based on the recorded one- and two-dimensional NMR spectra, the configuration of anomeric carbon atoms, α or β, of individual monosaccharides was determined, the sequence of sugar residues in the repeating unit was established, and structural elements of polysaccharides that had not previously been detected by chromatographic methods were also identified.

On the basis of the results obtained from chemical analyzes and spectroscopic methods, the chemical structures of the OPS of the tested bacterial strains were determined: *D. aquatica* IFB0154, *D. aquatica* IFB0694, *D. lacustris* IFB8647, *P. aquaticum* IFB5637 and *P. versatile* IFB5636.

Knowledge of the structure of the OPS may contribute to the understanding of the mechanisms of plant-bacteria interactions, which may lead to the development of new species-specific pathogen identification methods, e.g. rapid diagnostic tests, as well as effective methods of combating and preventing infection. The prospect of quick identification of the microorganism may contribute to reducing economic losses in potato and other crop plantations.