

Characterisation of the *Pectobacterium aquaticum* isolated from Polish water in addition to studying the selected virulence and environmental factors important for development of potato blackleg and soft rot diseases

MSc Weronika Babińska-Wensierska

Pectinolytic bacteria from the *Dickeya* and *Pectobacterium* genera belonging to the Soft Rot *Pectobacteriaceae* (SRP) cause substantial damage associated with soft rot in crops, vegetables, and ornamental plants, as well as blackleg in potatoes. *Dickeya* spp. and *Pectobacterium* spp. rank among the top ten most important plant pathogenic bacteria globally due to the huge losses they cause in potato production. Despite the considerable financial impact and the prevalent occurrence of pectinolytic bacteria, there are currently no efficient plant protection strategies available to combat these pathogens. Until now, screening of potato tubers for latent infections with *Dickeya* spp. and *Pectobacterium* spp. has been regarded as the most effective method for protecting this crop. Therefore, the aims of this thesis were set as follows: i) assessment whether Polish waterways may be the source of bacteria from the family *Pectobacteriaceae*, which can cause symptoms of soft rot and blackleg diseases on potato, ii) the search for virulence determinants in *Pectobacterium aquaticum* and *Dickeya solani*, iii) the application of metagenomics for elucidation of the differences in the structure of bacterial microbiota in suppressive and non-suppressive soils in relation to Soft Rot *Pectobacteriaceae*.

Pectobacterium spp. and *Dickeya* spp. are present in various environmental habitats, including water. In order to determine the abundance of SRP in Pomeranian lakes, water samples were collected from different depths during two years monitoring period. Subsequent isolation and comprehensive genotypic and phenotypic characterisation revealed the presence of bacteria from the family *Pectobacteriaceae* in the studied lakes. Interestingly, during this research, an isolate belonging to the recently established species, *Pectobacterium aquaticum* was acquired. The identified and characterized *P. aquaticum* IFB5637 strain exhibited the production of plant cell wall degrading enzymes (PCWDEs), including pectinases, cellulases and proteases. Moreover, it was able to macerate potato and chicory tissues under laboratory conditions. This data indicates that utilizing of surface waters for the irrigation of potato fields may lead to dissemination of pectinolytic bacteria and the consequent appearance of the symptoms of soft rot and blackleg diseases.

The next part of the study was devoted to the identification of the virulence factors of *P. aquaticum* and *Dickeya solani*. Among virulence factors of plant pathogenic bacteria there are lipopolysaccharides (LPS) which play an important role in the initial phase of host-pathogen recognition. Therefore, subsequent research was focused on elucidating the chemical composition of the O-polysaccharide (OPS) of the *P. aquaticum* strain IFB5637 LPS. The analysis that was performed indicated that the structure of the OPS consists of common sugars like mannose and glucose, along with abequose. Notably, the latter one was a newly discovered constituent of O-antigen among the members of the *Pectobacteriaceae* family.

The ability of *D. solani* to cause severe diseases is attributed to the effective production of

PCWDEs that break down the plant cell wall. Despite high genetic homogeneity of *D. solani* strains, some of them indicated extremely different virulence levels. Because there is still a lack of understanding of the principles behind the noted differences in the pathogenicity of *D. solani*, our study included investigation of the methylomes and transcriptomes of two *D. solani* strains: the highly virulent IFB0099 and the low virulent IFB0223. Examination of the DNA methylation patterns showed no noticeable differences between the profiles of the analysed strains. However, RNA-Seq analysis revealed differences between the compared *D. solani* strains. Some of the genes encoding components of the secretion system II (T2SS) (*gspJ*, *nipE*) and three endo-pectate lyases (*pelD*, *pelE*, *pelL*) exhibited higher stimulation of expression under induced conditions in strain IFB0099 compared to IFB0223, which may lead to an increased virulence. Additionally, upregulation of genes encoding the proteins building up flagella in IFB0099 (*flgC*, *flgB*, *flgD*, *fliG*, *flgG*, *flgF*, *flhA*, *fliA*) in contrast to IFB0223 might result in increased mobility of the highly virulent strain.

The severity of disease caused by plant pathogenic bacteria depends on plant the host resistance, the bacterial virulence and environmental conditions. Therefore, it was examined whether the development of disease symptoms caused by *Dickeya* spp. and *Pectobacterium* spp. could be linked to the specific features of the soil, in particular the composition of the bacterial soil microbiota. According to the literature, the term ‘suppressive soil’ is used to describe soil in which the growing plants show no or minimal disease symptoms, even in the presence of a virulent pathogen. In contrast to the ‘suppressive soil’, a ‘non-suppressive soil’ is described as soil, whose microbiological composition favours development of disease symptoms. On the basis of an earlier monitoring of the occurrence of soft rot and blackleg on potato plantations, suppressive and non-suppressive soils towards the above-mentioned diseases were collected from two different locations. The performed analyses showed that suppressive and non-suppressive soils exhibited similar physical and chemical properties. However, 16S rRNA gene amplicon sequencing demonstrated differences in the composition of the bacterial microbiota between the two analysed soil types, *i.e.* representatives of the genera *Bacillus*, *Acidobacterium*, and *Gaiella* turned out to be much more abundant in the studied suppressive in contrast to the non-suppressive soil.

In summary, the performed research enriches knowledge on the complexity of interactions between economically significant plant pathogens from the genera *Dickeya* and *Pectobacterium*, the environment and host plants. It open a future perspective for better disease management and enhancing crop protection strategies. Though, further studies and advancements in understanding the virulence mechanisms and interactions involving SRP are essential for the development of efficient control measures and, by these means, boosting the sustainability of agricultural systems.