Characteristics of the molecular mechanism involved in the pathogenicity of bacteria from the genus *Dickeya* on plants.

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Pectinolytic bacteria from the genus *Dickeya* (previously *Erwinia chrysanthemi*) and *Pectobacterium* (previously *Erwinia carotovora*) cause blackleg and soft rot on potato and numerous plants including crops, vegetables, ornamentals and herbs (Toth *et al.* 2011). The bacteria cause economic losses to crop production. The bacteria can survive on the plant tissue as saprophytes (latent infection) what makes their detection difficult without the use of immunological or molecular methods. Bacteria from the *Dickeya* genus produce a large number of virulence factors, among which the most important are the pectinolytic enzymes. These enzymes degrade polygalacturonic acid and pectin, which are main components of the plant cell wall. The long-term studies conducted with the use of *Dickeya dadantii* 3937 strain, led to identification of a dozen genes coding for pectate lyases (e. g. *pelA, pelB, pelC, pelD, pelE, pelI, pelL, pelN, pelX, pelZ*) in its genome (Hugouvieux-Cotte-Pattat *et al.* 2014). Moreover, more than 10 global regulators were identified (e. g. KdgR, PecS, PecT, Fur, Fis, H-NS, CRP, MfbR) (Charkowski *et al.* 2012) that influence the expression of pectate lyase genes and the secretion of the plant cell wall degrading enzymes (Hugouvieux-Cotte-Pattat *et al.* 1996; Hassan *et al*. 2013; Charkowski *et al*. 2012; Nasser *et al*. 2013).

In 2005 for the first time in Poland, bacteria from the *Dickeya* genus were detected in symptomatic potato plant coming from seed potato plantation (Slawiak *et al.* 2009b). Identification and characterization of the pectinolytic strains isolated in recent years in Poland and other European countries led to the distinction of a new group among *Dickeya* species (Slawiak *et al.* 2009a) and recently classification of this group to a new species *Dickeya solani* (Wolf *et al.* 2014). Strains from this group were more virulent and moved more easily along the plant vascular tissue than *D. dianthicola* which was previously isolated from symptomatic potato plants (Czajkowski *et al.* 2010; Toth *et al.* 2011).

I performed genetic and phenotypic characterization of four *D. solani* strains differing in their virulence level and I constructed mutants of these strains in the genes encoding the global transcriptional regulators KdgR, PecS, PecT and in the genes coding for the QS elements ExpR and ExpI. Thanks to extensive phenotypic characterization of the selected strains and their mutants, I determined the influence of these regulators on the *D. solani* virulence on potato (Potrykus *et al.* 2014a). These studies showed that the global negative regulators PecS, PecT and KdgR inhibit the virulence factor gene expression, both in *D. solani* and *D. dadantii* 3937. My results indicated that the PecT regulator significantly influences the expression of the virulence factors in *D. solani* and is the most important regulator in *D. solani* among those tested. Moreover, the QS mechanism in *D. solani* seems to be important for its virulence. The strains with either *expR* or *expI* mutation

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demonstrated a lower virulence in the potato maceration assay than the corresponding wild type strains. The results suggest that the regulation of virulence by the QS mechanism is slightly different in *D. solani* and *D. dadantii* 3937.

Moreover, I optimized the method suitable for detection and identification of pectinolytic bacteria causing blackleg and soft rot diseases on potato plants (Potrykus *et al.* 2014b). The proposed method enabled fast detection of the bacteria in the potato plants with or without disease symptoms. The method aims for detection of bacteria from the *Dickeya* genus and bacteria from the *Pectobacterium atrosepticum* and *Pectobacterium carotovorum* subsp. *carotovorum*/*Pectobacterium wasabiae* species in one multiplex PCR reaction. The reaction is preceded by the isolation of total genomic DNA from the plant tissue. Thanks to implementation of total genomic DNA isolation from the plant homogenate combined with the multiplex PCR, both cost and time of the pathogens detection and identification were substantially decreased and the isolation of bacteria is no more compulsory.

Finally, in cooperation with a Finnish research center, I conducted the genotypic analysis of 46 Finnish strains isolated from potato plants with the use of rep-PCR and sequencing of *dnaX* gene, that led to the confirmation of *D. solani* presence in the high grade regions of agriculture in this country (Degefu *et al.* 2013).

The presented doctoral thesis provides new significant data improving our knowledge about the molecular mechanisms of *D. solani* pathogenicity and enables the monitoring of the presence of chosen groups of pectinolytic pathogens in plant tissue and in waterways.

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