Characterization of Dickeya solani strains and identification of bacterial and plant signals involved in the induction of virulence.

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Pectinolytic bacteria from the genera *Pectobacterium* (former *Erwinia carotovora*) and *Dickeya* (former *Erwinia chrysanthemi*) are casual agents of blackleg and soft rot diseases. They cause crop damages and high economic losses. For example, losses caused by pectinolytic bacteria are evaluated at about 2 to 10% of the potato yield, depending on the year. In 2009, the value of potato losses in Europe was estimated to reach 250 million Euros. During the last years, *Dickeya* strains have been more and more often isolated from diseased plants in Poland, France and other European countries. The genus *Dickeya* is a highly diverse group, which according to the present nomenclature contains seven species: *D. aquatica*, *D. chrysanthemi*, *D. dadantii*, *D. dianthicola*, *D. paradisiaca*, *D. solani* and*D. zeae*. Recent results, obtained in different European countries, indicate that a new species – *Dickeya solani* can efficiently infect potato plants and cause disease symptoms in temperate climate. The *D. solani* strains are considered as more aggressive than other blackleg causing bacteria. Preliminary analysis suggested that they need lower optimal temperatures for disease development as well as lower inoculum levels for infection spreading. They seem to have a greater ability to colonize potato plants roots and to spread through the plants' vascular system. *D. solani* strains produce a wide range of plant cell-wall degrading enzymes which are the main virulence factors.

The aims of the study were: 1) phenotypic and genotypic characterization of the *D. solani* strains isolated in countries with different climatic conditions: Poland, Finland and Israel; 2) study of the potato tuber extract influence on the expression of selected *D. solani* genes: *pelD, pelL, tssK, lfaA;* 3) comparative genomics of ten *D. solani* strains, performed on 4 genomes sequenced for this study and 6 genome sequences available in the GenBank databases.

The results showed that the strains from different climatic conditions have identical rep-PCR profiles (three different primers sets were used – ERIC, REP and BOX) and Restriction Fragments Length Polymorphism-Pulse Field Gel Electrophoresis (RFLP – PFGE) profiles, but they do differ

phenotypically, especially in the activity of plant cell-wall degrading enzymes. Polish strains have higher activities of pectinolytic, cellulolytic and proteolytic enzymes than Finnish and Israeli strains.

D. solani mutants in the *pelD, pelL, tssK, lfaA* genes were obtained by the means of site-specific mutagenesis. The highest induction by plant extracts was observed for the *lfaA* gene. The *pelL* expression was also induced by plant derived signal(s), contrary to *pelD* and *tssK*.

Comparative genomics analysis has elucidated the pangenome shape of 10 *D. solani* strains. Ten *D. solani* genomes are encoding 41 947 proteins. The *D. solani* genes form 10 analysed genomes were grouped into 5 045 Orthologous Groups, 3 809 belonging to the core genome, 413 to the accessory genome and 823 to the unique genome. The analysis of the protein sequences of pathogenicity-related genes (9 cell wall – degrading enzymes) as well as their 19 regulators (chosen on the basis of knowledge available for *D. dadantii* 3937, the most studied *Dickeya* strain belonging to a closely related species) showed 100 % sequence homology within the 10 genomes.

All genenomic studies proved that *D. solani* strains form a very homogenous group. On the other hand, the phenotypic analysis showed some variability among strains from different climatic conditions. The key to the observed variations in the phenotypic traits can be a different regulation of the expression of the genes encoding virulence factors, which can be influenced by temperature, pH, iron deprivation or oxygen and nitrogen availability, as well as the presence of specific plant tissue compounds.