Molecular determinants in the interaction of plant pathogenic bacteria from the species *Dickeya solani* and *Pectobacterium atrosepticum* under different temperatures

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Pectinolytic bacteria *Pectobacterium* spp. and *Dickeya* spp. that cause blackleg and soft rot are responsible for high economic losses in potato production under different climate conditions worldwide. *Pectobacterium atrosepticum* typically causes blackleg and soft rot on potato plants in a temperate climate, whereas *Dickeya* spp. are responsible for these diseases in a warm climate, although "cold-tolerant" strains of *Dickeya dianthicola* have been isolated from the blackleg-diseased plants in Western Europe. During the last decade new *Dickeya* species named *Dickeya solani* have been isolated from potato in many European countries. *D. solani* is more virulent and aggressive than other *Dickeya* spp. and *P. atrosepticum* isolated from potato in Europe in the past. The aggressiveness of *D. solani* appears to increase at high temperatures. An increase in temperature due to climate change may be partly responsible for the faster spreading of this pathogen through Europe and could shift the distribution of *Dickeya* spp. and *Pectobacterium* spp. in Europe. The temperature may act as a signal that activates the expression of specific factors in plant pathogens during an infection. Several studies have reported thermoregulation of gene expression in plant pathogenic bacteria, but little is known about the influence of temperature on metabolism and host-adaptive processes in pectinolytic bacteria.

The main objective of the study was to identify and characterize genes up-regulated in *P. atrosepticum* strain SCRI1043 and *D. solani* strain IFB0099 at low (18 °C) and high temperatures (28 and 37 °C, respectively). Random mutagenesis of *D. solani* IFB0099 and *P. atrosepticum* SCRI1043 was carried out using mini-Tn*5gusA* transposon containing promoterless β-glucuronidase reporter gene (*gusA*). In total, 8000 transposon mutants of *D. solani* and 5775 transposon mutants of *P. atrosepticum* were generated. Next, a three-step screening GUS procedure (a qualitative and two quantitative GUS assays) was performed to analyze transposon mutants of *D. solani* and *P. atrosepticum* for temperature-dependent reporter gene expression. 46 transposon mutants of *D. solani* and 40 transposon mutants of *P. atrosepticum* exhibited an evidently temperature-dependent GUS activity and were selected

for further investigation. Seven transposon mutants of *D. solani* and 20 transposon mutants of *P. atrosepticum* showed a higher GUS activity at a low temperature – 18 °C, whereas 20 transposon mutants of *P. atrosepticum* and 39 transposon mutants of *D. solani* demonstrated a greater GUS activity at a high temperature – 28 °C and 37 °C, respectively. A function for temperature-regulated genetic loci of *D. solani* and *P. atrosepticum* targeted by transposon insertion was determined. These genetic loci encoded proteins involved in primary bacterial metabolism, membrane transporters, regulatory proteins, virulence factors as well as hypothetical proteins. The transposon mutants of *D. solani* and *P. atrosepticum* were tested to investigate whether mutations in the identified genetic loci affected their phenotypic characteristics associated with virulence *viz*. production of pectinases, cellulases, proteases, phospholipases, siderophores, and auxins as well as the ability to form biofilm and macerate potato tuber and chicory leaves tissues. Eight transposon mutants of *D. solani* IFB0099, four with an induced increase in GUS activity at 18 °C and four – at 37 °C, and five transposon mutants of *P. atrosepticum* SCRI1043, three with an induced GUS activity at 18 °C and two – at 28 °C, expressed visible phenotypes different than the wild-type *D. solani* strain IFB0099 and *P. atrosepticum* strain SCRI1043, respectively, including the decreased ability to macerate potato tubers or chicory leaves or impaired biofilm formation.

It can be hypothesized that the temperature-regulated loci identified in the presented study could contribute to the improved fitness of pectinolytic bacteria in different ecological niches and under changing environmental conditions.