Importance of the selected elements of the extracytoplasmic protein quality control system (DsbA, DegP, DegS, FkpA) for virulence and survival under stress conditions of the potato pathogen, *Dickeya solani*

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Phytopathogenic bacteria are an important factor limiting production efficiency in the agricultural sector. The potato (*Solanum tuberosum*), which is one of the crucial crops, is susceptible to infection with the *'Soft Rot Pectobacteriaceae*' (SRP) bacteria, which include species from the genera *Pectobacterium* and *Dickeya*. The newly distinguished species, *Dickeya solani*, is a significant cause of potato crop losses in many European countries, as well as in Georgia, Turkey, and Israel. Symptoms of the disease caused by the SRP group's members, including *D. solani*, are tuber soft rot, resulting from cell wall degradation and disintegration of plant tissue, and the black leg, that is stem rot progressing from the side of the tuber. Plant cell wall degrading enzymes (CWDEs) are key factors in the virulence of SRP bacteria. *D. solani* has a wide arsenal of pectinolytic enzymes. Their activity is directed at pectins and polygalacturonic acid, which build the host's cell walls. Other enzymes produced by *Dickeya* and *Pectobacterium* species that are involved in the breakdown of cell walls are cellulases and proteases. Motility and chemotaxis as well as the ability to uptake metal ions, mainly iron, are also required for efficient infection of the host organism.

During the infection cycle, *D. solani* cells are exposed to many stress factors, including, i.e. elevated temperature, the presence of reactive oxygen species, changes in environmental pH, and osmotic stress. The peripheral areas of the bacterial cell - the periplasm and cell membranes are particularly exposed to environmental stress. The consequences of unfavourable factors include disturbances in the structure of proteins which may lead to a loss of protein function, and accumulation of protein aggregates in the subsequent stages. To counteract this phenomenon, the elements of the protein quality control system (PQCS) are induced. These include protein folding helpers (chaperones and protein folding catalysts) and proteases that degrade irreversibly damaged proteins. The periplasmic elements of PQCS have been thoroughly characterized in *Escherichia coli*, a model bacterium. However, there is much less knowledge about the role of these proteins in phytopathogenic bacteria. In the case of *D. solani*, there were no reports on this topic.

This study aimed to determine the significance of selected periplasmic PQCS elements, including DsbA disulfide oxidoreductase, DegP and DegS proteases, and FkpA peptidyl-proline isomerase, for the ability of *D. solani* to grow under stress conditions, produce virulence determinants, efficiently infect and develop disease symptoms in the plant.

First, the mutated strains of *D. solani* were obtained. The genes encoding the mentioned proteins were inactivated by disrupting their sequence with the antibiotic resistance cassette. In the case of most of the tested mutants, no significant differences in sensitivity to single stress factors were observed as compared to the wild-type strain. Only

the use of a combination of stresses, e.g. ionic osmotic and thermal stresses, had a more negative effect on bacteria lacking PQCS elements. However, the observed differences were not large. That results may indicate the possibility of mutual replacement of individual elements of PQCS in *D. solani*. Then, using a variety of plant models (whole potato tubers, chicory leaves, *in vitro* potato plants, and potted potato plants), the ability of the mutant strains to infect and cause disease effects were investigated. Among the tested mutant strains, only *D. solani dsbA::cm* showed significantly reduced virulence. The mutation in the *degS* gene slightly decreased the infectivity while the mutations in the *degP* and *fkpA* genes did not seem to affect virulence of *D. solani* at all. Therefore, further studies focused on the effects of the lack of DsbA protein in *D. solani*. The ability to produce functional virulence factors was thoroughly carried out by the activity of secreted CWDEs assays, cell motility assays, and the amount of extracellular siderophores analysis. It turned out that *D. solani dsbA::cm* strain was characterized by a very low activity of pectinases and cellulases, strongly restricted motility, and negligible production of siderophores.

For a better understanding of the mechanisms in which the DsbA protein is involved, the quantitative SWATH-MS proteomics analyses with the use of liquid chromatography coupled with mass spectrometry were performed. It turned out that a lack of DsbA led to the induction of pleiotropic changes in *D. solani* cells. That effect was not only a reduction in the level of secreted virulence factors (the elements of motility apparatus, and CWDEs) but also the induction of stress response systems. The observed changes indicated disturbances in the homeostasis of the periplasm and cell membranes in *the D. solani dsbA::cm* strain. That may be evidenced by an increased level of stress proteins associated with stress systems dependent on factors σ^E, Cpx, and Rcs. Other pieces of evidence are changes in the level of some enzymes in the main metabolic pathways which are providing energy, reducing force, and biosynthetic precursors to restore the balance in this area of the cell. An interesting observation in the *dsbA* mutant was the increased levels of a group of proteins associated with the stress response within the cytoplasm. This indicates that the effects of the lack of DsbA protein are not limited to the extra-cytoplasmic regions, but affect the functioning of the entire bacterial cell. Moreover, the analysis of the *dsbA* mutant cells' secretome composition revealed the presence of a significant amount of cellular proteins, suggesting disturbance of cell membrane integrity. This assumption was confirmed, *D. solani dsbA::cm* cells were more sensitive to sodium dodecyl sulfate detergent.

To sum up, the results obtained in this study indicate that the lack of a single of the tested periplasmic PQCS elements does not significantly affect the ability to grow and survive stressful conditions by *D. solani*. Among the protein folding factors investigated, the functions performed by DsbA disulfide oxidoreductase turned out to be essential for efficient infection and induction of disease symptoms in plant hosts by *D. solani*.