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Review of the doctoral dissertation by Małgorzata Golanowska entitled:

„Characterization of *Dickeya solani* strains and identification of bacterial and plant signals involved in the induction of virulence"

prepared in the Laboratory of Plant Protection, Department of Biotechnology and Biotechnology, Intercollegiate Faculty of Biotechnology University of Gdansk and Medical University of Gdansk, (Poland) and Laboratory of Microbiology, Adaptation and Pathogenesis, National Institute of Applied Sciences of Lyon, France (L'Institut National des Sciences Apliquées de Lyon Laboratoire de Microbiologie, Adaptation et Pathogénie) under supervision of Prof. dr hab. Ewa Łojkowska and Dr Nicole Hugouvieux-Cotte-Pattat

Plant—microbe encounters can be both friendly and hostile. The soil environment contains numerous beneficial mycorrhizal fungi and rhizobia, which associate with roots and provide plants with mineral nutrients and fixed nitrogen, respectively, in exchange for carbon. By contrast, plants are constantly exposed to a range of fungal, bacterial, and viral pathogens, and have evolved unique defense mechanisms to struggle with these infections.

The knowledge related to various aspects of plant microbe interactions is still growing and its importance will strongly increase due to political and public pressure. The world population is growing and the estimated amount of food needed by 2050 will be double of what is produced now whereas the area of agricultural land is decreasing. We must increase crop yields in a sustainable way. To achieve this goal, we must first learn how to make use of "good" microbes to promote plant growth (e.g. as bio-fertilizers) and how to fight with "bad" microbes to provide successful plant protection.

The doctoral thesis, which I have received for review, fits well into this research topic, as it concerns characterization of *Dickeya solani* strains. It should be emphasized that the research constituting the thesis has been cooperatively conducted in two leading research centers having expertise in the field of plant protection and biocontrol against pathogens.

In my opinion, the choice of *D. solani* as a research model was very accurate. Pectinolytic bacteria from the genera *Dickeya* (former *Erwinia chrysanthemi*) and *Pectobacterium* (former *Erwinia carotovora*) form a group called Soft Rot Erwiniae (SRE). The SRE are found worldwide, they produce a wide range of plant cell-wall degrading enzymes (PCWDE) and cause diseases such as blackleg and soft rot affecting a wide range of host plants during their growth, storage, or marketing. The SRE belong to the group of top ten bacterial pathogens causing great damage to different crops. *Dickeya* spp. affect a wide diversity of tropical and subtropical plants, including potatoes, many ornamental plants, maize, rice, and pineapple. *Dickeya solani* can efficiently infect potato plants causing disease symptoms in temperate climate and leading to huge economic losses reaching, only in Europe, hundreds of millions of Euros. Since potatoes are among the top ten commodities produced in the world and potato losses caused in the recent years by *Dickeya* spp. have increased significantly in a number of European countries and in Israel (a major importer of European potato seed tubers), the choice of *D. solani* as a research model is fully legitimated, especially that *D. solani* strains are considered more aggressive than other blackleg causing bacteria. The choice of such a research model, appropriate research objectives, and methodology comprising microbiological, genetics, and genomics approaches allowed the Author to make important observations, which significantly broaden our knowledge about plant-pathogen interactions.

The reviewed doctoral thesis comprises in total 163 pages of the manuscript and contains 49 figures and 24 tables. In general, the manuscript was prepared very carefully with respect to the language and editorial style and the few minor typing errors do not affect significantly its high scientific value. In general, the dissertation has the layout typical for most experimental papers and the content of each subsection is proportionally balanced. Moreover, the short summaries of the thesis in English, French, and Polish, the list of abbreviations, tables, and figures, as well as the conclusion section, summarizing the main achievements of the thesis were provided.

In the introduction section, several parts can be distinguished in which the Soft Rot Enterobacteriaceae, the symptoms and course of diseases caused by SRE, the SRE virulence factors, secretion systems and their role in SRE cells, as well as issues related to complex regulation of SRE virulence determinant expression were presented. This information is followed by a subsection devoted to methods of detection and identification of SRE. The final part of the introduction provides important information about the significance of the research on *Dickeya* spp., which strongly justifies undertaking a scientific project like the one constituting the reviewed thesis. In conclusion, the introduction is concise and clearly written; it provides all necessary information required for interpretation of results presented in later chapters of the thesis. My small remark concerns the description of the H-NS protein

presented on page 37, which in respect to bacteria should be rather described as a histone-like nucleoid structuring protein instead of “a protein that influences the chromatin structure”.

The aim of the thesis was clearly defined and almost fully addressed in the result section except for identification of potato signals inducing the expression of *D. solani* virulence factors. Of course, I do not consider this a failure: this often happens in experimental work. Moreover, the huge amount of other data presented in the thesis quite clearly verifies the scientific hypothesis of the dissertation.

Materials and Methods were presented in separate sections. The strains chosen for the analyses comprised the type, reference strains, and a set of strains that differed in their origin and were isolated in different climate zones: in Poland, Finland, and Israel. The methods used for the analyses comprised various classical microbiological and genetic techniques as well as modern genomic and bioinformatics approaches. All of them were described with enough precision allowing for independent repetition of the analysis in any other laboratory.

The research described in the result section was started from analyses of the phenotypic traits of *Dickeya* spp. It was a trail to answer the question why the *D. solani* is more aggressive than the other SRE species even at low inoculum levels and why it is able to cause severe disease symptoms in a wide range of climates. Given the availability of preliminary evidence that strains originating from different climatic zones differ in their ability to cause disease symptoms, the Author asked a more detailed question regarding the influence of temperature on the fitness of the strains originating from different climatic zones. To answer this question, several phenotypic and genotypic traits of strains originating from Poland, Finland, and Israel were studied. The results obtained by the PhD student allowed her to observe that *D. solani* strains have higher pectinolytic, cellulolytic, and proteolytic activities at 18 and 28°C than *D. dadantii* and *D. dianthicola*. At 37°C, *D. solani* strains also have the highest cellulolytic activity. The strains of *D. solani* have a higher total pectate lyase activity in induced conditions than *D. dianthicola*.

Among the tested *D. solani* strains originating from different climatic conditions, the Polish strains have the highest pectinolytic, cellulolytic, and proteolytic activity at all the tested temperatures. They also have a higher total pectate lyase activity in induced conditions than the Finnish and Israeli strains. The author rightly concluded that the highest activity of the PCWDE of *D. solani* strains isolated in Poland can explain their highest ability to macerate potato tubers tissue. The more general conclusion drawn from this part of the research was that the high level of pectinolytic, cellulolytic, and proteolytic activities was strongly correlated with the bacterial species while the incubation temperature, although significant, affects them to a lesser extent, and that the tested features are not strongly dependent on the origin of the strains. All the data presented in this section are well

supported by statistical analyses and I my opinion the conclusions are fully legitimated. However, I feel a little bit confused about the conclusion presented on page 106 and 107, in which the author states that: “the **genus** has a higher impact on the strain phenotypic features”. Furthermore, in the description of the graphs presented in fig. 11, 14, 17, 20, 23, the x axis is denoted as “genus”; however, in these graphs we are able to see the features of various *Dickeya* species. During the course of this research, the author used various species belonging to the *Dickeya* genus and various strains of *D. solani* species. Therefore, my question to the Author is: did you mean in the conclusion on page 107 that you observed the differences with respect to the tested features within the *Dickeya* genus or it is just a mistake and it should be stated that the tested phenotypic features are correlated with the species?

My further remark related to this section concerns the way of description of the Author’s conclusions. In my opinion, it is not proper to say that the “genus or species” has an impact on the phenotypic features of strains. It should be rather stated that those features are correlated (as indicated by the observed statistical significance) with the species used in the study.

The *D. solani* strains of various geographical origin displaying differential expression of plant cell wall degrading enzymes were found to be strongly conserved at the genetic level: their molecular profiling revealed that they are actually identical with respect to the REP, ERIC, BOX, and RFLP-PFGE profile. Thus, I agree with the Author that the differential phenotypes of the strains cannot be simply explained by their genome diversity. Nevertheless, such genetic conservation is surprising and I would like to hear the Author’s opinion about this curiosity. Is such conservation also the case in other Dickey species?

In the next part of the results, the influence of potato tissue extract on the pectinolytic activity of *D. solani* strains was studied in order to find the plant tissue compound/s inducing pectinolytic activity, important for development of disease symptoms. For this purposes, mutants of *D. solani* were obtained carrying the GUS encoding cassette in the well-chosen genes encoding pectinolytic enzymes *pelD* and *pelL* representing the main and secondary pectate lyases, respectively, T6SS structural genes – *tssK*, and the *lfaA* gene encoding a regulatory protein of LacI family regulators. The potato tuber extract induced the expression of a secondary pectate lyase gene *pelL* and regulator protein encoding the *lfaA* gene, but not *pelD* (major pectate lyase) and *tssK*. This result is interesting and important, and in my opinion stays in a good agreement with Author’s previous observation, allowing to conclude that a complex regulatory network must exist in highly genetically conserved strains displaying differential expression of PCWDE, and induction of the *lfaA* gene seems to point to such a possibility. Therefore, having the fully sequenced genomes available, the Author may try to answer such a question by global transcriptome profiling of *Dickeya* strains in the future

perspective. My minor remark to this part concerns the lack of a figure demonstrating the constructed mutants with their genetic content, which is usually helpful in such a case.

The final part of the result section was dedicated to the comparative genomics of the selected *D. solani* strains. Ten *D. solani* genomic sequences were analyzed, four of which were *de novo* sequenced. Those comprised *D. solani* strains differing in the virulence levels (2 strains showed a high level of virulence and 2 strains exhibited a low level of virulence). The analyzed genomes were well-conserved (confirming previous observation), and had an apparently pangenomic structure with a clearly distinguishable core and an accessory genome, as well as a pool of unique genes. The core genome of *D. solani* contained genes coding for main virulence factors and genes coding for their regulators. The strains with high and low virulence were identical in 99.98 %, according to average nucleotide identity (ANI) values. In conclusion, this part provides a very nice, modern overview of *D. solani* genome structure and evolution and constitutes an excellent starting point for future analyses and sequence data mining. I have a few comments and remarks related to this part of thesis.

First, on page 145, while summarizing the comparative genomic analyses, the Author states that the genes show 100% homology. I have found similar phrases also in the summaries and introduction. This is not proper and should be avoided. Homology by definition is a binary value and does not display intermediate states: homology, indicating a common ancestor, exists or not and can be observed on the basis of gene/protein sequence similarity/identity, and the similarity/identity may be expressed in percents, but not the homology itself.

Second, in my opinion, in the genome scale comparisons, beside syntenty (which reflects only the conserved gene content), genome colinearity (reflecting also preservation of gene order) should be described, as this term is more sensitive to genome structural variation. For example, the inversion of part of the chromosome preserves the synteny but disturbs the colinearity, and provides useful information about genome evolution.

Third, do the genes (both structural and regulatory) related to *D. solani* virulence cluster together and form any kind of a pathogenicity island or are they dispersed in the genome?

The results obtained during the preparation of the thesis were objectively discussed in the discussion section. Having read this chapter, I became convinced that the Author is a mature investigator, able not only to perform complex experimental and bioinformatics analyses but also capable of proper interpretation of the results obtained as well as presentation thereof against the current state of knowledge in the field (in the thesis more than two hundred literature positions have been cited).

In summary, I would like to state that doctoral thesis by Małgorzata Golanowska deserves a very high mark. The Author presented numerous original and valuable results

concerning the phenotype and genome structure of *Dickeya solani*. The Author demonstrated that the *D. solani* strains have higher activities of plant cell-wall degrading enzymes (such as pectinases, cellulases, and proteases) than *D. dianthicola* strains, regardless of the temperature of incubation and that the Polish *D. solani* strains are superior to strains originating from Finland and Israel in regard to production of PCWDE and ability to macerate potato tissue, presumably contributing to their higher virulence. Moreover, the highly conserved pangenomic structure of the *D. solani* strains was demonstrated with gene-related virulence factors located in the core genome. The interdisciplinary character of the research performed in cooperation with other scientific groups should be emphasized.

In a final conclusion, I am deeply convinced that the reviewed doctoral thesis by M. Golanowska fully meets the conditions required for doctoral dissertations. I submit a request to the Scientific Board of Intercollegiate Faculty of Biotechnology University of Gdansk and Medical University of Gdansk for admission of Małgorzata Golanowska to further stages of the doctoral procedures. Concomitantly, taking into account the high scientific value of the thesis, I suggest awarding the thesis with an appropriate prize.

Andrzej Mazur