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Review of the doctoral dissertation by Sabina Żołędowska entitled:

„Characterization of biodiversity and pan-genome of plant pathogenic bacteria from *Pectobacterium parmentieri species*"

prepared in the Laboratory of Plant Protection and Biotechnology, Department of Biotechnology, Intercollegiate Faculty of Biotechnology University of Gdansk and Medical University of Gdansk, (Poland) under supervision of Prof. dr hab. Ewa Łojkowska. Co-supervisor of the thesis was prof. Alessio Mengoni (University of Florence, Italy), and the function of auxiliary supervisor was performed by Dr Wojciech Śledź (Intercollegiate Faculty of Biotechnology University of Gdansk and Medical University of Gdansk)

Interactions between plants and microorganisms occur in many different ways and on many different levels. Virtually all organs of the plant interact with some microorganisms at a certain stage of their life. Plants may both benefit and suffer from the microbes either through direct or indirect effects of the associated microbes, as well as serve as habitats for microbial communities.

Development of modern molecular techniques such as Next Generation Sequencing (NGS) allow to identify and quantify the diversity of microorganisms interacting with plants. Complete microbiomes associated with different parts of the plant can be evaluated and such knowledge in necessary step towards understanding functional effects of these multidimensional interactions. The knowledge related to various aspects of plant microbe interactions is still growing and one can assume that its importance will strongly increase in the near future.

The doctoral thesis prepared by Sabina Żołędowska, which I have received for review, fits well into this research topic. It should be emphasized that the research constituting the thesis has been conducted in the leading research group supervised by prof. Ewa Łojkowska, having expertise in the field of plant protection and biocontrol against

pathogens. Moreover, the interdisciplinary character of the research performed in cooperation with other scientific group should be noticed.

Pectobacterium parmentieri is a newly established bacterial species within the plant pathogenic family *Pectobacteriaceae*. Bacteria belonging to a group of Soft Rot *Pectobacteriaceae* (SRP), are causative agents of diseases such as soft rot and blackleg in a wide range of host plants including important crops of high economic impact (e.g. potatoes), during their growth, storage or marketing. The SRP bacteria are found worldwide: in Europe, North America, Africa and Oceania, in different environmental conditions. The success of *Pectobacteriaceae* spp. in the field of plant pathogenesis is mainly attributed to the ability of production of portfolio of plant cell-wall degrading enzymes (PCWDE) including pectinases, cellulases and proteases secreted through type I or type II secretion systems. In my opinion inclusion of *Pectobacteriaceae* spp. into the top 10 bacterial pathogens, fully legitimate the choice of *P. parmentieri* as a research model.

The reviewed doctoral dissertation by S. Żołędowska comprises in total 87 pages and it was presented in a form of two original research articles already published in journals noted in Journal Citation Report (JCR): one in Plant Disease (2018) and one in BMC Genomics (2018) as well as six reports presented at national and international scientific conferences. It should be emphasized that S. Żołędowska is the first author in all of these publications, which indicates her dominant and significant role in the planning and conducting the experiments, as well as data analyses and writing the manuscripts, which was confirmed by relevant author contribution statements. Besides the copies of mentioned above publications covering the PhD thesis, in the dissertation the abstract (prepared in English and Polish), description of Candidate scientific achievements as well as summary of obtained results and conclusions were provided.

As mentioned above *P. parmentieri* species appeared recently as a result of reclassification of potato-originated *P. wasabiae* isolates. The complex taxonomic history of this species was clearly described in both research papers as well as in the introduction section provided by the Candidate. Despite the prevalence of *P. parmentieri* in numerous countries comprehensive studies related to biodiversity of this species, especially based on full genomes sequences has not been previously reported.

The aims of the thesis were clearly defined. Firstly, it was intended to asses of the structure of pectinolytic bacteria population in Poland with special emphasis of biodiversity of *P. parmentieri* strains isolated from potato fields. Secondly, the Author aimed to reveal potential pan-genomic structure of this species and determine the range of genome plasticity focusing on the genetic traits affecting virulence and pathogenicity of the tested strains. After reading both publication covering S. Żołędowska thesis I'm fully convinced that these goals have been fully accomplished.

The choice of appropriate methodology comprising microbiological, biochemical, genetics, genomics and bioinformatics approaches allowed the Author to make important observations, which significantly broaden our knowledge about plant-pathogen interactions.

In the first publication (Plant Disease, 2018) included by S. Żołędowska into her PhD thesis, the results of 2-years survey (2013-2014) of the *P. parmentieri* occurrence on seed potato plantation in Poland were presented. The authors collected about 450 samples of diseased potato tubers, plants or accompanying weeds and demonstrated that under studied condition (i.e. a temperate climate) *P. parmentieri* strains were fairly abundant and were detected in almost all regions of Poland. As proved by multiplex PCR analysis the *P. parmentieri* isolates constituted significant fraction of pectinolytic bacteria from seed potato fields (16% in 2013 and 13% in 2014, respectively). Here, my first minor remark appear, **concerning discrepancy between the percentage of detected *P. parmentieri* strains among the field isolates, which in the publication was specified as above, while in the summary included in dissertation these values were presented as almost twice as large for respective years.** The total number of 85 *P. parmentieri* strains were further analyzed with respect to genetic variability. The application of REP-PCR and *recA* gene-based phylogenetic analysis revealed substantial genetic diversity of obtained population. In the phylogenetic tree constructed on the basis of sequence alignment of 590 bp *recA* gene fragment, the strains were grouped in two separate clades containing *P. parmentieri* strain regardless of the year of isolation. **My another minor remark concern the reasons of application of solely *recA* gene fragment in phylogenetic analyses. According to my knowledge, to reveal the genetic diversity and taxonomic position of any isolates, a multigenic (also known as multilocus sequence analysis - MLSA) approach based on analyses of several conserved genetic *loci* (including e.g. 16S rRNA, *atpD*, *glnII*, *recA*) is usually applied. The use of several genes scattered across the bacterial genome increases the informative level of phylogenetic analysis and improves its discriminatory power.**

The isolates differed with respect to phenotypic traits crucial for virulence of pectinolytic bacteria. It was observed that *P. parmentieri* population obtained in 2013 was genetically more heterogeneous than the one from 2014. However, when both genetic and phenotypic traits were taken into consideration strains obtained in 2014 year differed more significantly from the ones isolated in 2013.

In my opinion this observation is important, because it shows significance of phenotypic data included into all kind of phylogenetic analyses. The genetic potential encoded in the genome, does not fully reflect the real metabolic and physiological abilities of the strains, which are revealed by phenotypic analyses. The biodiversity observed in two-year period was discussed in regard to changing weather conditions. I wonder to what extent

the changing weather conditions directly affect the structure of the soil bacteria population and what role does the plant play in the process of shaping the bacterial community? A plant sensing climatic changes must also make the selective pressure to interacting bacteria. **This is just thought-provoking comment and I would like to hear Candidate opinion about my speculations.** Undoubtedly, the comprehensive data gathered during the studies described in the first publication constituted well starting point for further analyses and allowed to select the most divergent representatives of *P. parmentieri*, which were subjected to complete genome sequencing.

The second publication by S. Żołędowska (BMC Genomics 2018) was an attempt to interpret the genomic basis of the wide host range and widespread occurrence of *P. parmentieri* in different climatic and geographic zones. The previously isolated strains differing with respect to PCWDE secretion, potato maceration activity and fingerprinting profile as well as reference strains (of different geographic origin) were included into comparative genomic analysis. The choice of strains was well thought. I think, that repeated and expanded phenotypic evaluation of strain selected for genomic analyses was also well-reasoned. The authors have gained the confidence that at the beginning of "deep genome mining" analyses by NGS the strains are well defined with respect to crucial phenotypic traits. Moreover these analyses lead to important conclusion that the most severe disease symptoms on plants were raised by isolates having moderate activity of virulence factors, the effects of various PCDWEs were synergistic and that activity of each enzyme tends to complement each other. This result shows interesting adaptive strategy employed by *P. parmentieri* strains (also observed in other plant associated soil bacteria, e.g. symbiotic rhizobia), in which "moderate" frequently means the "most successful" in pathogenesis or symbiosis with plants.

Complete genome sequences allowed to investigate structural variation of *P. parmentieri* leading to the conclusion that the species has open pan-genomic structure with recognizable core (>52%), accessory (>20%) and unique genome fractions (>26%), respectively. The relatedness among the strains measured by ANI values was high, however dendrogram based on all proteins sequences, allowed grouping of the strains into two main clades, differing with respect to the content of accessory genes. No grouping regarding geographical origin was demonstrated. In my opinion, interesting conclusion were drawn from the analysis of dispensable genome fraction and pathogenome of *P. parmentieri*. First, the high genomic diversity and plasticity could be attributed to the location of almost halves of the genes (47%) in predicted dispensable pan-genomic fraction. **Of course real dispensability of those chromosomally located genes needs to be verified experimentally: it is known that some extrachromosomal bacterial genes are indispensable for cell survival with relation to growth conditions)**. The highly abundant

MGE (profages sequences, CRISPR-Cas elements) might have further contributed to the considerable genome rearrangements influencing *P. parmentieri* genome plasticity and allowing for adaptation to different environmental niches. Second, it was confirmed that major virulence genes were located in the core genome, while some variation in the pathogenome structure may be reflected in the different abilities of *P. parmentieri* strain to macerate potato tissue. **My another remark related to the mentioned above results concern LPS biosynthesis genes detected in dispensable pan-genomic fraction.** The postulated correlation between their location in this fraction and different degree of pathogenicity related traits of tested strains is not fully clear to me. The exemplified strain IFB5408 was in fact the most outstanding in biofilm formation and swimming motility. I agree that both of these phenotypes may be related to the membrane structure and influenced by lipopolysaccharide: an essential component of the outer membrane in most gram-negative bacteria. However, the role of additional copies of individual genes of LPS biosynthetic pathway in observed phenomenon is speculative. I could argue that the additional copies may be just example of genetic redundancy typical for plastic bacterial genomes. Moreover, according to my knowledge neither LptA nor LptC proteins possess the ABC signature. LptA family members are periplasmic proteins, which may form a bridge between inner and outer membrane, via interactions with LptC and LptD, thereby facilitating LPS transfer across the periplasm, while LpsC is inner membrane located protein facing towards periplasmic space.

I also have several questions concerning the plasmid uniquely detected in the genome of IFB5427 strain. The plasmid contain some genes related to conjugal transfer, however it was not widespread among the highly related strains, possibly occupying the same ecological niche? What can find out about the evolutionary history of pPAR01? Was it recently acquired by the strain? It has GC content similar to the genome, but is it well adapted to the genome (for example what is codon adaptation index, CAI, for plasmid encoded genes, in relation to the genes encoding ribosomal proteins? CAI value may be a measure of replicon adaptation to host genome, reflecting indirectly the time spent by the plasmid in the cell). The described plasmid encodes numerous potentially advantageous genes, however the strain possessing this replicon belongs to rather moderate ones with respect to pathogenicity related traits, raising the question about the putative function of plasmid? Finally, I wonder whether the source of the strain (it is the only sequenced strain, isolated from the weeds accompanying potato plantation in Poland) may have something to do with unique presence of the plasmid?

In general huge amount of the data presented in S. Żołędowska papers clearly verifies the scientific hypothesis of the PhD thesis. Each time the obtained results were objectively discussed in the relevant discussion sections. Having read the papers, I became fully convinced that the first Author is a mature investigator, able not only to perform complex

experimental and bioinformatics analyses but also capable of proper interpretation of the obtained results as well as presentation thereof against the current state of knowledge in the field.

In summary, I would like to state that doctoral thesis by Sabina Żołędowska deserves a very high mark. The PhD Candidate presented numerous original and valuable results concerning the biodiversity and genome structure of *P. parmentieri* leading to the conclusion that genomic variation and plasticity observed for the species contributed to the wide spreading and ability of bacteria to cause disease symptoms in various climatic zones.

The overall scientific achievements of the Candidate is also substantial, especially as for young scientist. Despite two articles included into PhD thesis, S. Żołędowska is coauthor of nine original research articles, she participated in several research project both as investigator or leader and she took two international internships supported by Erasmus+ project.

In a final conclusion, I am deeply convinced that the reviewed doctoral thesis by Sabina Żołędowska 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 Sabina Żołędowska to further stages of the doctoral procedures. Concomitantly, taking into account the high scientific value of the thesis, I recommend awarding the thesis with an appropriate prize.

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