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Formal basis of the opinion

The opinion was prepared for the Biotechnology Discipline Council of the University of Gdańsk, in the proceedings for awarding the degree of doctor in the field of exact and natural sciences in the discipline of biotechnology in accordance with the decision taken at the meeting on 17/05/2024.

REVIEW OF DOCTORAL DISSERTATION

mgr Roberta Gatta

under the title "**Social interactions between *Bacillus subtilis* and *Dickeya solani***"

in the proceedings concerning the award of the academic degree of doctor

in the discipline of biotechnology

The review has been prepared in accordance with the requirements of the Act of 20 July 2018. (art. 187) Dz.U. 2018, item 1668 and based on the reviewer's opinion on the admission of mgr Roberta Gatta to the further stages of the proceedings for the award of the doctoral degree.

1. General description

The doctoral dissertation of mgr Roberta Gatta submitted for review was written under the supervision of the supervisor - dr hab. Adam Iwanicki, from the Department of Molecular Bacteriology, University of Gdańsk. The dissertation is a monograph written in English and meets the general rules and requirements. It consists of seven main chapters: introduction, aim of the work, materials and methods, preliminary research, results and discussion of results, conclusions and bibliography. The doctoral dissertation also includes an

abstract in Polish and English. The above components of the doctoral dissertation were presented on 146 pages.

The doctoral thesis submitted for review concerns the molecular basis of interactions that may occur between the plant pathogenic bacterium *Dickeya solani* and the environmental isolate *Bacillus subtilis* MB73/2. The main objective and specific objectives of the doctoral thesis (in the form of 3 points) have been correctly defined (p. 57). Unfortunately, the dissertation lacks a clearly distinguishable section presenting the hypotheses. On pages 132-135 there is a chapter "Conclusions", which in my opinion is rather a summary of the results obtained in the doctoral thesis. The doctoral student used a significant number of literature items to write the dissertation, most of them published in the last 10 years, in recognized scientific journals and closely related to the described research problems.

2. The significance and relevance of the issues presented in the doctoral dissertation

Bacteria of the *Dickeya* genus belong to Gram-negative pectinolytic bacteria of the family Pectobacteriaceae, which cause Soft Rot Pectobacteriaceae (SRP). Strains of *D. solani* are among the dominant bacterial pathogens of potato in Europe. They are considered highly aggressive, because they have a relatively wide temperature range required for disease development, a low level of inoculum necessary for the spread of infection, the ability to colonize potato plant roots and an efficient system of spread through the plant vascular system. The results obtained in this study extend the knowledge of ecological dynamics in the plant rhizosphere and may contribute to the development of sustainable and environmentally friendly management strategies for *D. solani* infections in agriculture. The search for natural antagonists, e.g. *Bacillus* strains, is a very promising branch in control of plant disease and may provide a new and more effective solution to this problem.

3. Comments on the doctoral dissertation

In the introduction, the PhD student presented a review of the literature on the classification of the *Dickeya* genus (especially in the context of recently introduced changes), the problem of infection with this pathogen in various European countries and around the world, and the economic losses associated with the global reduction in potato yields. In the following sections, the PhD student presents strategies for managing and controlling infections caused by SRP. Bacterial pathogens of *Dickeya* are transmitted mainly by tubers, which poses a high risk of very rapid transmission to subsequent generations of plants and through export/import between even distant countries. Table 1 (pp. 19-20) summarizes the physical and chemical methods of combating diseases caused by SRP and their limitations that have been studied so far. The summary presented by the PhD student shows that so far there is no effective method of combating the rapidly spreading pathogen and searching for other, alternative solutions is an important issue that should be effectively introduced into standard practices in potato cultivation. The doctoral thesis also addresses the problem of fertilizing plant crops (e.g. calcium and nitrogen) and the correlation of these agrotechnical treatments with the occurrence of diseases caused by *Dickeya*, as well as the possibilities of introducing new potato varieties to the agricultural market, which could be characterized by increased resistance to infections with this pathogen. The Introduction also describes the

role of elicitors as a defense mechanism of plants against bacterial infections, which can activate induced systemic resistance (ISR) and systemic acquired resistance (SAR). The following chapters also describe factors that may affect the biocontrol of pathogens belonging to the SRP, as well as diseases caused by *Dickeya* sp. and infection life cycle of this pathogen. A very comprehensive chapter is dedicated to the factors regulating the virulence of the *Dickeya*. The PhD student describes the genus *Bacillus* and its antagonistic potential in detail.

In conclusion, in my opinion the Introduction is too long and in many paragraphs too far away from the main purpose of the dissertation. There is also a lack of a clear introduction to the research problem.

The 'Materials and methods' section of the dissertation was prepared correctly. The methods were correctly described. What is missing is information on the statistical analyses that were used to present the results, e.g. paragraph 8.8 (Results and discussion). There is also a lack of connection of the individual parts of the methods to the main objectives of the dissertation.

The experiments carried out on optimizing the swarming motility test of *D. solani* are interesting and important for laboratory work, but no numerical/statistical results summarizing the results obtained are presented in the dissertation. Table 2 (p. 87) and Table 3 (p. 90) summarize the results of swarming and AHL production by *D. solani*, but the results are presented by the number of corresponding '+' referring to centimeters, and no statistical analyses were applied. During the implementation of the project and the conduct of the experiments, the PhD student encountered many technical difficulties as well as those arising from the biological properties of the microorganisms. She described these problems and pointed out potential causes of failure. I very positively evaluate the contribution of the PhD student to the performed experiments, patiently searching for new/alternative solutions, which allowed to explore the basics of interactions between the studied microorganisms. Chapter 8.8 (Results and discussion, pages 122-124), which describes the ability of *D. solani* to induce soft-rot symptoms on potato slices in co-inoculation with *B. subtilis*, was very well presented in terms of the results obtained. Very interesting are the results of an experiment in which it was shown that the LysR-regulator may be involved in the regulation of motility and secretion of antimicrobial compounds (Chapter 9.4). Further research in this area is needed.

In conclusion, the results were presented in an interesting way and, although not in all experiments the expected outcome was positive, they represent a great value for future research. Part of results presented in this dissertation was already published: Gatta R, Wiese A, Iwanicki A, Obuchowski M. Influence of glucose on swarming and quorum sensing of *Dickeya solani*. *PLoS One*. 2022 17:e0263124. doi: 10.1371/journal.pone.0263124.

4. Key results of the doctoral thesis and their significance

Among the most important achievements presented in the dissertation of mgr R. Gatta, I include:

- (i) Demonstrating that medium type, volume, and humidity levels play a key role in inducing swarming motility. These factors affect the accumulation and detection of AHLs signaling molecules in the vicinity of the colony, thereby facilitating or limiting the expression of swarming motility.
- (ii) Demonstration of a strong interaction between glucose concentration, carbon catabolite repression, AHL production and swarming motility.

- (iii) Confirmation of a complex prey-predator antagonism rather than a simple avoidance mechanism, e.g. by the formation of a zone of inhibition between the interacting bacteria, a sharp front that *B. subtilis* is unable to penetrate and the coordinated and directional escape of *D. solani*.
- (iv) An indication that surfactin may facilitate the diffusion of antimicrobials produced by *B. subtilis* in the culture medium, while increasing membrane permeability to AHLs, thus favouring faster and coordinated escape of *D. solani*.

In conclusion, the dissertation of mgr R. Gatta presents a number of important scientific achievements presenting new and valuable knowledge in the field of interactions between rhizosphere bacteria and crop pathogens, which can contribute to the introduction of new solutions applied in sustainable agriculture. The experiments, which were carried out using diverse research techniques, present a broad set of research results that are well presented.

5. Critical comments

- (i) The dissertation contains shortcomings related to the Latin names of the organisms studied or cited, e.g. italics are not always used, abbreviations of Latin names are incorrectly used when names are described/cited again, Latin names of bacteria are sometimes misspelled, e.g. page 15, 21, 22, 24, 40, 78, 87, 92,
- (ii) The first information that relates to the hypothesis of this dissertation is on page 78 (preliminary results) and is very general, indicating that *B. subtilis* strain MB73/2 can produce an unidentified antimicrobial compound(s) with potential antagonistic activity to *D. solani* sp. More precise hypotheses do not appear until pages 105 and 108 (Results and discussion).
- (iii) Editorial comments: (i) on page 70 (line 2) there is no reference to the relevant paragraph or citation; (ii) on page 115 (line 17) there is no indication of the relevant Figure.

6. Conclusions

In conclusion, the dissertation of mgr Roberta Gatta, presents important and well-presented research results, which contributes to its overall good scientific level. The number of my comments is minor and does not affect the overall good evaluation of the dissertation. I conclude that the dissertation submitted for review meets the conditions set out in the Act of 20 July 2018. (art. 187) Dz.U. 2018, item 1668. In view of the above, I request the Council of the Biotechnology Discipline of the University of Gdańsk to admit mgr Roberta Gatta to the further stages of the proceedings for the doctoral degree in the discipline of biotechnology.

Prof. dr hab. Katarzyna Hrynkiewicz

