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Review

of the doctoral dissertation of Mrs Roberta Gatta, entitled "Social interactions between *Bacillus subtilis* and *Dickeya solani*".

The following review was carried out in response to the decision of the Biotechnology Discipline Council of the University of Gdańsk, dated April 26, 2024, appointing me to the doctoral committee as a reviewer, based on the following documents:

- 1) a copy of the doctoral dissertation,
- 2) regulations for awarding doctoral theses.

I unequivocally state that the documents made available to me are complete, both in terms of form and content. Familiarization with the above materials allowed me to prepare the submitted review.

Assessment of the form of the work

The dissertation submitted for assessment was carried out at the Division of Molecular Bacteriology of the Intercollegiate Faculty of Biotechnology of the University of Gdańsk and the Medical University of Gdańsk under the supervision of dr hab. Adam Iwanicki. The work has the form of a doctoral dissertation with a total number of pages: 146, the dissertation was written in English. The main part of the work consists of an abstract, summary in Polish, introduction, purpose of the work, description of the materials and methods used, preliminary results, results with discussion, summary and bibliography. The work contains 40 figures, 4 tables and a bibliographic list in alphabetical order. The work is written in an excellent way, which is due to several factors. 1) Introduction, which contains a very concise presentation of the subject of the

work with particular emphasis on the *Dickeya solani* as a very important plant pathogen, *Bacillus subtilis* as a potential environmental predator of *D. solani* and the complex social interactions observed in the bacterial world with particular emphasis on *D. solani* and *B. subtilis*. 2) The methodological part fully refers to all the experiments described in the Results and Discussion section. The methodology concerning the swarming motility is particularly valuable. 3) The Results and Discussion are written in a cause-effect arrangement. The results and conclusions from one experiment are the basis for planning the next experiment. The results part reads like a good novel describing the social interactions between *D. solani* and *B. subtilis*. The reader moves on with curiosity to each subsequent part of the results, which brings him closer to understanding the complex interactions between these two bacteria. 4) The conclusions regarding each of the experiments, as well as the final summary, are objective and balanced. According to the reviewer, Roberta Gatta's doctoral dissertation is exemplary. The reviewer does not undertake to evaluate the grammar of the English language used in the work.

Evaluation of the scientific achievement

The scientific objectives included in the work were fully implemented by the PhD student and described in the dissertation.

Roberta Gatta has undertaken a very interesting research topic. This is determined by the following facts: 1) *D. solani* is a significant plant pathogen, especially potato crops. This bacterium is responsible for huge losses in the global economy. 2) *D. solani* is an example of a bacterium whose significant spread, especially in Europe, is most likely caused by climate warming. 3) *D. solani* is also an example of a bacterium that has become a significant plant pathogen worldwide as a result of the globalization of crops. 4) The use of *B. subtilis* as a potential natural tool in combating plant infections caused by *D. solani*. 5) Conducting research based on environmental strains of *D. solani* IBF102 and *B. subtilis* MB73/2 isolated from domestic potato crops. 6) Roberta Gatta has taken on a very interesting scientific topic, which is an attempt to describe the social interactions between *D. solani* IBF102 and *B. subtilis* MB73/2. The PhD student begins with a macroscopic description of these interactions and ends with an attempt to explain their molecular basis. This aspect of the work determines its great value.

Of particular scientific interest is the study of social interactions between *D. solani* IBF102 and *B. subtilis* MB73/2 in environmental conditions very similar to the natural. For this reason, Roberta devoted a very large part of her work to the optimization of experiments involving the study of the swarming motility, in relation to pure cultures of *D. solani* IBF102 and *B. subtilis* MB73/2 and finally both of them in one culture. The optimization included parameters such as: type of medium, method and time of drying bacteriological medium, humidity of the environment during incubation, volume of the medium used, concentration of the medium used, the effect of glucose concentration, spatial distance of inoculation of both bacteria on the plate and the effect of time between inoculation of one and the other bacteria. The optimization of these parameters finally allowed for obtaining high repeatability for swarming motility experiments. At the same time, the obtained macroscopic and molecular image of social interactions between *D. solani* IBF102 and *B. subtilis* MB73/2 is of great scientific importance, and the PhD student was able to avoid recording and describing artifactual phenomena.

Roberta approached the study of social interactions between *D. solani* IBF102 and *B. subtilis* MB73/2 in a very systematic way. Step by step, she discovered subsequent aspects underlying the antagonism between these two bacteria. For this purpose, the PhD student used many experimental techniques, including: chemical mutagenesis, mutagenesis using transposons, molecular cloning techniques, genome sequencing and analysis of sequencing results, fluorescence and white light microscopy, advanced methodology of culturing bacteria on solid media. As a result, Roberta Gatta obtained the following main conclusions: 1) there is a very well-defined growth inhibition zone between the interacting bacteria *D. solani* IBF102 and *B. subtilis* MB73/2, 2) there is a sharp border near the area occupied by *D. solani* IBF102, which *B. subtilis* is unable to cross, 3) *D. solani* IBF102 bacteria 'escape' in a directional and coordinated manner from the aggressor *B. subtilis* MB73/2, 4) surfactin secreted by *B. subtilis* MB73/2 is a factor that significantly facilitates the directed migration of *D. solani* IBF102. Getting closer to explaining the mechanism of the formation of the inhibition zone by *D. solani* IBF102, which *B. subtilis* MB73/2 could not cross, was possible thanks to the comparison of the behavior of two *D. solani* strains: IBF102 and IPO2222. These strains differ from each other only by 6 mutations in their genomes. Studies

indicate that a mutation in the gene encoding the LysR-type transcription regulator is responsible for the specific behavior of the *D. solani* IBF102 strain grown in the presence of the *B. subtilis* MB73/2 strain. It remains to be investigated how the change in the LysR repressor affects the expression of the transcripts regulated by it and, ultimately, the observed phenomenon of the formation of an inhibition zone by *D. solani* IBF102.

The conducted studies are very comprehensive. As a reviewer I would like to suggest the possibility of investigating the following aspects of the interaction between *D. solani* IBF102 and *B. subtilis* MB73/2 in solid culture conditions: 1) the change of pH distribution in the plate during the co-cultivation of both strains, 2) the change of glucose content distribution in the medium during the co-cultivation of both strains. However, the most important is the analysis of the metabolome and proteome using mass spectrometry for pure cultures of *D. solani* IBF102 and *B. subtilis* MB73/2 and co-cultivation for macroscopically distinctive areas of the plates. It seems that this type of analysis, perhaps in combination with transcriptomic analysis, will allow for the final identification of molecular factors responsible for the observed interactions between *D. solani* IBF102 and *B. subtilis* MB73/2.

Editorial evaluation of the work

The work is written very well. As a reviewer, I do not want to focus on individual editorial errors that have no impact on the substantive value of the dissertation. Out of obligation I will mention only a few of them: typos in protein names, e.g. 'Kdgr' on page 36, spelling errors in the names of bacterial species, e.g. *Pseudomonas Syringae* – page 45, Polish inclusions in the text of the paper, e.g. '...od fourteen to eighteen carbons.' – page 45, errors in chemical formulas, e.g. NaCl₂ – page 63, no spaces between the number and the unit of volume, e.g. '25ml, 50ml' – page 67, citing the method with the number XXX – page 70, citing the figure with the number X – page 115, inconsistent signing of panels in the legend to figure no. 11, incorrect designation of panels B and C in figure 15, inconsistent introduction of figure 36 first, and then figure 35, in the case of figures 35 and 36 the use of two different units of length.

Application for distinction of doctoral dissertation

As a reviewer, I apply to the Biotechnology Discipline Council of the University of Gdańsk to distinguish the submitted doctoral dissertation of Mrs Roberta Gatta. Justification of the application: 1) The dissertation describes a huge amount of experimental work of very high scientific value and great publication potential. 2) The work is a model example of scientific inquiry, the initial point of which is the observation of a macroscopic phenomenon, and the final effect is its explanation at the molecular level. This is all the more important because it concerns very complex phenomena of interactions between bacteria constituting environmental strains. 3) The work has significant application potential, drawing attention to the possibility of using the *B. subtilis* MB73/2 strain in combating a globally important pathogen such as *D. solani*.

Roberta Gatta is a co-author of one publication. The reviewer understands, however, that the PhD student and the supervisor decided not to publish the current results due to the lack of a final experiment determining the effect of LysR protein mutations on the observed interaction phenomenon between *D. solani* IBF102 and *B. subtilis* MB73/2. Performing this experiment, e.g. by using mass spectrometry, will allow for an outstanding publication of the entire results.

Final conclusion

I declare that the doctoral thesis of Mrs Roberta Gatta is innovative and has significant scientific value. In connection with the above, I declare that the dissertation submitted for evaluation meets all the conditions specified in art. 13 of the Act of 14 March 2003 on academic degrees and academic title and on degrees and title in the field of art (Journal of Laws 2003 No. 65 item 595, as amended) and at the same time included in the provisions introducing the Act – Law on Higher Education and Science (art. 179. 1. of the Act of 3 July 2018). The research conducted by the PhD student may be of significant importance in a broadly understood cognitive and practical nature. At the same time, I request the Biotechnology Discipline Council of the University of Gdańsk to admit Mrs. Roberta Gatta to further stages of the doctoral procedure.

Gdańsk, 15.08.2024

Rafał Rafpiatek