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**Review report on PhD Thesis of mgr Roberta Gatta**  
**“Social interactions between *Bacillus subtilis* and *Dickeya solani*”**

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Mgr Roberta Gatta submitted the doctoral thesis dealing with the investigation of the environmental strain *B. subtilis* MB73/2 for its efficacy in controlling *Dickeya solani* growth. Moreover, the PhD thesis concerning the assessment of the relationships between these microbes and screening of single gene deletion mutants of *B. subtilis* and *D. solani* allowed to explain mechanism of a pray-predator interactions in the context of bacterial community.

**The background and the scope of the thesis**

Soft rot Pectobacteriaceae including *Dickeya* genus are responsible for diseases of wide range of crops. *D. solani* compared to the other species of that genus has a very narrow host range and is dominated potato pathogen in Europe, and in some countries *D. solani* caused up to 25% of the potato blackleg incidents. Moreover this pathogen can colonize the roots of potato plants and contaminate plants by the vascular system of crops. What is important *D. solani* is resistant to antimicrobial metabolites synthesized by saprotrophic bacteria of potato holobiont and was identified also in healthy potatoes rhizosphere. Economic impact of *Dickeya solani* is significant, as this pathogen threat global production of potato, constituting one of the four the most important crop in the world. However, the yield reduction and financial losses caused by *Dickeya* species are difficult to assess due to similar symptoms of diseases generated by other soft rot Pectobacteriaceae.

It is known that calcium and nitrogen have a crucial role in plant resistance, therefore balanced fertilization can be a part of integrated control strategy against blackleg and soft rot

pathogens, together with breeding cultivar resistant to these plant health problems. Moreover, microbial control agents are used to protect plants against pests and diseases. Soil, rhizosphere and endophytic microbiomes are recognized as rich sources of microbial communities with representatives of *Bacillus*, *Pseudomonas* and *Serratia* genera relevant to protect plants against pathogens. These microbes belongs to plant growth promoting bacteria and in recent years they becoming very valuable alternative to the use of chemicals and pesticides in sustainable agriculture enhancing plant growth and fighting against phytopathogens. It is worth to mention that bacteria belonging to the *Bacillus* genus have GRAS (Generally Recognized as Safe) status, therefore have great potential from biotechnological perspective. Bacterial social interactions are complicated and are connected with signal molecules secretion and depends on single-cell gene expression. However, there are many knowledge gaps concerning interactions between beneficial and pathogenic microorganisms in order to understand mechanisms of actions important from practical, biotechnological point of view.

**Therefore, in this doctoral dissertation, in order to deepen knowledge and to find solution for *Dickeya solani* control, mgr Roberta Gatta performed experiments investigating the competitive dynamics between *Bacillus subtilis* MB73/2 and *Dickeya solani* IFB102. These research are relevant for understanding the ecological dynamics of microbial communities in plant rhizosphere and contributing to the development of sustainable strategies to control *D. solani* infections in agriculture.**

**The novelty and scientific level of the thesis are very good, considering the investigation of competitive strategies concerning species interactions, elucidation of the molecular mechanisms recognition important in antagonistic interactions through understanding involved signaling pathways, as well as evaluation of biotechnological potential of *B. subtilis* as biocontrol agent against *D. solani*. These research are important for development of environmental friendly solutions allowing many applications for science and new practical biotechnological future products. This research are in line with one of the goals of European Commission to decrease mineral fertilization and chemical products use in agriculture. In my opinion doctoral dissertation background is up to date, innovative and relevant for biotechnology and sustainable agriculture development.**

#### **General description and the structure of the thesis**

The thesis presents original research results in the field of biological sciences, agricultural and environmental biotechnology. The content of the thesis include

**introduction with three main chapters concerning characterization of soft rot Pectobacteriaceae and *Dickeya* sp., description of plant growth promoting rhizobacteria and state of art on bacteria social interactions. All of them include numerous sub-chapters providing very comprehensive background into dissertation topic to the reader.**

This PhD thesis is clearly presented and well structured. It consists of 7 main chapters including Introduction, Aim of the study, Materials and methods, Preliminary results, Results and discussion, Conclusions, and Bibliography. The thesis is written on 146 pages and enriched by schemes, figures and tables supporting reader to follow the topic and results. At the end PhD student placed numerous, recent, valuable references confirming that mgr Roberta Gatta made deep recognition of presented topic.

In the first introduction chapter PhD Candidate includes description of *Dickeya* genus taking attention to *D. solani* potato pathogen with description of economic losses and potential strategies of managing and controlling infections caused by soft rot Pectobacteriaceae. Mgr Roberta Gatta discusses economic impact of potato-diseases pathogens. In sub-chapter concerning management control strategies PhD Candidate presents physical and chemical treatments, role of calcium and nitrogen in plant resistance, breeding resistance strategies, application of elicitors inducing plant defense response and biocontrol agents. Then mgr Roberta Gatta introduces reader to the diseases description caused by *Dickeya* sp., as well as in infection cycle of this pathogen. She presents all steps of this process including adhesion to the plant surface, apoplast invasion and cell wall degradation. Very important sub-chapter concerns plant cell wall degrading enzymes, secretion system of this pathogen and virulence regulatory network. PhD student clearly presents and underlines relevance of plant growth promoting rhizobacteria with characterization of *Bacillus* genus and *B. subtilis* species with mechanisms of action of this beneficial bacteria as phytopathogens antagonist, including nutrient availability improvement, plant hormone regulation or antibiosis, underlines the role of regulatory network and antimicrobials secreted by this species. In last introduction chapter PhD Candidate presents social interactions of bacteria taking into account *quorum sensing* phenomenon and biofilm formation.

In chapter II PhD Candidate underlined that the main goal of her research was to investigate the competitive dynamics between *Bacillus subtilis* MB73/2 and *Dickeya solani* IFB102, two putative antagonistic species naturally inhabiting the plant rhizosphere. The objectives are clear and are connected with the title and content of the dissertation.

Chapter III includes the description of the used methodology to perform research presented in PhD thesis. Mgr Roberta Gatta presented detailed description of strains, primers, reagents, media and buffers used in the research, as well as described wide range of methods, many different techniques and software used in the investigations, including microbiological, biochemical, molecular, as well as bioinformatical and statistical approaches. This confirms that PhD Candidate is well prepared to scientific work and has wide skills to carried out own research as scientist.

The next part of dissertation (chapters IV and V) is focused on results presentation and discussion in the state of art. The preliminary results obtained by mgr Roberta Gatta were used as a base to further main experiments, because they confirmed antagonism between tested *B. subtilis* and *D. solani* species leading to formulate hypothesis that *B. subtilis* MB73/2 can produce an unidentified antimicrobials with strong activity against *D. solani*. The main motivation of the research was the need to better understand the social dynamics between tested microbes. The discussion section of the thesis presents comparison of obtained results with literature. Author in this part of the thesis demonstrates and underlines that the swarming motility and *quorum sensing* intensity were dependent on volume of the medium and its concentration. Moreover, the results confirmed that the diffusion of N-Acyl homoserine lactones (AHLs) in the medium plays a crucial role in inducing swarming behavior. She also observed the inhibitory effect of glucose on swarming motility of *Dickeya solani*. In order to investigate the social dynamics at play, PhD Candidate performed a series of experiments to analyse how the interacting populations respond to the presence of their competitor when they establish social interactions in co-culture on a semi-solid medium, as well as in liquid co-culture. She obtained also the results indicating that the presence of surfactin produced by *B. subtilis* MB73/2 may facilitate the swarming motility of *D. solani* by releasing the surface tension and by facilitating the formation of membrane pores, enhancing permeability to quorum sensing molecules. The results of mgr Roberta Gatta showed that both tested microbes secrete antimicrobials. However, an adaptative response may allow *D. solani* to initially resist to *B. subtilis*, until the accumulation of the antimicrobial is sufficient to inhibit the growth and cause the lysis of the bacterium. She indicated that presence of *B. subtilis* can significantly reduce the maceration ability of *D. solani*, preventing rot symptoms on potato slices.

At the end of dissertation the general conclusions basing on the performed research were formulated.

**The dissertation is well structured, following logically topics to receive the results of all planned objectives. The results are presented transparently, facilitating their analysis. All the assays were conducted correctly and results were elaborated statistically. The undoubted advantage of the research conducted by PhD Candidate is the use of classical microbiological methods and advanced molecular analyses. This approach allows to obtain more complete results and interpret the outcomes. Very good critical analyses are made in results and discussion section. Very relevant conclusions can be also used as future needs presented at the end of dissertation become a base for future contributions.**

The most important achievements of mgr Roberta Gatta dissertation in my opinion are the following:

- the findings concerning a close association between glucose concentration, swarming behavior, and *quorum sensing* in *Dickeya solani*;
- medium type, volume, and humidity levels belong to critical determinants impacting the accumulation and detection of *quorum sensing* signaling molecules around the colony, supporting or reducing the expression of swarming motility;
- the revealing a complex prey-predator antagonism rather than a simple avoidance mechanism;
- discovering that surfactin plays a crucial role in promoting the swarming behavior of the pathogen, while also being essential for its directional escape.

### **Summary and Conclusions**

**The dissertation is a complex response to reduce knowledge gaps on relevant potato pathogen *D. solani* and its interaction with antagonistic bacterial strains. This thesis is comprehensive, well written and documented. The text is prepared in clear and concise manner. The conclusions confirm that the main objectives of the dissertation have been achieved. The experiments were well arranged and measurements methods and techniques were correctly applied. The results were elaborated and their interpretation was at very good scientific level. The strengths of the doctoral dissertation include the consistency and novelty of the presented research, the high quality of the obtained results and high biotechnological potential of application developed strategies and approaches. The weaker points are mainly connected with too general formulation of some of the conclusions based on rather on literature than own research, only laboratory testing of developed solutions without real-life field studies and lack of results publication. However,**

**it is worth to mention that the topic is very relevant not only for science but also from biotechnology perspective for many practical approaches, mainly in agriculture.**

The following points can be stressed:

- The research topic is relevant for the development of bio-based product for agriculture, horticulture and forestry.
- The provided analyses fully correspond to all objectives of the thesis.
- The research subject, the methodology and methods are consistent, corrected and used appropriate.
- The results are analyzed, interpreted and discussed in accordance with the scientific standards in the manner of environmental microbiologists community.
- The literature references are correctly selected and cited.
- The thesis clearly demonstrates that mgr Roberta Gatta can conduct independent scientific research and future research work carried out by him independently will meet the standards of her scientific community.

The following remarks and comments related to the thesis should be explained in details:

- I didn't find information about inoculum preparation for this research – especially it is interesting to know number of CFU (colony forming unit) per 1 ml of inoculum and survival in different media or carriers, These information are important especially from practical point of view when biopreparations are developed.
- Please justify the main reasons of the selection of *Bacillus subtilis* species used in your study.
- Are there any biopesticides, biofertilizers, bioproducts based on *Bacillus subtilis* strains used in agriculture sector?
- I would like to ask the PhD Candidate if he can point main practical applications of the achievements presented in the dissertation.

The above comments don't affect the positive evaluation of the entire dissertation, which fulfills requirements set on PhD theses.

**The PhD dissertation of mgr Roberta Gatta undoubtedly brings significant input into the knowledge on the *D. solani* pathogen and its interactions with beneficial antagonistic *B. subtilis* bacteria. Notably significant and innovative are the results of the**

**relationships concerning swarming motility and *quorum sensing* phenomenon. Overall, I consider the research and PhD dissertation as valuable study, which present novelty and high scientific level. The PhD Candidate has proven her scientific skills by designing and conducting advanced research and analyzing the results.**

**The PhD thesis of mgr Roberta Gatta covers interesting and very relevant topic, contains original and valuable scientific results in biotechnology discipline. The thesis meets the requirements imposed on a PhD dissertation (art. 187 of Act of 20 July 2018, The Law on Higher Education and Science) and I recommend to admit mgr Roberta Gatta to public defense of her thesis.**

Sincerely,

Magdalena Frąc

Lublin, 18.08.2024