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Report on the dissertation entitled „Investigation of the influence of glucose, glycerol, and iron ions on the synthesis of antimicrobials and formation of biofilms by *Pseudomonas donghuensis* P482" presented to the University of Gdansk by MSc Marta Matuszewska in order to obtain the Dr. degree.

This dissertation was presented as a monography-type, under the supervision of Prof. Dr. Sylwia Jafra and Dr. Magdalena Rajewska from the Intercollegiate Faculty of Biotechnology of the University of Gdańsk and Medical University of Gdańsk, Poland.

Microorganisms associated with plants play a key role in their fitness, some may influence the uptake of nutrients, others may enhance resistance to biotic and abiotic challenges. The interaction between plant defense mechanisms and beneficial bacteria depends on several direct and indirect effects. In a direct system, an efficient colonization of the host plant by beneficial bacteria is an essential step. Beneficial bacteria can compete for the ecological niche (space and nutrients) with pathogens or inhibit pathogen's growth through secretion of antimicrobial compounds (e.g. antimicrobials). Plant growth-promoting bacteria (PGPB) can also reduce the effects of an infection with pathogens. On the other hand, induced systemic resistance (ISR) is a canonical, indirect phenomenon in which beneficial bacteria enhance plant defenses in systemic tissues. Ms Matuszewska presented here a very interesting dissertation on a previously identified bacterial from the *Pseudomonas* genus and named in previous reports *Pseudomonas donghuensis* P482. The first isolation was in Gdansk in 2012 and in parallel in Donghu lake (Gao et al., 2012). The genetic characterization of this bacterium, which was performed in the same lab and manuscript was coauthored by the PhD supervisor: Krzyżanowska DM, Iwanicki A, Czajkowski R, Jafra S. (2021) High-Quality Complete Genome Resource of Tomato Rhizosphere Strain *Pseudomonas donghuensis* P482, a Representative of a Species with Biocontrol Activity Against Plant Pathogens. Mol Plant Microbe Interact. 2021 Dec;34(12):1450-1454. Here, Ms Matuszewska continues the challenging work towards characterization and understanding the possible mode of action as well as the possible use of *Pseudomonas donghuensis* P482 in biocontrol (Krzyzanowska et al., 2016; Ossowicki et al., 2017) approaches.

The use of biocontrol strains in plant protection approaches is very popular nowadays. Its popularity is driven by the trend towards sustainable agriculture and lowering the impact on environment by diminished use of pesticides. However, diverse characteristics of potential biocontrol strains pose challenges and sometimes even risks for the growers, the consumers or even the native microbial community. Therefore, before its use, such strains require a throughout characterization. Thus, along these preconditions, Ms. Matuszewska performed her work and presented her dissertation.

The main premise or working hypothesis, was that bacterial strains adapt to the ecological niche in which they persist or even multiply. This adaptation depends on the available nutrients, in this case carbon and iron were studies, and will affect the impact of the particular bacterium on other microorganism as well as the plant host(s). The tested bacterium, *Pseudomonas donghuensis* P482, was originally isolated from tomato rhizosphere and the general aim was to test if its antagonistic activity against several plant pathogenic bacteria including: *Dickeya solani, Pectobacterium Brasiliense* and *Pseudomonas syringae*, changes on different carbon sources and with different iron bioavailability. It is important to notice that the work presented here was performed on different levels: physiological, transcriptional activity as well as genetic.

The dissertation opens with two abstracts, the usual one introducing the reader to the subject and presenting shortly the main finding, and a second one, which present the topic of biocontrol dependency of growth media and particular gene (clusters) to broader public.

Those are followed with three introductory chapters: introduction, general research hypothesis and aims of the study. The introduction is a good compendium of information required to comprehend the following chapters and the results of the presented work. It presents nicely the concept of PGPR, the diversity of *Pseudomonas* spp. associated with plants. The introduction gives also a very nice overview on the studies bacterium including, isolation data, previous reports from different places and host plants as well as already observed activities. Those activities, reported already, are the base of this dissertation. Very good is the comparison with previous studies and the focus on growing conditions, which were used. Those have namely, in general, tremendous impact on the production of secondary metabolites. The introduction continues with the introduction of carbon metabolism, iron homeostasis and the production of biofilms, all of which are subject of the following experiments. Taking together, this chapter introduces very precisely the subject.
Nonetheless, the introduction could benefit from more precise wording. For example, PGPRs are not necessarily biocontrol agents (page 14). Many supports plant growth by auxin production and nutrient mobilization, without having a negative effect on other microorganisms. Also, the phenomenon of Induced Systemic Resistance (ISR) is not equal to antagonistic activity towards pathogens.
The following chapters 6 and 7 (general hypothesis and aims) present in a concise manner the thesis.

The following chapter 8, presents material and methods used in this study. It is rather well presented and should allow to repeat the experiments without problems. This chapter contain also

the probably most important table of this dissertation, Table 2. It lists the mutant strain used in this study. Ms Matuszewska, continued to use the mutant's KN numbers, this and the Supplementary Table S1 keeps the reader focused during the lecture. The mistake in figure and tables numbering contributes to this focus request.

The main findings are presented in chapter 9. The results are divided into thematical blocks, which allow to comprehend the experimental setup and the presented results.
The data are presented as a matrix of three interacting groups of factors or levels: a) genetic; Three groups of mutants were tested along with the wild-type strain. Mutants related to pyoverdine (PVD) synthesis and mutants in biosynthesis of 7-hydroxytropolone (7-HT) were generated for this thesis. Mutants in an interesting cluster discovered previously (cluster 17) were generated by T. Maciag during his master thesis; b) carbon source; minimal medium was complimented with either glucose or glycerol to verify the impact of different carbon sources on bacterial phenotype and; c) iron content: two different Fe oxidation level, and no Fe control, were chosen to verify the impact of Fe-bioavailability on the performance of bacteria.
This setup makes for, at time, quite complex matrix and the reviewer acknowledges the difficulties to present the results in a simple manner. Generally, the experimental setup, the used controls and verification, statistical analysis and data interpretation follow the good scientific practice and will be acceptable for publication.

The results itself are indeed challenging and too complex for a discussion at this point. The reviewer is looking forward to an interesting discussion during the disputation.

Briefly, the impact of carbon source on the production of PVD in particular mutants is particularly interesting and could contribute to our understanding of antimicrobial activity of different strains as well as on the requirements in respect to the inhabited ecological niche (e.g. host plant or soil), which such strains have. Similarly, the effect of Fe ion-type (Fe(II) vs Fe(III)), is very informative. The two different Fe ions require different uptake mechanisms and are very important also for the host plant. The presented results indeed, can offer only the first insights into the tripartite interaction between the *P. donghuensis* P482, plant pathogens and nutrient (carbon or Fe) present in the same environment. A follow-up studies are necessary.

The chapter 9 is concluded with 3 verifications paragraphs. First, describes the secondary metabolites, including PVD and 7-HT, produced by *P. donghuensis* P482 wild-type strain and the investigated mutants. This section however, maybe because of the missing MS/MS analysis somehow lacks the ambition to present fully annotated pathways, which could have been identified in KEGG or similar. The following qPCR analysis is proceeded with and extraordinary analysis of house-keeping genes and the question which and how many to choose. This deserves a mention. The results confirmed, in general, the estimated regulation. As the last part of results Ms Matuszewska, studies the production of biofilms. Here, the Fe-bioavailability seems to play the most important role.

Important to notice, part of the results was published already:
Matuszewska M, Maciąg T, Rajewska M, Wierzbicka A, Jafra S. The carbon source-dependent pattern of antimicrobial activity and gene expression in *Pseudomonas donghuensis* P482. Sci Rep. 2021 May 26;11(1):10994. doi: 10.1038/s41598-021-90488-w. PMID: 34040089; PMCID: PMC8154892.

The following discussion concludes this thesis. This part recapitulates mostly the results however it gives also a nice background to the already published situation in other bacterial species. Ms Matuszewska presents and discuss very honestly the obtain data, also the negative once, which is not always the case, and provides indication on the possible mode of action. This obviously depends on the different nutrient availability, e.g. the inhibition of PVD production under Fe-sufficient conditions, which inhibits the antibacterial activity of *P. donghuensis* P482, or the downregulation of the 7-TH genes in the presence of glycerol as the only carbon source.

In general, this dissertation displays the deep understanding of Ms Matuszewska in respect to the nature of bacterial physiology and their interaction with other microorganisms. She assessed the questions on different levels seeking verification of her hypothesis using different experimental approaches.

Ms Matuszewska used original experimental solutions to carry out subsequent research tasks. In doing so, she demonstrated the ability to conduct scientific work independently, properly analyze the data obtained and draw legitimate conclusions. There is no doubt that the planning of this research and the discussion of its results required in-depth theoretical knowledge on the subject as well as the combination of already available with new tools.

In my opinion, this dissertation fully meets the conditions for a doctoral thesis (dissertation). I propose to the Scientific Council of the Inter-University Faculty of Biotechnology of the University of Gdansk and the Medical University of Gdansk to admit Ms Matuszewska, M.Sc. to further stages of the doctoral dissertation.

With best greetings

PD Dr. Adam Schikora