



Opponent's review of the doctoral dissertation of Ing. Daria Rapoport

"Bacterial interactions and secondary metabolites in plant and soil environment"

The evaluated dissertation of Ing. Daria Rapoport was conceived under the supervision of RNDr. Markéta Marečková, Ph.D. at the Faculty of Science of the Charles University in Prague.

The dissertation is focused on the study of an environmentally important phylum of *Actinobacteriota*. These microorganisms, with their significant ability to produce secondary metabolites, very significantly affect soil microbiota and plants. The first part of the thesis is devoted to this issue. In the second part, the author looks at the phylum from a different perspective and studies its phytopathogenic and biocontrol representatives. The used molecular-biological methods presented in the thesis are an important tool for getting insight into the secrets of the laws of interactions and characterization of the observed microorganisms.

In terms of content, I rate the dissertation as above average and very comprehensive. Out of 155 pages, 21 pages are devoted to a very straightforward and readable introduction about the current state of the art; the author describes mutual microbial interactions, analyzes the process of quorum sensing and introduces the reader to the main secondary metabolites produced by actinobacteria. The next chapter is devoted to taxonomically novel Trebon Clade. The last part of the theoretical introduction discusses the role of actinobacteria in plants, while comparing the PGP activities with their possible pathogenicity, which is mainly focused on potato scab.

The first part of the actual thesis (approx. 60 pages) is dedicated to monitoring of the interaction of actinobacteria isolated from acidic soil with other bacteria and provides a large number of results, which were obtained in an experimental trial of a very unique arrangement. In this part, methodologies from the fields of microbiology, genetics, and chemical analyses are used. The statistical analyses are very thorough and use many models. The results are presented as bar charts and phylogenetic trees (unfortunately, some are hard to read Fig. 1-11). A large part of the results is devoted to the genetic and biochemical characterization of the new isolate 15TR 583 which was designated as *Trebonia kvetii*. These results were published in *International Journal of Systematic and Evolutionary Microbiology*, IF 2.747.

The second part (approx. 24 pages) presents the results of the study of phytopathogenic actinobacteria isolated from potatoes from different geographical locations. The presence/absence of pathogenicity genes was confirmed in the isolates.

The cluster of isolates with the detected txtBC region was mostly identified in known scab-causing streptomycetes; on the other hand, the cluster of isolates without amplified txtBC had a subset of isolates lacking other genes and was represented mostly by known phytopathogens and sub-clusters with different gene combinations represented only partially by known scab-causing streptomycetes. These results are part of a publication in *Applied Soil Ecology*, IF 4,046, and other papers to be published.

The results of the experiments are presented as form graphs, bar charts, tables, and phylogenetic trees. Obtained results are discussed in the context of relevant data from the literature. The extensive list of used literature includes approximately 321 items.

I consider the focus of the dissertation, its conception, and final drafting to be of high value and scientific outreach. In particular, I appreciated the fact that the doctoral candidate provided a much more complex insight into the issue than is usual, basing her conclusions on a rich set of molecular biological approaches. The work brings new scientific knowledge and will certainly be well received by the scientific community.

Ing. Daria Rapoport fulfilled all tasks assigned and there is no doubt about the quality of her results. She presented her results at seminars in the Czech Republic and abroad as both lectures and posters. She has won several awards. She is the author of 4 scientific publications as the first author (two in high-impact journals) and co-author of other five publications and a member of the teams of several research projects.

The work is in English with many formal errors.

In the theoretical part, from a formal point of view, I can criticize the nonuniform labeling of microorganisms (incorrect use of italics or missing italics). The use of one-letter abbreviations for microorganisms is not always unambiguous.

Methodological part and results - formal flaws:

Most of the pictures lack an explanation of abbreviations (fig. 1-0, 1-6, 1-7, 1-9, 1-20, 1-21, 1-18, 1-19, 1-20, 1-21, 1-22, 1-23, 1-24, 1-25, 1-26, 1-27, tab. 1-5, 1-6, 1-7).

Figure 1-11 is more of an artist's graphic rather than a figure, similarly Figure 1-17.

In Table 1-13, there are decimal points instead of periods, as is correct in an English text.

Inhibition test - not clear what the OD was for the overnight culture. Furthermore, there is no indication of how much water the chemicals are added to.

There are several questions for discussion:

Can you describe the mode of horizontal transfer in the genus *Streptomyces*. Does the conjugation occur both ways?

What role does the addition of achromopeptidase play in the isolation of DNA from a TC isolate?

Do you think 16S rRNA amplicons are suitable for determining the relative abundance of taxa? What are the main disadvantages of this approach?

Explain what the numbers in Table 1-1 mean, including color intensity?

Doctoral dissertation of Ing. Daria Rapoport brings a number of results, her goals have been met and the candidate has demonstrated the ability to independently work in science and to use logic and critical thinking. Therefore, I recommend that the committee accepts the proposed dissertation, which meets the requirements of § 47, paragraph 4 of Act No. 111/1998 coll., and, after its successful defense, **awards the degree of Ph.D.**