A review of dissertation thesis

Thesis title: Diversity and Ecological Role of Cyanobacterial Lipopeptides

Thesis author: Mgr. Tomáš Galica

In the presented thesis, the author summarizes his contribution to research on lipopeptides produced by cyanobacteria. The first part of the thesis provides a brief introduction to cyanobacteria and to microbial lipopeptides including their biological function, biosynthesis, and an overview of cyanobacterial lipopeptides. The introduction contains relevant information to help the reader get quickly oriented in the research field and understand the ongoing text. In addition, the overview of cyanobacterial lipopeptides might be a base for an interesting review paper. The core of the thesis is represented by the second chapter, which contains author´s research achievements in the form of comments to one manuscript in revision and four papers published in impacted journals. Tomáš Galica is a first author of two of them and a co-author of the remaining ones. Full texts of the papers and the manuscript, and CV of the author are attached at the end of the thesis. The literature sources used throughout the thesis are appropriately cited and are listed using a consistent formatting. The thesis is written in English.

Considering the research quality, I appreciate the LC-MS/MS method developed and successfully applied for detection and (tentative) identification of lipopeptides. Particularly the idea of using two different collision energies to detect fragments originating from amino acid as well as fatty acid moieties is very elegant and it is noteworthy that this piece of research was published in one of best chromatography journals. The genome mining applied in the paper II represents an up-to-date approach for targeted search of new specialized metabolites. Its application facilitated to identify tens of new biosynthetic gene clusters of lipopeptides, which is a great starting point towards discovery of new microbial specialized metabolites. Interesting is also the effort to find a correlation between the presence of the lipopeptide gene clusters and the life style of the studied cyanobacteria – it would be exciting to confirm the conclusions in upcoming research. Lastly, I would like to highlight the fourth paper, which is in my opinion the most intriguing one. It describes discovery of a new lipopeptide, cyanochelin A and its analogs. This work involved genome mining, metabolite purification and its structure elucidation, and experiments uncovering its biological role, making this paper a complex research story.

The presented work contains all parts formally required for the short version of a PhD thesis. The text is well organized and easy to follow. The quality of the work is well documented by the fact that four out of five presented publications already successfully passed the independent scientific review in decent scientific journals. I appreciate also the wide scope of methodological approaches used by the author (cyanobacteria culturing, LC-MS/MS analyses, metabolite purification by HPLC, bioinformatics - genome mining in particular, silent gene cluster activation, labelling studies, photolytic experiments). **To conclude, I am convinced that this thesis should be accepted for the defense and if that is successful, I recommend Mgr. Tomáš Galica to be awarded the Ph.D. degree.**

Out of my interest and curiosity, I have the following questions for the discussion:

Question to paper I.

The MS/MS spectra on p. 74 contain a lot of peaks; while it may be still possible to easily detect the fragments belonging to an expected lipopeptide, is it not too challenging to identify fragments of a new/unexpected lipopeptide? What could be the reason for so many peaks?

Question to paper II.

In this study, lipopeptide gene clusters were identified in only 16% strains (out of 376). Could this information be biased for instance because the lipopeptide biosynthesis employs an alternative to the canonical fatty acid-AMP ligase (FAAL)? Would not you expect the lipopeptide cluster to be in nearly all substrate-associated strains, i.e., that for these strains lipopeptides are more of primary rather than secondary metabolites? Would you be able to use an alternative gene to FAAL for the targeted search of lipopeptides?

Question to paper III.

Here you provide details on the biosynthesis of two lipopeptides, in which two different types of fatty acid-AMP ligases are employed in order to incorporate fatty acids with different side chains. Are some of the strains accessible to basic genetic engineering methods? Would it be possible to increase the number of produced variants by heterologous expression of eg. FAAL type II into a strain with FAAL type I only?

Question to paper V.

In this paper, you analyzed the biosynthetic gene cluster of muscotoxin and provided convincing evidence using labelling studies that 4-methyl-L-proline is derived from Leu. However, you also considered that it might be derived from Pro. Are you aware of any biosynthetically reasonable way to derive 4-methyl-L-proline from Pro?

In Prague, September 10, 2021 Zdeněk Kameník, PhD.