

Review report for Frantisek Jirik's thesis: The evaluation of the photochemical activity of cyanochelin 1026 and its photolytic fragments.

The present master thesis work performed by Frantisek Jirik for the partial fulfillment of the master of science award from the Faculty of Science, University of South Bohemia is aimed to evaluate the photochemical activity of a siderophore Cyanochelin 1026 and identify its photolytic fragments. The thesis is written in 104 pages and contains all the required elements, the results are illustrated by well elaborated graphs suitably illustrating the results obtained. I evaluate the assignment of the topic positively, since despite the fact that the siderophores have been studied extensively in past, study on siderophores from cyanobacteria is still scant. Also, identifying the photolytic fragment of cyanochelin 1026 is one of novel aspect of the current work. The methodological approach was appropriately chosen and there is no doubt that the results obtained are relevant. The student has certainly got an insight into general microbiology and analytical chemistry.

General Comment:

I have some major concern related to the background organization, repetition of sentences from methods and results into discussion section and typological error occurring throughout the thesis.

Introduction:

- Thesis should be checked thoroughly for basic typological errors like spacing after the full stops. And most importantly the first sentence of the section 2, gram-negative should change to Gram-negative.
- I personally feel more detailed introduction should be provided for cyanobacterial lipopeptides (with some structures) especially the siderophore studies in the current work belongs to that class and also considering the host laboratory expertise in cyanobacterial lipopeptides.
- Biosynthesis of lipopeptides are not that relevant for current study but still would like to see more general cyanobacterial lipopeptide structural variations.

Materials and methods:

- In general avoid using comma (,) instead of decimal (.), especially if the thesis is written in English.
- Proper and more accurate citations should be provided, for example *Cyanobacterium Leptolyngbya* sp. NIES-3755 growth was reported in Galica et al., 2021, then why to cite Mares et al., 2019. Growth parameters are very general, but if the same strain has been reported with standard culturing conditions then its proper to cite most suitable one.
- Section 4.2.1, why CAS assay was used? Was it used for monitoring the siderophore production while growth? If not then it should be placed somewhere else rather than in section 4.2. If yes then add a general paragraph stating the purpose of this assay, which is currently placed in the section 4.1.
- Tab 2: also provide the flow rate.
- More details on CID should be provided as in the current state it is not clear that a ramping of eV was used, and if so what was the collision energy (eV) for obtaining the MS/MS spectra for cyanochelin 1026.

Results:

- I don't have much comments on this section as the results are presented very well and illustratively. Only thing I would like to mention here is to maintain a uniform and correct

representation for presenting m/z values. For example sometimes its presented as m/z633 and sometimes 633m/z.

- One of the main objective of the current work is to optimize the isolation procedure in order to obtain sufficient amount of Cyanocelin 1026 for NMR spectroscopy. But I am missing the detailed results of NMR spectroscopy and also details about recording NMR spectra is missing in methodology section.
- Fig 34: What was the reason to identify the fragments m/z 633 and m/z 634 at 0 minute? Is it a real fragment or a precursor molecule. Was it detected in the crude extract too? If so, more work should be needed to understand that if these fragments are also a precursor molecule. Please mention this in discussion for future work.
- It would be also great to see the isolation of various photolytic fragments of cyanochelin 1026 and evaluate its biological activity or determine the ecological role in its native environment.

Discussion:

- This statement is incorrect “The isolation procedures, which were carried out percussively during the work on the thesis, utilized approximately same among of biomass for each batch with, which has resulted in variable yields of cyanochelin 1026 as can be seen in Tab. 11.” As the first batch used for isolation was much lower than other batches.

All the above mentioned comments do not undermine the fact that the student certainly did a good job in the lab. I think that in this respect he has fully met the requirements for a master thesis, but he need to spend more time on formatting and organizing the thesis. Also, thesis being too long makes it bit hard to follow but considering his height, he tried best to fit with his own personality. Hence, I recommend the thesis under the evaluation category of excellent and for organizing the defense.