Statement of the bachelor thesis reviewer

Name of the student: Zuzana Gajarská

Thesis title: Influence of cultivation conditions and age of the culture on the production of cytostatic secondary metabolite 2505 and its natural analogues

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Review of the bachelor thesis "**Influence of cultivation conditions and age of the culture on the production of cytostatic secondary metabolite 2505 and its natural analogues**" by **Zuzana Gajarská.**

The submitted thesis by Z. Gajarská aims to study the cultivation conditions of a selected cyanobacterial strain and to evaluate its production of a selected secondary metabolite with cytostatic activity. The author had to learn sterile cultivation methods, large-scale cultivation, methods of analysis and evaluation of microbial growth and LC-MS methods used in secondary metabolite analysis. Three growth experiment designs were tested and the obtained cells were analyzed. The thesis has 39 pages with one-thirds dedicated to *Introduction* and *Results and discussion* each which can be viewed as a very balanced layout.

The thesis is very nicely prepared, with clear writing, clean graphic design and no significant errors or omissions in technical quality. I have found only a few minor issues such as: 1) on page 5, the citation to Méjean & Ploux, 2013 is typed erroneously as "Mejeán et al." and in the list of references it is cited as a journal paper while it seems to be a book chapter; 2) on page 9, top line, "... light energy are saturated ..." - a word is likely missing here; 3) likewise, on page 33, top line, "production was might have been".

The section *Introduction* covers the topic very nicely, explaining broader background on secondary metabolites in cyanobacteria, principles of used methods of cultivation and analysis but also potential use of the selected metabolite 2505. Personally, I would have welcome some figures in the *Introduction* of e.g. the chemical structures discussed and the structures of the multi-enzyme complexes responsible for secondary metabolite synthesis (if known). The *Introduction* actually contains no figures whatsoever which makes it a bit of a dry read. Some more details of the mass spectrometry topics such as 2+, 3+ ions, isotope clusters or the deconvolution method and its use could have been added to the introduction. Three of the nine figures in the thesis come from MS

data and inclusion of these descriptions would show better that the student understands the method well.

The section *Results and discussion* is written in an understandable, clear style and is reasonably easy to follow, considering the amount and type of data that had to be described. My comments here are limited to suggestions aiming to make the text easier to follow to a more general reader. 1) Where applicable, the number of replicates could be mentioned in figure captions, for example of Fig. 6. Likewise, the metric used for error bar plotting is not described here (one assumes standard deviation). 2) The y-axis units of Figs. 2 and 3 could have been better explained although they are clear after considering the method. 3) The exponents (e+5, e+6) in y-axis labels of Figs. 5, 6 and 8 could have been factored out to make the plots nicer and cleaner. I like the colour choice in Figures it shows that the writer gave some attention to details (or used a good software for plotting).

Considering the amount of work dedicated to growing the cultures, the discussion could have been longer, with inclusion of more extensive comparison with published results. On the other hand I know from my own experience that high quality comparative data of this kind are in many cases rare and not easy to find. The results presented in Table 6 and their discussion appear a bit strange and confusing. The growth rates of L1/N+ and L2/N+ cultures seem quite similar (Fig. 6) yet there is a big difference in the calculated doubling times which do not seem correct. Similarly, on page 33, it is commented that the N+ condition showed faster growth than the N- condition which fits the lines in Fig. 6 but Table 6 shows the rates quite similar and L2/N+ is actually the slowest there.

In conclusion, it is my opinion that the submitted thesis by Z. Gajarská fulfills all criteria required of such work and **I recommend the thesis for the defense**.

Questions to the student, to be answered during the defense:

Q1) It is mentioned on p. 15 that the cultivation medium was autoclaved for 20 minutes. Is this duration independent of the volume autoclaved? What was the volume here? It would be also interesting to hear what is the best way of sterilizing the tens of liters of medium needed for Dynamic experiment II.

Q2) If I understand correctly, the cross-gradient experiment was only done in one repetition. Could you comment on the expected variability should the experiment be repeated? Are the results robust and stable?

Q3) In Fig. 3, the compound 2477 shows higher intensities than the titular 2505 but they are both apparently just minor contaminants among the major components of the base peak chromatogram. The compounds 2505 and 2491 seem to elute very closely to an undescribed compound of much higher abundance (just before the 15 min point). What is the identity of the major components of the base peak chromatogram? How would you proceed with purification of compound 2505?

Q4) Based on your data and experience, what growth conditions would you use for 2505 production and why? What would be the target culture volume for efficient production?

(bonus) Q5) It has been my understanding that NMR is a relatively insensitive method, what is the sensitivity of NMR when coupled with HPLC as mentioned on p.6?

RNDr. Radek Litvín, Ph.D.

In České Budějovice, 13 June 2017