Přírodovědecká fakulta
Faculty of Science

Jihočeská univerzita v Českých Budějovicích
University of South Bohemia in České Budějovice

OPPONENT'S REVIEW ON BACHELOR/DIPLOMA* THESIS

Name of the student: Lisa Pulferer
Thesis title: Occurrence of the cALAs gene in the BCCO Actinomycetes collection and cultivation improvements for the overproduction of secondary metabolites in soil associated Actinomycetes

Supervisor: Erika Corretto, Ph.D., Ana Catalina Lara Rodriguez, Ph.D.
Referee: Ing. Miroslav Petříček, Ph.D.
Referee`s affiliation: 1st Faculty of Medicine, Charles University, Prague

	Point scale[1]	Points
(1) FORMAL REQUIREMENTS		
Extent of the thesis (for bachelor theses min. 18 pages, for masters theses min. 25 pages), **balanced length of the thesis parts** (recommended length of the theoretical part is max. 1/3 of the total length), **logical structure of the thesis**	0-3	2
Quality of the theoretical part (review) (number and relevancy of the references, recency of the references)	0-3	2
Accuracy in citing of the references (presence of uncited sources, uniform style of the references, use of correct journal titles and abbreviations)	0-3	2
Graphic layout of the text and of the figures/tables	0-3	2
Quality of the annotation	0-3	2
Language and stylistics, complying with the valid terminology	0-3	2
Accuracy and completeness of figures/tables legends (clarity without reading the rest of the text, explanation of the symbols and labeling, indication of the units)	0-3	1
Formal requirements – points in total		13
(2) PRACTICAL REQUIREMENTS		
Clarity and fulfillment of the aims	0-3	2
Ability to understand the results, their interpretation, and clarity of the results, discussion, and conclusions	0-3	1
Discussion quality – interpretation of the results and their discussion with the literature (absence of discussion with the literature is not acceptable)	0-3	1
Logic in the course of the experimental work	0-3	2
Completeness of the description of the used techniques	0-3	3

* Choose one

1 Mark as: 0-unsatisfactory, 1-satisfactory, 2-average, 3-excellent.

Experimental difficulty of the thesis, independence in experimental work	0-3	2
Quality of experimental data presentation	0-3	1
The use of up-to-date techniques	0-3	2
Contribution of the thesis to the knowledge in the field and possibility to publish the results (after eventual supplementary experiments)	0-3	1
Practical requirements – points in total		15
POINTS IN TOTAL (MAX/AWARDED)	**48**	**28**

Comments of the reviewer on the student and the thesis:

The presented thesis, dealing with the screening of streptomycetes strains for the production of one group of secondary metabolites containing C_5N moiety in their structure and cultivation improvements for the overproduction of secondary metabolites in soil associated Actinomycetes, is divided into two major themes as there is already obvious from the title. The first one concerns the genetic approach to find a specific gene for the biosynthesis of C_5N unit, the second one intends to make the first preliminary study on optimization of cultivation conditions leading to production of sufficient amount of secondary metabolites that could be further analyzed chemically and by the activity spectra.

As to the formal aspects, the work is fairly well written and fulfills the necessary standards. The **literature review part** of the work, written on 9 pages, is logically arranged but I feel an urgent need to add several comments to it:

- Introduction of the sub-chapter 1.3 Polymerase chain reaction (PCR) was not necessary since this technique is commonly known and does not need to be explained in such a detail.
- In 1.2.Bacterial secondary metabolites there are mentioned only macrolides as secondary metabolites and antibiotics. Why? There exists the whole array of antibiotics and compounds with anti-inflammatory activities and there should be mentioned at least several major groups of these metabolites in this place. Now it looks as if only macrolides were the one. In the last sentence of this chapter there are mixed up together two different things: precursor feeding that is applied for the production of modified metabolites and feeding with labeled intermediates (^{13}C) usually used in study of biosynthetic routes.
- In 1.2.1. Manumycins at the end of the first paragraph there is claimed that mC_7N unit plays an important role in the activity of these antibiotics and as an example it is mentioned farnesyltransferase activity of manumycin A. This is not true, since the upper chain is very likely engaged in this activity. mC_7N structure plays a major role in the antibiotic (antibacterial) activity. Next, I did not understand to the last sentence of this sub-chapter.

I have also several questions and comment to the part **Materials and methods**:

- Why there is used strain with hemA gene as a positive control in 16S RNA analysis as is stated in sub-chapter 3.2.16S rRNA gene PCR analysis.
- There must be some mistakes in tables 2 and 5 as adding 0.75 µl and 0.2 µl, respectively of the stock 100 µmol/L to the mixture of final volume 25 µl does not afford the stated concentration 10 µmol/L.
- In the sub-chapter 3.6.Growth and fermentation media there is wrongly referred table 9 as Table 10 in the text.
- Manumycin fermentation media contained pentane as is stated in Table 9. How did you manage to dissolve this highly flammable alkane in water?
- In the beginning of sub-chapter 3.7.Fermentation of Kutzneria sp.... there was explained that four flasks were prepared for three types of media. That is a bit

awkward statement since it probably should mean that each of three types of media were placed in four flasks.

As to the chapter **Results and Discussion**, I have the following comments:

- It is not surprising that hemA gene can be detected only in one strain from the group of other closely related strains as was mentioned in sub-chapter 4.3.Phylogenetic analysis based on the 16S rRNA gene, since biosynthetic gene clusters are usually transferred horizontally.
- I have a substantial reproach to the method of pH measurement in the cultures. It was obviously done only once and from only one flask even though that four flasks were available. Such measurements should be done at least in triplicates with some statistical evaluation. The fact that cultures did not grow the same way in all four flasks is well demonstrated in the Fig. 9 where in case of cultures without agar only two had some antibiotic activity and in case of culture with 1% agar only one out from three.
- How do you explain that cultures with 0.5% agar did not have any activity in contrast to cultures without agar and cultures with 1% agar?
- I do not agree that the bioassay test in Fig. 10 shows 3 inhibition zones. I can see clearly four with the biggest one at the chromatography start. Why you did not use also another solvent system in order to fractionate these very likely polar compounds?

In the **Conclusions** there is correctly stated that in case of pH measurements 'more data should be gathered'… 'to gain an in-depth knowledge about the optimum pH range'. This is a correct notion, but as I mentioned above the measurements should be done in multiplicates while the conclusion presented in this place on more frequent measurements will not probably lead to more convincing results. According to the results shown in the previous chapter, one can assume that in some media (e.g. in most media without agar) a production of some antibiotics occurred. Therefore it sounds a bit odd that the final conclusion is: … either no secondary metabolites were produced or the extracts were contaminated with the agar...

In spite of the above listed comments, I consider the quality of presented thesis as fairly good and sufficient to be recommended for the Bc. degree defense.

Suggestions and questions, to which the student has to answer during the defense. Mistakes, which the students should avoid in the future:

Please see the above.

Conclusion:

In conclusion, I

recommend/ ~~do not recommend*~~

the thesis for the defense and I suggest the grade .[2]

In Praha date September 13, 2021

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signature

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[2] You can suggest a grade, which can be modified during the defense based on the presentation. However, if the reviewer is not present at the defense, the grade will not be counted. Grades: excellent (1). Very good (2), Good (3), Unsatisfactory/failed (4).