



University of South Bohemia České Budějovice
Faculty of Science

Branišovská 31, 370 05 České Budějovice, Czech Republic

Opponent Report on

Bachelor Thesis of Carmen Ziebermayr

The Bachelor thesis of Ms. Carmen Ziebermayr investigates the permeation of salivation-stimulating agent pilocarpine into tick saliva and the effects of pilocarpine on cytokine production by immuner cells of the host. As such, it is a highly needed work for all experimentators using tick saliva.

The thesis is divided into usual chapters with one exception – chapter Methods usually describes the actual performed experiments. In this thesis, Carmen used it to describe the basics of the used methods and the real experiments are explained in next chapter Experiment. Even though this is unusual, I do not see any problem in such a thesis division. However, the problem is that methods description is shallow, and thus I recommend Carmen either not to include this special chapter in her future Master's thesis or to really describe the methods in depth.

The thesis has 22 pages, which is acceptable for this kind of work. However, I have got an impression that Carmen used bigger dimensions of some images to extend the document (example page 8).

In the introductory part of the thesis, Carmen tries to describe the main aim of the work, pilocarpine and its effects on immune cells, as well as the studied organism, ticks. In my opinion, this section is too short and does not include basic informations, especially on cytokines and their importance for the study. Also the section devoted to description of ticks is insufficient. For example, the third family of ticks, *Nuttallidae*, is left out; only limited information is provided on pharmacologically important molecules in tick saliva and their effect. In this regard, I have my first question for Carmen:

1. why did you choose only Salp15 as an example of salivary proteins and what other molecules are present in tick saliva and what are their effects?

The description of saliva-activated transmission is also poor – inclusion of two more sentences would be enough to have a nice definition of this phenomenon.

Another problem is the usage of literature. First of all, it is unusual to use references in the titles of sections – rather, they should be used in the text itself to help the reader understand which part of the text was inspired by which source. Here are just some of my comments:

I am missing information on the sources of information for histamine action, pathogen invasion of host etc.

In my opinion, reference numbered as “8” is not really dealing with tick attachment to the host (page 3).

Reference numbered as “4” is also not cited correctly, as it does not describe the pharmacologically active molecules (page 3).

The use of webpages should be limited and while some articles on Wikipedia are based on scientific papers, the source numbered “9” (picture on page 3) is not acceptable at all.

The chapter Aim does not contain specific aims and it is written as a combination of introduction, discussion, and aims description. One of the main aims – the quantification of pilocarpine in saliva – is not mentioned here at all. Again, the sources cited in this section are not satisfactory.

In the Materials and Instrumentation, Carmen names five instruments. Among them also so common instruments as incubator or microscope. Therefore, I do not understand why other instruments are not included – I am sure, you used also flow-box, vortex mixer, centrifuges ...

What is more surprising, I did not find the description of HPLC – only the description of the column itself is included. Therefore,

2. Carmen should provide the description of the HPLC instrument during the defense of her thesis.

The Methods are also written in a hurry. For example, only one type of mass analyzer is described – so, my next question is:

3. Are there also other mass analyzers? What kind of mass analyzer was used in this work?

In this section, we can find two figures. The source for both of them is Wikipedia. For future work, I would recommend Carmen to use figures from scientific articles (sometimes even the Wikipedia articles use scientific articles as a source, so you might consider looking them up and using them). Also, both of these pictures are so simple that it would be possible to prepare them by yourself in several minutes. And both of these pictures are examples for how you tried to extend your thesis by including inappropriately big pictures.

4. During the defence, Carmen should include also correct description to these pictures. For example on the Fig. 2 there IS NOT a mass spectrometer. Mass spectrometer is an actual instrument, so, there should be a picture of such an instrument.

Even though there are two sections describing methods, I did not find some elementary informations not only on the principle but also on the real experiments which you did perform. One of many questions to these sections:

5. How did you calculate the peak area for pilocarpine? Manually or you used a software?

Also in the section Experiment, we can find an example of useless filling up space – Table 3 on page 11. The experiment which it describes is so simple that there is no need to include a whole table for it and if you are doing so, it is totally useless to include a table describing the whole 96-well plate if you used only 21 of the wells

One more question to this section:

6. Did you examine also the effects of pure pilocarpine, without LPS added, on splenocytes? If yes, what did you find; if not, why not?

Section Results is also poorly written and should deserve more attention. Again, the descriptions of figures are unsatisfactory. I have many questions and comments, here are some of them:

7. What do you think, what caused the shift in retention time of pilocarpine in your samples compared to the standard?

8. Are the chromatograms included in Fig 5 real chromatograms of your samples?

9. Fragments of parent ions in MS/MS experiments allow to determine the structure of the molecule – however, in your MS/MS spectra the ions are not assigned to fragments, which could confirm the identification of pilocarpine. So, how did you confirm that the studied molecule is pilocarpine?

10. Figure 12 – it seems like low concentration of pilocarpine enhances the production of TNFa – how can you explain this fact?

11. The use of terms like “a lot”, “rather below”, “quite above” are not really scientific and exact – you should not use them. You should either include an x-fold increase/decrease, or you can use percent of a control, etc. So, please for the purpose of the defence, include more rigorous description of effects of pilocarpine on the cytokine production.

One comment – You were not examining the effect of lowering the pilocarpine concentration on cytokine production, but the exact opposite (Page 17, sentence “At a concentration ...”).

Discussion is written similarly to other parts of the thesis. So, only one questions:

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12. You write that the concentration of pilocarpine is “quite well within this range”. In my opinion, it is not. You've used pilocarpine salivary concentration from two different ticks (*A. americanum* and *I. scapularis*) for comparison with your results. However, *I. ricinus* is a tick closely related to *I. scapularis*, not *A. americanum*. So, *I. scapularis* is the organisms to which you should compare your results above all, the values from *A. americanum* are supplementary for the discussion. And for *I. scapularis*, the concentrations of pilocarpine in saliva are much higher than in your experiments from *I. ricinus*. Please, discuss this difference during the defence.

I have also three Biological chemistry students defending in this term, therefore I understand, that Carmen had only limited time available for writing her thesis. However, this cannot explain a number of mistakes, missing information, incorrect information, etc. which I did not even try to include in my opponent report. I hope, her next (masters) thesis will be of much higher quality.

Bachelor thesis is a way how to prepare students for their real scientific work in their following studies. Carmen Ziebermayr fulfilled this purpose and even though the thesis she submitted is not perfect, it fulfills the requirements for bachelor thesis on our faculty. I recommend Carmen Ziebermayr's thesis for defence and I will look forward to her answers.

In České Budějovice, June 14th 2010



Mgr. Ján Štěrba