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Review of Diploma thesis – Bc. Tomáš Knobloch

In his thesis, Tomáš studied the role of bacteria as a source of energy and nutrients for an emerging model diplonemid, *Hemistasia phaeocysticola*. He used three main approaches – cell culture and measurement of growth curves, immunofluorescence and RNA-seq data analysis, which is sufficient for Master thesis, especially during pandemics. The thesis references 44 sources, comprising both reviews and original research manuscripts, and is relatively short with 42 pages. In principle, this is not a problem, but in this case the thesis would benefit from better explanations and additional information in some sections. In past, I was the opponent for the Bachelor thesis of Tomáš, and I think he made a good progress during the course of his Master thesis research.

The formal side of the thesis is fine – it has all the required chapters, sources are referenced, figures and tables are referenced in the text and suitably complement the text of the thesis, graphs contain the information they could contain. I appreciate the effort of the author to write the thesis in English. Although there are some typos (free instead of three, deap instead of deep etc.) and stylistically incorrect or suboptimal phrases, the overall level is acceptable. Nevertheless, I have few comments regarding the formal side of the thesis pointing out what could be improved:

i.) some Latin names are not in italics

ii.) sometimes, shortened informal versions of words are used, such as doesn't instead of does not

iii.) author uses rather unusual reference style – references are inserted after the punctuation mark at the end of the sentence, and in case of multiple references, each is inside an individual pair of brackets

iv.) I would find it better if tables which are part of an electronic supplement were marked as appendices and were listed as electronic appendices, or if at least these were marked as not being part of the thesis, just included in the electronic supplement.

In my opinion, the main weakness of the thesis is the insufficient amount information in the individual results sections. I admit it might be to some extent because of my lack of expertise in the field of lower eukaryotes. It is often unclear why the described experiments were done, what exactly was done, what was the purpose of the experiment. Some of the information could be obtained by going back to methods section, but by far not all, and I believe that the main information necessary for understanding the purpose of the experiment should be repeated in result chapter, even if it is described in more details in methods. In addition, the thesis also suffers from using different names for the same things – this can be the best exemplified by the fact that the author sometimes named and described the media according to their composition, and sometimes as medium with bacteria, positive control and negative control, without explaining which control is which medium. In some results chapters, the results were nicely presented in graphs or figures, but insufficiently described in the text. Despite the criticism, Tomáš learnt and employed several important and widely used techniques in his field, appropriately presented what he achieved, summarised his findings and properly discussed them in the discussion chapter, and I therefore recommend his thesis for the defence.

My questions for the author are:

I.) Even when the libraries were prepared commercially, it would be good to mention the kit that was used for library preparation. Do you have the information which kit from which company was used?

II.) Result chapter 4.1: It is unclear why you were not aware of bacteria species present in the medium, I would assume that available media are of known composition. Or is it not a classical medium, just a sample of ocean water? Why did you assume that detected bacteria species were suitable as prey for *Hemistasia phaeocysticola*?

III.) Result chapter 4.1: Are the bands in figure 4 of expected size? Why there are two bands of PCR product in transformed E. coli lanes

IV.) Result chapter 4.2: Did you explore whether the same phenotype occurs if *Hemistasia phaeocysticola* feeds on something else? How often such phenotype occurs with different nutrient sources?

V.) Result chapter 4.5: What did you mean by the sentence: "As seen from the Heatmap, samples cluster according respective sampling condition, suggesting that the distribution of differentially expressed genes is sufficiently similar between triplicates"?

VI.) Result chapter 4.5: What did you mean by annotated (37392) and non-annotated (5702) eukaryotic transcripts? How was this analysed?

VII.) Discussion: Why dead bacteria can be a better source of nutrients than live bacteria? Could there be differences between bacterial species, e.g., that other species would be a better source of nutrients than studied bacteria species?

VIII.) Discussion: Why do you mention carotenoid content of Paracoccus bacteria in the discussion? Could be related to the observed problems with division?

In České Budějovice, on 12/05/2021

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