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Review of Master thesis of Bc. Kateřina Vejvodová „Recombinant production and characterization of protease domain from TBEV nonstructural protein NS3

The thesis of Kateřina Vejvodová is a work of molecular biology. The goal was to produce recombinant protein complex of NS3 protease domain with its cofactor and evaluate its function. Overall, Kateřina succeeded in this aim, despite the testing would need more time to obtain more data. On the other hand, I can imagine that the cloning and production itself was not without problems and could take a lot of time and effort.

The work has 74 pages and has classical division. All parts have appropriate length and content. I appreciate detailed list of abbreviations. Also the supplement is properly used to show supporting, but necessary information. Text is accompanied by adequate figures that could be sometimes bigger and more self-explanatory, especially in the introduction (e.g. figure 9 and 11). Writing and English are good, the text reads well, sometimes with mistakes and typos, but nothing that would make the reading difficult. Now to individual parts:

Introduction describes TBEV from general questions such as the transmission, life cycle and pathogenesis, followed by the description at the cellular and molecular level – replication in cells, genome organization and proteins, finished with the description of the protein of interest – NS3. Introduction is nicely written, statements are supported by sufficient amount of references. A little flaw I see in the small size of figures with protein structures and the lack of sufficient description. For example figure 11 – the models are too small and I find the rainbow coloring confusing. In Figure 1, when you want to refer to a source article, it is better to mention the names, such as "adapted from Pulkkinen et al., 2018 (citation)".

Questions 1: Author writes that flaviviruses are transmitted by arthropods, i.e. mosquitoes and ticks. Are there some flaviviruses transmitted by other arthropods or without the vector at all?

Question 2: Authors writes that in Europe, *Ixodes ricinus* and *I. scapularis* are vectors of TBEV. Is this correct?

Material and methods are described with enough details and I do not have any comments to the text. However, I have some comments and questions to the factual and formal side: The composition of buffers and solutions should be described in unified style, with concentrations usually in molarity rather than in grams per some volume. Often even the volume is missing (TBS, transfer buffer, etc.). This should have been done more carefully. In table 2, BamHI is incorrectly highlighted in the sequence. In table 3 and 4, the concentration of the template DNA seems about 3 orders higher than usual. As such, you would put 25 and 50 ug of DNA in the reaction. Is it correct information? Table 5 – concentrations/ enzyme units should be stated.

Results are described clearly, supported by adequate figures. Results are described without speculations or discussion. I appreciate big number of pilot expression experiments (Table 8) in order to optimize the yield.

Question 3: In figure 19 and 20 the arrows and asterisks point at the size 20-23 kDa, but the complex should have app. 28 kDa. Are you sure with the presence of cofactor? Or why there is this discrepancy? Similar in figure 23...

Question 4: How do you explain so much impurity after IMAC (figure 23A). One would expect that affinity chromatography should yield very pure protein.

To me, it seems that in almost all gel and Western blot figures with NS2Bcof-NS3proH, the cofactor is missing. According to figure 14, both parts of the complex should bear His-tag in this construct. However, it is detected only in figure 25 and 27, maybe in fraction 8 in figure 23B. You write that you confirmed the presence of the complex by mass spectrometry (data not shown). **Can you confirm and/or show the results of the analysis to show that both parts are present?¨**

The evaluation of activity seems to be at the beginning, which is fine. The recombinant is very active, comparable to previous studies with NS3 proteases from other flaviviruses.

In discussion, author compares her results with the literature. I see one discrepancy there: Author writes in the results that glycerol had no effect on the activity of the protease, but in the discussion she states that there was a linear concentration dependency on glycerol. **Which statement is right?**

Overall, I find the work of Kateřina Vejvodová very good. I see a big question mark over the success of co-expression as the size of anti-Histag detected band is lower than it should be. Moreover, if there was the cofactor outside the complex, it should be detected as smaller band on the gels and Western blots (at least for NS2Bcof-NS3proH). I would like to hear a discussion on this topic during the defense.

Anyway, the thesis is very good and I recommend it for the defense. I would suggest grade between 1 and 2 and final grade will be dependent on the presentation during the defense.

In Hrdějovice, 20.5.2021

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