Evaluation of the master thesis

Author: Bc. Kateřina Vejvodová

Title: Recombinant production and characterization of protease domain from TBEV nonstructural protein NS3

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Reviewed by: Radek Šíma, Ph.D., Institute of Parasitology, BC ASCR

The master thesis of Kateřina Vejvodová aims to recombinant production and biochemical characterization of protease domain from TBE virus protein NS3.

The master thesis is written in English and extents 74 printed pages, including the list of references and supplements. Formally, the thesis follows the common structure required by the Faculty of Science. It is composed in a logical manner and subdivided into sensible sections.

The **Introduction** is very well written and introduces the reader to the topic. The author describes in detail the life cycle, pathogenesis, and structure of the TBE virus. In the following chapters, the author summarizes current knowledge about the TBE virus replication, with a special focus on polyprotein processing by viral and host enzymes. Viral proteases are discussed as promising therapeutic targets for newly developed inhibitors. Currently, there are no approved therapeutics to treat TBE. Therefore, the characterization of novel targets and design of inhibitors has great scientific merit. I appreciate the author's ability to present these complex topics in a clear and readable form.

Materials and Methods are clearly structured, and individual methods are explained sufficiently. I have just a few minor comments:

Pages 25-27: Numbers in chemical formulas should be written in lower case (e.g., $MgCl_2$, $MgSO_4$, KH_2PO_4)

Page 28: Primers are amplifying (not encoding) the hydrophilic region of the NS2B. Both genes (not proteins) were cloned into pETDuet-1 expression vector.

Page 29: The title of the 3.4.2 chapter should be chosen better.

The author mixes using decimal separators (e.g., page 35: 0,1mM IPTG, 37,5 µL of RNase-A, etc.). This should be unified throughout the text.

Results are summarized on 15 pages, including 1 table and 18 figures. Kateřina successfully cloned and expressed both recombinant proteins, NS2 and NS3. She tested various cell lines and cultivation parameters to find the best conditions for producing a sufficient amount of recombinant proteins. She further tested the effect of glycerol, ionic strength, and pH range on enzymatic activity and defined optimal conditions for subsequent verification of proteolytic activity of the NS3 protease. Kateřina had to master a broad spectrum of methods which is a strong part of her master thesis and important input for her future career.

Comments:

Page 44: Panels in Figure 20 should be labeled A, B, C, D and not E, F, G, H.

Figures 22, 24, and 26: In chromatograms, it would be better to display fraction numbers rather than volume in ml. It will help orientation in corresponding protein gels and Western blots.

In the **Discussion**, Kateřina critically evaluates her results and compares them with previously published works. I haven't major comments on this chapter.

Final evaluation

Kateřina Vejvodová prepared a high-quality master thesis. It contains a minimum of mistakes, and I appreciate that she decided to write her thesis in English. Kateřina proved her talent for scientific work and I am sure that she will employ gained experience in her future research career. I'm happy to **recommend** her master thesis for defense.

Questions

1. On page 11, the author mentions that: "in Europe, *Ixodes ricinus* and *Ixodes scapularis* serve as vectors of TBEV, which is not correct. What are the vectors of the TBE virus?

2. In Figures 17 and 18 (representing PCR verification of cloning), we can see two bands. One at 500 bp, which corresponds to the NS3 gene, and the second at about 200 bp. Moreover, in Figure 18, we can see a 500 bp band also in the negative control. Please could you clarify what these bands are?

3. Figure 28: It is interesting that enzyme activity peaks at pH9, which is quite high and far from physiological pH. Do you have any explanation for this finding? Are there any cell compartments with such a high pH?

4. In figures 28, 29, and 30, I miss any information about the statistics used in the graphs. Please could you explain what the individual columns and error bars represent?

5. Presented work is a solid basis for subsequent research. The next logical step would be a specific inhibition of NS3. Please could you briefly suggest the following steps leading to a design of an inhibitor of NS3?

In České Budějovice, May 18th, 2021

Radek Síma