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Bachelor thesis evaluation: Simone Röhrnbacher
Theme: The production and purification of tick salivary serpins in bacterial expression system

Simone Röhrnbacher focuses in her thesis on production of several serine protease inhibitors (serpins) from the hard tick *Ixodes ricinus* and evaluation of the inhibitory effect of recombinant serpins against various proteases. Thesis has 50 pages consisting of 7 chapters (introduction, aims, materials and methods, results, discussion, conclusion and references) and contains only a few typos.

The first chapter, named Introduction has 8 pages. It starts with very short introduction to ticks and goes directly to the problematic of tick-host interaction and detailed description of serine protease inhibitors. Both subchapters are well written and contain enough details for the reader to get the research overview. What I would really appreciate in this chapter is the scheme of clotting cascade, which would help the reader to get much better overview of this complicated process. I would also like to get more information about the chimeric IRS-2, which is only briefly mentioned at the end of this chapter.
I have two questions to the author:
1 – On page 6 you say, that tick salivary proteins show high level of pluripotency and redundancy. Do you believe, that targeting of these proteins could lead to the efficient anti-tick vaccine? Which tick proteins, could be in your opinion, the best targets for the functional anti-tick vaccine?
2 – Is there any record for metalloprotease inhibitors in the tick saliva?

Chapter two: Materials and methods starts with the big table 1 containing all substances, buffers and chemical that were used by the author during her work. It is clear from the chapter that the author managed many molecular approaches and biochemical methods.
My questions/suggestion to the candidate:
1 – Figure 2. Shows the DNA and protein markers used during your work. I would suggest to rather include these directly to the pictures. It is than easier to get the "size" information from the picture
2 – When you are using any kit e.g. GeneJET gel extraction (subchapter 3.2.1.2 Agarose gel extraction) it is enough to write that the extraction was performed according manufactures instructions. You do not need to write the whole procedure unless you modify anything.
3 – subchapter 3.2.1.3 Plasmid transformation shall include the exact type of used plasmid.
4 – Why did you use both ampicillin and chloramphenicol for the expression experiments?
5 – Subchapter 3.2.2.1 contains sequences of all primers used for the study. I am missing more primer details here e.g. (annealing temperature, length of amplicon, restriction sites)
6 – In subchapter 3.2.3.2 you state that the IB were isolated from cells by freezing and thawing. However, by this procedure, you isolate the complete insoluble cell fraction. Is this just a wrong formulation or did you perform any additional steps to isolate pure IB?
7 – Why did you shake only at 100rpm for the large-scale production? Could not this be one of the reasons for the slow cell grow?
8 – Did you really sonicate for 2 minutes without any brake? It seems to me too much and I would be worry about overheating of your sample? Did you somehow cool the sample?

9 – Subchapter 3.2.5.2 is called Ion exchange chromatography, but the column used (superdex 75 10/300 GL) is for gel filtration. Could you comment on this?

Results chapter has 13 pages and highlights all the experimental data acquired during the lab work. Large-scale production, refolding and purification are important parts of this work and although the author managed to get active inhibitors, the procedure still needs to be optimized due to very low yield (ca 0,8g of wet IB from 8L of media and only ca 250μg after refolding). What was the final yield of individual recombinant proteins after the purification?

My questions/suggestion to the candidate:
1 – In figure 3, you show the gradient PCR for various primer pairs, but the negative control belongs only to one of these. You shall include negative control to every primer pair you use.
2 – What cDNA did you use? Did you try nested PCR to get stronger signal?
3 – How would you interpret 3 bands in line 7 in picture 5?
4 – You ligation success is quite low (about 20%). I would recommend you to dephosphorylate you vector prior ligation next time.
5 – in chapter 4.2.1 Sequencing results you say that the sequences of IRS-7 and IRS-10 did not match the predicted sequences. What was the difference? How many clones did you sequence?
6 – Figure 5 shows the optimization of IRS-2 chimera and IRS-10-like production. It seems to me that especially in case of IRS-10-like the amount of produced protein is quite big. Why did you omit this protein for large-scale production?
7 – Could you please comment on the results in figure 10? It seems to me a bit odd that there are almost no proteins in cytosolic as well as membrane fractions.
8 – What was the negative control for your inhibitory experiments? Did you include buffer after purification?

In the next chapter, (discussion) author compares her results to the recent literature. The biggest part of this chapter deals with the possible optimization of protein production, refolding and purification as author realized are the most problematic part of her thesis. Author successfully identifies several problems causing small protein production (eg leaky expression, too large media volume or various additives to refolding buffers) and brings some idea how to improve them. I would recommend to the author, as an additional strategy to optimization process, to reclone proteins of interest into another expression vector and try to get the soluble protein from cytoplasmic fraction instead of IB production.
My questions/suggestion to the candidate:
1 – you state that the toxicity and leaky expression could be the reason for low protein production, but you are already using *E.coli* BL21(DE3)pLysS cell line, which reduces the leaky expression. Did you compare with *E.coli* BL21(DE3)

Despite of all my questions and comments I think that the Bachelor thesis of Simone Röhrnbacher fully meets the requirements of Faculty of Science at University of South Bohemia and therefore I recommend it for the defense.

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