Referee's report

Bachelor thesis written by Adriana Walnerová:
Cloning of the gene coding for Outer surface protein C from the Lyme borreliosis spirochetes.

This bachelor thesis is focused on Outer surface protein C (OspC) from *Borrelia burgdorferi*, a pathogene causing Lyme disease. Theoretical part contains brief but sufficient literature search. It gives essential information about Lyme disease, *Borrelia burgdorferi* and the protein of interest. Practical part demonstrates that many experimental methods were utilized in this work. Discussion is sufficient and comprehensive.

Some information in the theoretical part should be better organized (text in chapter *3.4. Outer surface proteins*). It would be more suitable to place the chapter *3.7. The genome of B. burgdorferi* prior to chapters describing outer surface proteins and OspC. It would be suitable to attach illustrative figures to some information described in the text (text describing VlsE protein on page 11 and text describing crystal structure of OspC on page 13). Then this text would be more understandable for people who are not familiar with this topic.

Some abbreviations are not explained sufficiently and they are not listed in the List of abbreviations (abbreviation „GFP" on page 14, abbreviations of PCR products „STREP-Ex-OspC" on page 15, etc.). Theoretical sizes of PCR products are missing in descriptions of agarose gel pictures. Theoretical size of rOspC is also missing in descriptions of SDS-PAGE and blotting membrane figures (theoretical size of rOspC is mentioned only in the discussion part *6.5 Affinity chromatography*).

In the figure 11 on page 28 there is mentioned that „A small fraction of purified protein can be seen in eluate 3.", but there is no visible band in the gel.

In the discussion chapter *6.2 Transformation* there is mentioned that „All three PCR products were ligated into the pTriEx-5 Ek/LIC vector." But in the chapter *5. Materials and methods"* description of ligation procedure is missing.

Titles of tables should be written above tables. Line spacing is not uniform (up to page 23 is line spacing 1.5; from page 23 is line spacing 1.0).

Finally, I can conclude that the presented thesis nicely summarizes a large quantity of performed experimental work. There are no fatal mistakes and main goals of this work were accomplished. Therefore, I suggest classification: **excellent minus.**

Questions:

On page 11 you wrote: „VlsE is an outer surface lipoprotein which is very important in the bacterial invasion of a host. It has six variable regions on its outer surface which serve ... etc." Do you mean outer surface of bacterium in both cases?

Are OspA, OspB and OspC sequential or structural homologs? Or do they have only similar names and they are diverse proteins presented in outer surface?

Is the resolved crystal structure of OspC deposited in PDB database?

Why did you decide to utilize CoMAC for purification of recombinant protein? Did you expect higher amounts of the recombinant protein?

What was used for elution during purification by StrepTactin chromatography?

What are your future plans in OspC research?

What is the final phase of untreated infection caused by *Borrelia burgdorferi* in humans?

In Brno
11. 6. 2010

Jana Mrázková

Mgr. Jana Mrázková