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Bachelor thesis evaluation:

Generation of GFP producing *Borrelia afzelii*, the Lyme Disease pathogen, and its evaluation using a tick-mouse model

The bachelor thesis of Anne-Celine Danklmaier describes the methods of GFP and dsRed producing *Borrelia afzelii* strain and introducing of fluorescent *Borrelia* into tick-mouse infection laboratory model. The thesis consists of 6 chapters (introduction, aims, materials and methods, results, discussion and references) and has a good quality with minimum of typing errors.

In the first chapter (Introduction) the author characterizes the genus of *Borrelia*, its life cycle, the genome of spirochetes and genetic manipulation with *Borrelia*.

In the second chapter (Aims) the main objectives of the thesis are clearly defined.

The third chapter (Materials and Methods): This chapter is relatively extensive and the used methods and techniques are described understandably and in details. The author exactly describes methods used for *Borrelia* cultivation, the creation of a GFP and DsRed expressing *Borrelia* strains. Using of fluorescent *Borrelia* in tick-mouse model and detection of these spirochetes in the host is also well described.

The fourth chapter (Results): In this chapter author evaluates and compares acquired data and results. Here, I would like to ask a few questions.

- 1) Chapter 4.1. (page 26). The author verified if she obtained GFP *Borrelia*. She confirmed green fluorescence in quite high amount of *Borrelia*. What exactly mean "quite high" in percentage?
- 2) gDNA was isolated from mice infected with *Borrelia*. All samples were positive for kanamycin. Were these samples also positive for adeC (confirmation of the presence of *Borrelia*)?
- 3) Chapter 4.2. (page 30). Here, the author describes artificial infection of larvae. How did you verify that the larvae were really infected after artificial infection?
- 4) Page 28 and 33: The author checked plasmid profile of GFP and DsRed expressing clones. Plasmid cp32-5 was missing in both fluorescent strain of *Borrelia*. Is it possible that missing of this plasmid could have an impact on *Borrelia* infectivity for mice and ticks?
- 5) The last question, did the author perform all experiments by herself?

Chapter five (Discussion): In this chapter, the author discusses the advantage, which provides fluorescent strain of *Borrelia* in the tick-mouse model.

It is obvious from this thesis that Anne managed many methods of molecular biology and immunology and got relatively large amount of results. According my opinion, the Bachelor thesis meets all requirements of Faculty of Science at University of South Bohemia and therefore I recommend it for the defense.

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