UNIVERSITY OF CHEMISTRY AND TECHNOLOGY, PRAGUE
Faculty of Food and Biochemical Technology
Department of Biochemistry and Microbiology

Doctoral thesis

Author: Mgr. Jakub Držmíšek
Title: Functional characterization of the type III secretion system of the human pathogen *Bordetella pertussis*

The thesis was prepared within the framework of postgraduate studies at Charles University in Prague, in the field of mikrobiology

Opponent's report

This dissertation is part of an extensive basic research on the virulence of *Bordetella pertussis*. The dissertation itself deals with the importance of the T3SS secretion system in the pathogenesis of *B. pertussis*. The thesis is written as an annotated summary of the author's publications supplemented by 20 pages of literature introduction summarizing the current state of knowledge and another 8 pages of discussion. The work is supplemented by a number of excellent illustrative figures, apparently created by the author. I very much appreciate the way in which such a high quality work has been produced in a very reasonable 114 pages. The author used very advanced, sophisticated analytical methods, including artificial intelligence tools, to test his hypotheses.

The dissertation is written in the form of annotated publications, which the student has contributed to in varying degrees. Of the four cited publications, the student is the first author 2 times. According to WoS records, the student is also the (co-)author of three other publications. All seven publications have already been peer-reviewed and published in prestigious international journals. I am particularly positive about the fact that the author focuses on publishing mostly in the fields of Imunology and Infectious diseases, so it is obvious that he is focused on contributing to the development of these particular fields. Three of the papers were published in journals belonging to the first decile of the field, and the remaining four were published in the first or second quartile of the field. The average impact factor of the author's publications is 5.8 and the citation response is 54, despite the fact that many of them are recent publications. Although the author is a young researcher, his H-index has already reached the value of 4.

The formal quality of the submitted work is at a high level, the work is written in English with a minimum of typos or grammatical errors. As usual, bacterial genera and species are written in ittalics, similarly most Latin terms. I would just like to mention here that the terms "*via*" and "*vice versa*" are also of Latin origin and should therefore also be italicized in the scientific literature. A more comprehensive list of abbreviations would also be appreciated by many readers. It is clear from the present work that the student has also acquired a high level of professional communication during his studies.

Considering how interesting and stimulating I found the presented work, I have a few questions:

1. **Antibiotic treatment and resistance**
   What is the typical antibiotic treatment for whooping cough? Do the approaches recommended by The European Medicines Agency (EMA) and the US Food and Drug Administration (FDA) differ? How common is the emergence of resistance to this antibiotic therapy and by what mechanism? Were any of these mechanisms observed in the whole-genome sequencing data reported in the first publication (Dienstbier et al., 2021)? Does *Bordetella* use any of the described secretion systems for antibiotic transport?

2. **Quorum sensing**
Are any of the described transport systems directly involved in QS? Is the mechanism of QS in the genus *Bordetella* described, including signaling pathways and type/structures of signaling molecules?
3. **Intracellular phase**
The author states that *B. pertussis* can escape phagocytic killing and survive in macrophages upon internalization, and that the intracellular phase of infection may play a role in survival and persistence within the host. Previous work has described that, *e.g.*, *Staphylococcus* is able to survive internalization into macrophages and is even able to multiply in macrophages. Can *B. pertussis* also multiply in monocytes? Would some of your RNA-seq results, *e.g.*, from the second or third publication (Farman et al, 2023 or Drzmisek et al, 2021), suggest so? Or, on the contrary, would your data refute this possibility?
4. **Adhesins**
The author states on page 21 "*another adhesin that plays a role in resistance to host immune mechanisms*". Can I ask for clarification on how this particular adhesin may play a role in resistance? Isn't this structure antigenic? In contrast to the author's claim, it is often stated in the literature that interactions between microbial adhesins and host cell receptors leads to signalling events that trigger host inflammatory responses.
The author states that interaction with immunity cells was originally proposed to be mediated by CD11b/CD18, but this was ruled out by later studies. So what is the current understanding of the interaction between FHA and immune cells? Alternatively, what is the author's opinion?

Overall evaluation of the dissertation thesis

I recommend the thesis for defense, the author has clearly demonstrated that he is familiar with the scientific literature, is able to think about the issue, conduct his own experiments, interpret the results correctly and also subsequently publish the data in international impacted journals and write up in the form of a dissertation. I wish the author a successful defence and many more scientific successes.

In Prague on 10.1.2025

doc. Ing. Jitka Viktorová, Ph.D.
Ústav biochemie a mikrobiologie
Vysoká škola chemicko-technologická v Praze
Technická 3, 166 00 Praha 6
prokesoj@vscht.cz, +420 220 444 335