MUNI

Annex No. 10 to the MU Directive on Habilitation Procedures and Professor Appointment Procedures

HABILITATION THESIS REVIEWER'S REPORT

Masaryk University	
Applicant	Mgr. Petr Králík, Ph.D.
Habilitation thesis	Use of PCR-based methods in microbiology and their specific applications
Reviewer	doc. Ing. Marcela Pejchalová, Ph.D.
Reviewer's home unit, institution	University of Pardubice, Faculty of Chemical Technology, Department of Biological and Biochemical Sciences

The habilitation thesis of Mgr. Petr Králík, Ph.D., represents a commented collection of scientific publications published in peer-reviewed journals indexed in the Web of Science database. The thesis itself comprises a 38page annotated overview summarizing 17 scientific publications (15 original research articles and 2 review articles) out of the author's total of 75 publications listed in the WoS database. The overall length of the habilitation thesis is 204 pages. The articles included in the thesis were published between 2008 and 2022.

The author focused on three main areas of molecular diagnostics of microorganisms. The first section is devoted to the application of PCR methods for the detection and quantification of target DNA regions. Dr. Králík concentrated on the rapid detection and quantification of *Mycobacterium avium* subs. *paratuberculosis* (MAP) using quantitative PCR (qPCR) and digital PCR (dPCR). Detection of MAP, a major pathogen of ruminants,by classical cultivation is time-consuming and technically challenging.

The second section addresses the problem of MAP cell viability. Conventional PCR is not suitable for assessing the physiological state of viable cells, as it cannot distinguish between live and dead cells, or cells in a viable but non-culturable (VBNC) state. The author therefore employed viability PCR (vPCR), in which chemical dyes such as propidium monoazide or metal-binding compounds such as platinum or palladium salts inhibit PCR amplification of DNA originating from dead cells. The optimized protocol was applied in studies on MAP cell viability affected by antibiotics, disinfectants, bacteriocins, and supernatants of lactic acid bacteria. The differentiation of live and dead MAP cells was confirmed even in complex matrices such as raw and pasteurized milk.

The third research area of Dr. Králík's habilitation thesis concerns multiplex PCR diagnostics employing the most recent xMAP technology developed by Luminex Corporation, which enables simultaneous detection of a broad spectrum of microorganisms. In this part, the author does not focus on MAP but rather on pathogenic microorganisms in food, waterborne bacteria and viruses, as well as biological threat agents (*Bacillus anthracis, Brucella* spp., *Francisella tularensis, Yersinia pestis*). The MOL-PCR (multiple oligonucleotide ligation PCR) method is presented as a highly specific, flexible, and sensitive tool suitable for rapid monitoring of food and water safety as well as for surveillance and rapid detection of biological threats.

The issues addressed in this habilitation thesis represent a current direction in veterinary and food microbiology, as well as in biothreat surveillance systems, where advanced molecular biological methods are increasingly applied to enable rapid detection of a broad range of pathogens in complex real samples and environmental matrices.

In my opinion, the habilitation thesis is of high quality, well written, and logically structured. Mgr. Petr Králík, Ph.D., demonstrates a broad scientific scope and extensive experience in implementing innovative molecular biological methods. His scientific excellence is further evidenced by long-term collaborations with both Czech and international research institutions.

Reviewer's questions for the habilitation thesis defence (number of questions up to the reviewer)

1. In the context of several waterborne viral outbreaks in the Czech Republic, is the commercial application of the MOL-PCR method with xMAP technology being considered for the rapid detection of pathogenic microorganisms in drinking and recreational water?
2. What is your opinion on the possible association between MAP and Crohn's disease?

Conclusion

The habilitation thesis entitled "Use of PCR-based methods in microbiology and their specific applications" by Mgr. Petr Králík, Ph.D. **fulfils** requirements expected of a habilitation thesis in the field of Molecular biology and genetics.

Date: 25. 8. 2025 Signature: