

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

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The habilitation thesis of Mgr. Petr Králík, Ph.D., entitled "Use of PCR-based methods in microbiology and their specific applications" is a comprehensive overview of the most important results of his scientific and research work to date. The author focused primarily on the application of PCR-based methods in microbiology, specifically in three areas: quantification of target microbial DNA, viability assessment, and multiplex diagnostics. He typically used these approaches to detect and quantify *Mycobacterium avium* subsp. *paratuberculosis* (MAP) bacteria.

The habilitation thesis is presented as a commented collection of scientific papers published in peer-reviewed journals listed in the Web of Science database. The candidate's contribution and role in the listed works is always indicated. The thesis is presented in a 38-page annotated overview, followed by 17 scientific papers by the candidate (15 scientific papers and 2 reviews) published between 2008 and 2022. The submitted thesis contains a total of 203 pages.

The author focused primarily on detecting of the significant cattle pathogen *M. avium* subsp. *paratuberculosis*, which causes Johne's disease and subsequently economic losses in livestock farming, milk and meat production. The causative agent of the disease is classified as a very slow-growing microorganism, which makes its classical cultivation determination difficult. The use of molecular biological methods thus appears to be a suitable alternative that will speed up the diagnosis of this pathogen. In the first part of the submitted work, Dr. Králík deals with the determination of *M. avium* subsp. *paratuberculosis* using quantitative PCR (qPCR) and digital PCR. He also focused on the quantification of *M. avium* subsp. *paratuberculosis* in feces and the differentiation of truly infected animals from passive shedders, which do not pose such a significant risk in livestock. He successfully implemented this method across laboratories, significantly speeding up the detection of this bacterium.

The second part of the work deals with a modified qPCR method that allows the differentiation between live and dead cells of *M. avium* subsp. *paratuberculosis*. The author first used the intercalating dye propidium monoazide to evaluate the viability of bacteria, then an advanced alternative in the form of platinum and palladium salts. These methods were also used to study the viability of *M. avium* subsp. *paratuberculosis* exposed to various antimicrobial agents (antibiotics, disinfectants, bacteriocins, or supernatants from lactic acid bacteria). It is a great contribution that these methods have succeeded in distinguishing between viable and dead cells even in such a complex matrix as milk (raw or pasteurized).

In the last, third part of the habilitation thesis, the author no longer focuses on *M. avium* subsp. *paratuberculosis*. This part focuses on multiplex detection of microorganisms using xMAP technology, which was used to determine various pathogens. First, the principle of xMAP technology developed by Luminex Corporation is characterized. Subsequently, the use of MOL-PCR to determine food-borne bacteria and parasites, food- and water-borne viruses, and biothreat agents such as *Bacillus anthracis*, *Brucella* spp., *Francisella tularensis*, or *Yersinia pestis* is described. This method, which enables multiplex and simultaneous detection of different analytes within a single complex sample, is thus increasingly used in various microbiological, clinical, or research laboratories.

The text is well structured and of a high standard. It represents a comprehensive study on the current topic of using PCR methods to determine the amount of DNA in samples, the viability of microorganisms, and their multiplex detection. The habilitation thesis demonstrates the candidate's broad scope and ability to competently address the issue, as evidenced by his collaboration with many national and foreign institutions.

Questions for the habilitation thesis defence

1. How is it possible that passive shedders of *M. avium* subsp. *paratuberculosis* do not infect other animals?
2. How much higher is the price of analyzing one sample using the digital PCR method compared to "classic" quantitative PCR?
3. Based on your own experience, which PCR method for determining cell viability would you recommend?
4. For which probiotic bacteria in food supplements and pathogens transmitted by ticks is the MOL-PCR method also applied?

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: 14. 8. 2025

Signature: