

# EXAMINER'S ASSESSMENT

by Assoc. Prof. Dr Mihail Vladimirov Iliev

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**concerning:** thesis for the award of the educational and scientific degree "Doctor" (PhD), entitled "*Prevalence and characteristics of enteropathogenic Y. enterocolitica strains isolated from pigs*"

PhD candidate Maya Angelovska

## 1. General presentation of the procedure and the candidate

By decision appointed with the ordinance № I-115/2.10, 2023 (Director of Institute of Microbiology, BAS) I was elected as scientific jury member of a thesis entitled "*Prevalence and characteristics of enteropathogenic Y. enterocolitica strains isolated from pigs*" for the award of the educational and scientific degree "Doctor" (PhD) in the field of Higher education 4. Natural Sciences, Mathematics and Informatics, Professional field 4.3 Biological sciences (Microbiology) by Maya Angelovska, who is PhD student at the department Infectious Microbiology, Institute of Microbiology, BAS. Her supervisor is corresponding-member. prof. Hristo Najdenski.

The set of digital materials presented by Maya Angelovska **is in accordance** with Academic Staff Development Act in the Republic of Bulgaria and Regulations of the Institute of Microbiology, BAS, on the Implementation of the Academic Staff Development. Based on the documents enclosed it is clear that formal requirements for reaching educational and scientific degree "Doctor" have been fulfilled, which allows me to determine the procedure as lawful and to proceed to a detailed evaluation of the PhD thesis and the contributions presented by Maya Angelovska.

## 2. Actuality of the subject, aim and research tasks

The focuss of the examined research is layed over one of the most interesting and in the same time one of the most challenging topics in the field of foodborn zoonoses – the prevalence and characteristics of enteropathogenic *Y. enterocolitica strains* isolated from pigs. The challenging factors are as follows: 1) the importance of *Yersinia enterocolitica* as a zoonotic

pathogen causing a disease that is among the most reported foodborne zoonoses in the countries of the European Union.2) The extremely complicated taxonomic status of the genus *Yersinia*. According to Bergey's current edition, *Manual of Systematics of Archaea and Bacteria* (2015), the genus is composed of only 11 validated species. In recent years, this number has been revised many times. In 2020 alone, 6 new species were attributed to the species, which are phenotypically extremely difficult to differentiate from the species *Y. enterocolitica*. The species *Y. canariae* is particularly indicative in this regard, which is biochemically indistinguishable from *Y. enterocolitica* and only whole-genome alignment (OGRI) allows their distinction. 3) The difficult and slow isolation of pathogenic yersinia, which is one of the main reasons for the lack of clarity about their distribution and epidemiological significance. In this regard, the development of adequate protocols allowing rapid screening on the epidemiological dynamics of pathogenic strains of the *Y. enterocolitica* species is more than urgent.

The major goal of the present thesis is clear, but its scope is too wide for so called “PhD research” and unnecessarily increase the number of the research tasks.

### **3. PhD student knowledge on the topic**

The literature review is based on 303 scientific publications, pointing the major achievements in the studied problem, which allows me to determine the literature review as detailed and comprehensive. The scope and the way of organization and analytical discussion on the literature information allows me to conclude that Maya Angelovska is very well acquainted with worldwide achievements on the research problem in details.

### **4. Research methodology**

All the used materials and the applied methods are precisely listed. I highly evaluate the broad taxonomical scope of the previously identified strains (32 in total) used to optimize the subsequently applied LAMP protocol, which is a kind of methodological crown of the present thesis. The experimental design is based on an extremely large sampling campaign involving samples from 601 pigs. In this regard, the wide regional coverage of the selected locations should be highlighted, allowing the monitoring of the epidemiological dynamics at the national level, which directly affects the livestock industry in our country. Microbiological methods and techniques, as well as screening techniques for the target isolates, are adequately selected. The conditions for carrying out the individual stages of the taxonomic algorithms, the used nutrient media, the applied procedures for enrichment, isolation and cultivation are presented in a way that allows reproducibility and correctness of the obtained results.

The whole experimental scheme is correctly assembled, separate stages of the study are carried out in a logical sequence and in this way, it is a prerequisite for successful reaching of the major research goal. Maya Angelovska is acquainted and applies wide range of classical and modern microbiological and molecular methods, and in this way the educational goal of the PhD is successfully realized.

## **5. Characteristics and evaluation of the PhD thesis and contributions**

The thesis is structured in the accepted official order and includes *Introduction, Literature review, Goal and tasks, Methodology, Results, Discussions, Conclusions, Contributions and Reference list*. Ratios among separate chapters are optimal in their volume and ratio.

The Introduction, in a clear form, also highlights the main research accent related to the need for investigations on the prevalence and characteristics of enteropathogenic *Y. enterocolitica* strains isolated from pigs.

The Literature review section is informative enough. I highly appreciate the balance achieved in it between the classical microbiological reading of the available knowledge in the field and the molecular-based approaches for the identification and characterization of the target microorganisms. It is this balance that is mandatory for the purpose of the dissertation. A full description of phenotypic characterization, biotypic and serotypic differentiation, epidemiology and distribution, and virulence factors is available. The emphasis in the literature review is correctly placed on the microbiological proof of the target pathogens - with the comment on the conventional methods, the main difficulties and limitations related to their application are outlined. This is also the connecting part to the main contribution of the current dissertation, namely the molecular-based approaches.

It is extremely appropriate to include the so-called *Unsolved problems on the topic*. It summarizes the main difficulties and their causes. In this regard, the need for a combined algorithm for the needs of clinical medical and veterinary practice is clearly emphasized, in which the LAMP and PFGE protocols involved in the present work also have their place.

The Materials and Methods section fulfills its function, being sufficiently informative and well structured.

In the Results and Discussion sections, the results of the conducted experiments are described and discussed in a logical sequence.

The main value of the work, determining the realization of its ultimate goal, rests on the set sample collection campaign, which is impressive in volume and deserves recognition. Only thanks to an adequately applied pretyping scheme at the beginning of the experimental work, the subsequent molecular and phenotypic characterization of the target isolates is possible. These efforts are of high added value, resulting in a collection of 43 enteropathogenic *Y. enterocolitica* strains and their genomic material, providing an excellent basis for future research on the subject. Impressive in its details, work has been done regarding the optimization of the LAMP protocol for detecting the *phoP* gene of the pathogenic *Yersinia enterocolitica* and the evaluation of its efficiency. Precisely in this part, the achieved results are the undoubted contribution of the present dissertation work.

I fully accept the number and content of the derived conclusions and contributions.

## **6. Evaluation of the publications**

Two scientific publications are presented in connection with the dissertation work. The results are disseminated in journals whose focus fully covers the developed topic and are a guarantee for their adequate dissemination in scientific circles.

## **CONCLUSION**

The evaluated PhD thesis contains scientific and applied results, which represent an original contribution to science and meet the requirements of Academic Staff Development Act in the Republic of Bulgaria and Regulations of the Institute of Microbiology, BAS, on the Implementation of the Academic Staff Development. The PhD thesis shows that Maya Angelovska has theoretical knowledge and professional skills in the scientific specialty *Microbiology*.

Due to the above, **I give my positive assessment** of the research presented in the PhD thesis, summary, results, and contributions, and I propose to the esteemed jury to award the educational and scientific degree “Doctor” (PhD) to Maya Angelovska 4. Natural Sciences, Mathematics and Informatics, Professional field 4.3 Biological sciences, doctoral program Microbiology.

Prepared by:

Assoc. Prof. Dr. Mihail Iliev

02.10.2023