

НАЦИОНАЛЕН ЦЕНТЪР ПО ЗАРАЗНИ И ПАРАЗИТНИ БОЛЕСТИ	
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REPORT

on a procedure for the acquisition of Academic Degree "Doctor of Philosophy" (PhD)

Scientific field: 4. Natural sciences, mathematics and informatics

Professional Field: 4.3 Biological sciences

Scientific specialty: 01.06.12 Microbiology

Topic of the dissertation: "Microbiological, electron-microscopic and molecular-biological methods for the study of the pathogenesis of sarcoidosis"

Author of the dissertation: M.Sc. Borislava Ilieva Tsafarova,

NATIONAL CENTER FOR INFECTIOUS AND PARASITIC DISEASES (NCIPD),
Department "Microbiology", National Reference Laboratory "Especially Dangerous Bacterial Infections" (OBI), Sector "Microbiome"

Scientific supervisor: Prof. Dr. Stefan Panayotov

Reviewer: Prof. Dr. Daniela Bogdanova Karashanova , Institute of Optical Materials and Technologies, Bulgarian Academy of Sciences, appointed as a member of the scientific jury by order No. 582 of 01.12.2023 of the Director of the National Center for Infectious and Parasitic Diseases, Prof. Dr. Iva Hristova

1. Biographical data for the PhD student

Borislava Tsafarova is finishing her higher education at the Faculty of Biology of Sofia University "St. Kliment Ohridski". She received her BA in Molecular Biology in 2009 and two consecutive MScs in Cell Biology and Pathology in 2013 and Animal and Human Physiology in 2015. These influenced her interest to the topic of a rare disease in humans - sarcoidosis, by predetermining its further scientific development by preparing a thesis in the period 2020 - 2023 in the field of research into the pathogenesis of sarcoidosis.

2. Assessment for meeting the minimal criteria according to the specific rules for the acquisition of scientific degrees defined in the NCIPD.

During her doctoral studies, Borislava Tsafarova passed the necessary exams in the specialty (in 2 parts), English language and computer technologies. She successfully completed 6 specialized courses on topics related to the problems reflected in the dissertation, conducted an internship in transmission electron microscopy - one of the main methods for characterizing the examined samples in the dissertation, and regularly reported the work done to the Collegium of the Microbiology Department. She presented the results of her work at 16 international and 7 national forums and published 8 publications - independently (1 item) and co-authored (7 items), of which 5 in international journals and 3 in national ones, as well as one poster report. On 25.09.2023, she presented her thesis on internal defense to the Scientific Council of the National Center for Infectious and Parasitic Diseases and it was forwarded to a public defense.

From the presented report on the credit points collected from the activities during the three years of her doctoral studies, it can be seen that the PhD student fulfills the minimal requirements of ЗАРСРБ, the regulations for its application in the Republic of Bulgaria and NCIPD, exceeding more than three times the minimum number of 200 credit points, required by the Regulations of the National Center for Infectious and Parasitic Diseases - 747 items are presented. A positive certificate for the research conducted and published by the PhD student is the 13 citations received on her publications during the period of the doctoral studies.

3. Dissertation analysis.

The PhD thesis submitted to me for opinion is described in 169 standard pages and is structured in the following 5 main parts: Introduction, References' review, Materials and methods, Results and discussion and Conclusions. The aim and objectives of the thesis are formulated and a comprehensive bibliography is also attached 220 sources. The dissertation contains 42 figures, 11 tables and a list of abbreviations used.

The PhD study begins with a short introduction, in which the student presents the object of her research - a rare disease in humans - sarcoidosis. Here she reveals the motivation for his activity, undoubtedly related to the actuality of the topic, caused by the lack of a sufficient number of studies worldwide and clarity about the causes of this rare disease. Here, for the first time, the purpose of the dissertation work is formulated, namely the study of the microbiome in patients with sarcoidosis to establish the main factors determining the manifestation of the disease, although the purpose is also presented independently after the introduction, together with the tasks set for implementation. A comprehensive references' review follows, in which, based on literature data, important issues such as the epidemiology of sarcoidosis, its types and clinical forms, its manifestations, methods of detection and diagnosis, as well as applied therapies and hypotheses about its etiology are discussed and clarified. A place is devoted to

the Bulgarian contribution in establishing the causes of the disease, as well as to a discussion of the role of certain types of mycobacteria (*Mycobacterium tuberculosis* and *Cutibacterium acnes*) in it.

An important part of the dissertation describes the studied materials, the ways of their extraction, accumulation and sample preparation, and the methods applied to their analysis - microbiological, microscopic, molecular, etc. A valuable approach in the current research is the combination of different microscopic methods to extract maximum information about the morphology and microstructure of the observed objects and establish cell proliferation.

The most extensive part of the dissertation work, covering almost half of its volume, is devoted to the numerous results obtained from the microscopic, histological and immunohistochemical, as well as molecular (targeted metagenomic sequencing of blood and biopsy samples from patients with sarcoidosis; PCR studies for the detection of bacteria from the genus *Mycobacterium* , as well as *Toxoplasma gondii*) the research of tissue, blood samples, lavage and bronchoalveolar lavage of patients with sarcoidosis, tuberculosis, as well as some other diseases with similar symptoms.

In the course of the research, an interesting approach was applied by cultivation of blood from healthy individuals and patients diagnosed with sarcoidosis, which allows important conclusions to be drawn about the human blood microbiome.

Traditionally, the dissertation ends with conclusions drawn on the basis of the obtained experimental results, a list of literary sources and a reference with participations of the PhD student in conferences on the topic of the dissertation.

4. Is the abstract prepared according to the requirements, does it correctly reflect the main points and scientific contributions of DT?

The abstract is well-presented and fully meets the requirements for volume and content. It includes the main results of the research and the scientific contributions of the dissertation work.

5. Critical notes and motivated recommendations for future use of scientific and scientific-applied contributions.

I cannot fail to point out, first of all, that the dissertation makes an impression with the large volume of labor-intensive and time-consuming experiments carried out, its good style and language, the logical presentation of scientific facts and the formatting of the text. The only inconsistency I could point to is the lack of separately formulated contributions of the dissertation, although it abounds with such. In support of this is the very fact that an answer to the question of the relationship of sarcoidosis with the human microbiome is sought and

valuable results are presented for this rare and little-studied disease, as well as the applied modifications in some of the preparation procedures leading to an increase in efficiency of the study (p. 46: the modification of the DNA isolation method; p. 48: a method for the isolation of DNA from paraffin blocks, without the participation of DNA-glycosylase; p. 73: optimization of the PCR protocol for the identification of *Toxoplasma gondii* ; p .95: description of a novel matryoshka-like mechanism of microbial proliferation).

My remarks on the dissertation are few and mainly editorial and do not affect the essence, reliability and significance of the obtained results and the dissertation work as a whole:

- 1) In the caption of Fig. 12 the outer membrane (OM) is mistakenly labeled as the inner membrane.
- 2) The culture time of the cells presented in Fig. 15 A and 15 B differs in the text and in the description below the figure.
- 3) Fig. 15 D, E refer to SEM research, and TEM is noted in the text.
- 4) For the images in Figs. 18 and 19 it is written "electronograms". This name usually refers to the patterns obtained from the diffraction modes of TEM. In this case, it is more appropriate to call them "bright-field TEM micrographs".

I also have the following questions:

- 1) Why does the microscopic analysis (bright field optical microscopy on pp. 89-90) of stress-cultured lysed and filtered blood from healthy volunteers change the color of the cells with cultivation time?
- 2) Is there a relationship between the rod-like cell shape and the disease in cultivated whole blood from patients with sarcoidosis?

6. Conclusion with a clear opinion on whether or not to grant the scientific degree.

The PhD thesis submitted to me for evaluation is an extensive and detailed study of archival tissue materials as well as fresh biopsy, blood and BAL from patients with sarcoidosis and some other lung diseases. Analytical methods, sample preparation techniques and the type of materials used are precisely selected to obtain reliable and trustworthy results for the presence or absence of mycobacteria - primarily *Mycobacterium tuberculosis* and for *Cutibacterium acnes*, in the examined samples, with which to associate the origin of the disease sarcoidosis. The generated and still unanswered questions in this direction are a good prerequisite for the development and deepening of the future research on the subject.

The achieved results have been published in a sufficient number of scientific papers, some of which have a high impact factor. They have been presented in many scientific forums, which, together with the successfully completed educational program, provides more than three times higher credit points towards the minimal required in the Development of Academic Staff

in the Republic of Bulgaria Act (DASRB), the Rules for the Application of the DASRB Act, and with the Rules for its application in NCIPD. The remarks made do not affect the value of the PhD thesis. Therefore, I strongly **recommend to the respected members of the Scientific Jury to award Borislava Ilieva Tsafarova the PhD degree in professional field 4.3 Biological sciences, specialty 01.06.12 Microbiology.**

15.01.2024

Prepared the opinion:

/ Prof. Dr. Daniela Karashanova/