To the Chairman of the
Scientific Jury, appointed by Order
№ 439/20.12.2024 of the Director of the
National Center of Infectious and Parasitic Diseases – Sofia

R E V I E W

on dissertation work for the award of the educational and scientific degree "Doctor of Philosophy" in the PhD program "Microbiology", Scientific Field: 4. "Natural Sciences, Mathematics and Informatics", Branch 4.3.

Topic of the PhD thesis: "Metagenomic studies on the determinants of antibiotic resistance in model environmental samples" with author Deyan Valentinov Donchev.

Reviewer: Prof. DSc. Stefan Vacev Panaiotov

Scientific specialty: Microbiology

Institution: NCIPD - Sofia

I declare that I have no conflict of interest within the meaning of Art. 4, (5) of the Low for Development of the Academic Staff in the Republic of Bulgaria. I have no common publications with the PhD candidate Deyan Donchev.

The review has been prepared in accordance with the requirements of the Law for Development of the Academic Staff in the Republic of Bulgaria and the internal regulations for its application in the NCIPD adopted by the Scientific Board of the NCIPD.

The submitted documents are in accordance with the instructions published in the Regulations for the Implementation of the Law for Development of the Academic Staff in the Republic of Bulgaria and the Internal Regulations at NCIPD.

I. Analysis of the career profile of the PhD candidate.

Deyan Valentinov Donchev graduated from the University of Sofia, Faculty of Biology with a Master's degree in "Microbiology and Microbiological Control". He worked (09.2018 - 03.2021) as a biologist at the Laboratory "Microbiology and Virology", Lozenets University Hospital, Sofia. In 2021, he was enrolled as a full-time PhD student at the National Reference Laboratory for "Control and Monitoring of Antimicrobial Resistance" (NRL CMAR), Department of Microbiology, NCIPD. During his PhD studies, he showed a gradual and constant development. He passed several specializations in the country and abroad and participated in projects related to the topic of his doctoral studies. He speaks and uses English. His publications are in peer-reviewed international and national journals. He presents his results at scientific forums in Bulgaria and abroad.

His overall scientific performance is excellent: 27 publications, 22 of which are in journals with an impact factor (impact rank), 34 citations and a Hirsch index of 4. The publications are in journals such as Antibiotics, Viruses, Microorganisms, Int. J. Mol. Sci., Pathogens, J. Med. Virol., J. Theor. Appl. Phys., Biotechnol & Biotechnol Eq. The total impact factor is 79.87.

II. General description of the thesis for the educational and scientific degree of "Doctor of Philosophy".

The PhD candidate Deyan Donchev presents:

- A thesis written on 203 pages. Results and Discussion section is presented on 63 pages. The description contains 20 tables and 30 figures.

The research topic aims to study fundamental and practical tasks related to studies on the determinants of antibiotic resistance in model environmental samples.

The literature review provides an overview of world achievements in the field of metagenomics and bioinformatics analysis and their applications to the analysis of the spread of antibiotic resistance in the environment.

In the Literature Review section, the PhD candidate does a very good description of a wide range of interdisciplinary methods used in metagenomics and bioinformatics analysis to monitor antimicrobial resistance in nature. The description provides a detailed overview of all modern techniques and bioinformatics applications for the analysis of sequence data. A comparative analysis of the advantages and disadvantages of different bioinformatics tools and databases is made. Throughout the dissertation author expresses a personal opinion and comments on the problem discussed, which shows in-depth knowledge. The literature review is written in clear language and deserves to be published as a review article.

The aim of the study is concrete, timely and relevant to the country. The tasks set are well formulated and aimed at achieving the objectives of the dissertation. The objectives are realistic and achievable. Fundamental tasks can be clearly distinguished from those of a practical nature. The work plan is ambitious but not overloaded with unnecessary tasks.

The technical means and analytical methods used are described in detail in the Materials and Methods section. A wide range of laboratory and bioinformatics methods used and developed are described. The descriptions are detailed and can be easily reproduced for other similar studies.

The results and conclusions of the thesis include:

- Development of an in-house protocol for isolating DNA from water samples by comparing DNA yields with established commercial kits. The method has low levels of

variation in taxonomic composition compared to a microbial standard and is suitable for metagenomic studies.

- Six publicly available databases for bacterial identification based on the 16S rRNA gene were tested. Two of them, GSR and GTDB, are the most reliable for conducting a 16S metagenomic study.

- Two methods for concentrating bacterial mass from water samples were tested. The results of the thesis show that the method of flocculation with skim milk is not suitable for concentrating bacterial biomass for the purpose of metagenomic studies, due to the ability of some bacteria to multiply during incubation and metabolize skim milk.

- The discharge of recycled water from the Samokov Waste Water Treatment Plant leads to increased relative levels of resistance genes to clinically important antimicrobial agents (macrolides, tetracyclines, beta-lactams, carbapenems, sulfonamides, etc.) in the Iskar River.

- By studying the dynamics of resistance genes in the Stara Zagora WWTP, it was found that the plant is a source for the spread of the blaIMP carbapenemase gene.

- A significant proportion of the identified resistance genes are part of mobile genetic elements, which increases their potential for horizontal genetic transfer.

The discussion of the results and the conclusions of the thesis are a direct consequence of the results. Each of the results is discussed in a logical and consistent manner. Fundamental and practical conclusions are drawn. Since the majority of the tasks and results in the dissertation are related to testing and solving methodological problems, I believe that the fundamental conclusions are actually more. Through in-depth comparative analyses, the dissertation describes the variations introduced into the final taxonomic profile of microbial test standards by the use of different DNA isolation protocols, as well as by the use of different databases for taxonomic identification. The

dissertation tracks and describes a wide range of indicators that can be used to assess their performance, advantages and disadvantages. On the basis of this practical experience, it is possible to formulate other fundamental conclusions, rather than just the one given in the text.

In Table 15, p. 102, the measured ratio at 260/230 nm is "unusually" low considering that the DNA was isolated from a microbial test standard. The reasons for this result are not discussed in the text.

The "Conclusions" section describes the current prospects for the development of metagenomic studies for the monitoring of antimicrobial resistance in the environment.

All figures and graphs are presented in a very professional manner.

III. Evaluation of the candidate's scientific work on the thesis.

Deyan Donchev attached:

Scientific publications in international journals with impact factor - 2

Participation in national congresses - 4

Citations in international journals with impact factor - 1

Participation in national and international research projects - 4

The attached publications are directly related to the aims and results of the dissertation. The PhD studies were completed within three years.

IV. Comprehensive evaluation of the thesis.

Deyan Donchev conducted the first comprehensive metagenomic study of antibiotic resistance determinants in wastewater in Bulgaria. The results highlight the

impact of anthropogenic pollution on freshwater ecosystems through wastewater treatment plants. The results provide an assessment of the impact of WWTPs on the resistome, mobilome and microbiome of wastewater. The dissertation describes the modern approaches that should be used to identify faecal contaminants and acquired antimicrobial resistance genes, including those with clinical significance such as carbapenemase genes.

The dissertation explores fundamental tasks and solves specific practical problems. The literature review and methodological results provide valuable information for future broad-spectrum metagenomic studies.

VI. Critical comments and recommendations.

I have no significant comments or recommendations on the dissertation. The dissertation and all documents have been prepared in accordance with the requirements. The conclusions of a fundamental nature can be further developed.

VII. General assessment of the candidate's compliance with the mandatory conditions and mandatory quantitative criteria and scientific indicators according to the Law for the Development of the Academic Staff in the Republic of Bulgaria.

The documents submitted by the candidate cover all the requirements according to the Law on the Development of the Academic Staff in the Republic of Bulgaria for the educational and scientific degree of "Doctor of Philosophy". The reference to the requirements for obtaining the PhD degree significantly exceeds the threshold minimum of 200 credit points.

The results of the work demonstrate that the PhD candidate has worked exceptionally thoroughly and conscientiously. He has made great efforts to acquire

knowledge and laboratory methods and to describe his results in professionally written articles. This gives him the opportunity to be an innovative, sought-after specialist for collaborations and to have a successful scientific career.

The doctoral candidate Deyan Valentinov Donchev fulfils the mandatory conditions and scientific criteria for the acquisition of the scientific and educational degree "Doctor of Philosophy" according to the requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria and the Regulations on the Conditions and Procedures for Acquiring Scientific Degrees in the NCIPD.

I confidently give my positive assessment and recommend that the Scientific Jury award Deyan Donchev the scientific degree "Doctor of Philosophy" in the scientific field of higher education: 4. "Natural Sciences, Mathematics and Computer Science", Branch 4.3.

Reviewer

Prof. DSc Stefan Panaiotov