

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 "*Genome typing of probiotic microflora isolated from natural products*",

PhD candidate Anita Bozhidarova Gyurova

1. General presentation of the procedure and the candidate

By decision of the Scientific Jury, appointed by the ordinance № ? (Rector of Sofia University) I was elected as a head of the scientific jury of a thesis entitled "*Genome typing of probiotic microflora isolated from natural products*" 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 (Genetics – Bacterial genetics and molecular cloning) by Anita Bozhidarova Gyurova, who is PhD student at the department Genetics, Faculty of Biology, Sofia University. Her supervisors are assoc. prof. dr. Svetoslav Dimov (SU, Department of genetics) and acad. Draga Toncheva (MU, Department of Medical Genetics).

The set of digital materials presented by Anita Gyurova **is in accordance** with Academic Staff Development Act in the Republic of Bulgaria and Regulations of the Sofia University 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 Anita Gyurova.

2. Actuality of the subject, aim and research tasks

The interdisciplinary nature of the dissertation submitted to me for an opinion is a reflection of the current global trends related to the implementation of nutritional selection and control, while at the same time it is appropriately focused on research involving functional foods, which are permanently embedded in the eating habits of the Bulgarian

population - yogurt, cheeses, sausages, fermented vegetables and starters for the dough, as well as beekeeping, which is traditional for our country. Limitations caused by classical cultural methods for isolation, cultivation and characterization negatively reflect on the knowledge about the engaged microbial diversity. The successful implementation of classical microbiological methodology with modern bioinformatics and molecular methods for analysis and genomic typing of the lactic acid microbiota in traditional for our country food products and in bee products is the main certificate for the actuality of the topic. In this regard, in the focus of the research carried out by PhD student Anita Gyurova is essentially the identification of bacteriocin-producing enterococcal strains with the potential to inhibit the growth of the causative agent of the American disease foulbrood, as well as strains with probiotic potential. In view of the currently scarce information regarding representatives of the genus *Enterococcus* isolated from bees and bee products, the present dissertation represents a serious request to supplement scientific knowledge in this direction.

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 452 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 Anita Gyurova 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. The experimental scheme is based on an extremely large microbial collection, including 368 strains of lactic acid bacteria, isolated during the investigation of 31 target food products. The microbiological methods and techniques as well as screening techniques for selection of isolates showing prominent probiotic potential are adequately selected. Experimental conditions for carrying out the separate stages of metagenome studies, the used software products, the applied enrichment procedures, isolation, and cultivation procedures are explained in details, which allow reaching correct and reproducible 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. Anita Gyurova 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, highlights the main research accent related to the need for investigations on the lactobacillus component and the possibilities related to its biotechnological potential.

The *Literature review* section is informative enough. The current taxonomic status of the target microorganisms and their participation in fermentative processes in different types of foods is reflected. An excellent review has been made concerning the antibacterial potential with an emphasis on the genetic organization of the relevant determinants. My only remark to this chapter of the dissertation concerns the point *Molecular-genetic methods for the study of lactic acid bacteria*, namely: the description of the presented methodological arsenal could be successfully supplemented with more data from specific studies related to lactobacilli, in which the discussed molecular methods were engaged.

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 construction of experimental work-flow determining the achievement of its ultimate goal rests on the initial isolation campaign, which is impressive in scope and deserves recognition. Only due to an adequately implemented preliminary typing scheme at the beginning of the experimental work, the subsequent molecular and phenotypic characterization of purposefully selected strains is possible. A culmination of the efforts made in the part of the thesis concerning the microbial isolates from bees is the selection of the strain *Enterococcus durans* EDD2 and the characterization of the bacteriocins produced by it, exhibiting pronounced activity against *Paenibacillus larvae* by means of bioinformatic analysis of its genome sequence. Screening for the presence of genetic determinants in another isolate from the same group – *Enterococcus faecium* EFD, carried out after whole-genome sequencing, is also of interest and contributes to the scientific

significance of the thesis. In fact, this is also the first ever study of a beehive-derived bacterial isolate with valuable properties involving whole-genome sequencing. The part of the dissertation concerning the microbial isolates from the target food products is of considerable volume and substantial scientific value. In this part, the metagenomic studies carried out, revealing the real microbial diversity of the microbiota in investigated food products, also have a pioneering character, due to the endemic origin of the examined products and the specificities related to their preparation.

The results, in their entirety, strongly confirm the growing potential of whole-genome sequencing to characterize new microbial isolates possessing valuable probiotic properties.

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

6. Evaluation of the publications

Five scientific publications are presented in connection with the dissertation work. Four of them are in excellent reputable journals (one publication in a Q1 journal, three publications in Q2 journals). 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 Sofia University on the Implementation of the Academic Staff Development. The PhD thesis shows that Anita Gyurova has theoretical knowledge and professional skills in the scientific specialty Genetics (Bacterial genetics and molecular cloning).

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 **Anita Bozhidarova Gyurova** 4. Natural Sciences, Mathematics and Informatics, Professional field 4.3 Biological sciences, doctoral program (Genetics - Bacterial genetics and molecular cloning).

Prepared by:

Assoc. Prof. Dr. Mihail Iliev

12.11.2023