

# EXPERT OPINION

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**Concerning:** PhD thesis in the field of 4.3 biological science, scientific speciality /Genetics – Bacterial genetics and molecular cloning.

This opinion has been prepared in my capacity as a member of the scientific jury, determined by Order N RD-38-107/28.02. 2023.

Candidate for the procedure: **Anita Bozhidarova Gyurova**

Topic of the dissertation: “***Genomic typing of the probiotic microflora isolated from natural products***”.

Microorganisms are an integral part of the history and function of life on earth. Approximately 60% of the biomass of our planet consists of microorganisms, without which the ecological cycle of the three elements important for the existence of life - carbon, nitrogen and sulfur - is unthinkable. They outnumber all other organisms on earth. Over 10,000 species of bacteria have been identified so far. Due to their metabolic diversity, they play an essential role in natural processes such as decomposition and nutrient cycling. Increasing efforts worldwide are being made towards the development of molecular methods for the analysis and genomic typing of various microorganisms. A large proportion of these genomes are derived from lactic acid bacteria used as probiotics or starter cultures in food fermentation.

The PhD thesis of Anita Bozhidarova Gyurova is dedicated to an up-to-date and significant fundamental problem - genetics and genomics, which are gaining more and more importance for the development of modern biological science. In the current dissertation, the doctoral student sets herself the very important task of isolate and taxonomically characterize lactic acid bacteria, which are traditional for Bulgaria naturally fermented products, as well as from bee products harvested in different ecological regions of the country. In this regard, A. Gyurova, as well as her supervisors, deserve admiration for the focus of the topic and especially for its practical aspect, which is valuable and necessary for our country.

In the current PhD thesis, the doctoral student focuses on the isolation and taxonomic characterization of lactic acid bacteria, both from traditional for Bulgaria, naturally fermented products, and from bee products harvested from different ecological regions of the country. In the process of investigation, A. Gyurrova collected a total of 348 isolates isolated from various food products, as well as from bees and bee products which were tested for the presence of antimicrobial activity, and the strains with such availability were determined taxonomically by classical and molecular genetic methods. Some of the most promising isolates have been analyzed at the genomic level by applying NGS (Next-Generation Sequencing). In this way, the potential of the lactic acid bacteria in the production of bacteriocins, which appear as natural agents against bacterial infections, is realized.

From a scientific point of view, it is worth mentioning the NGS-based metagenomic study of the traditional Bulgarian cheese produced in the village of Tcherni Vit which was carried out by the doctoral student. This turned out to be the first scientific study of this unique for our country dairy product. The results clearly show that the Bulgarian green cheese has an extremely rich microbiome, as well as the only Bulgarian noble mold.

Among the some of genomes of selected LAB isolated from bee products, the presence of bacteriocin genes was found, which, according to the doctoral student, could be an explanation for the antimicrobial properties of the strains. This was confirmed in the doctoral student's subsequent studies of *Enterococcus faecium* strains, in which antibacterial activity was found against the honeybee pathogen *Paenibacillus larvae*, as its presence appeared to be a natural protection of hives. It has been established that this bacterium causes a contagious disease, the American foulbrood disease, which is characterized by high mortality of bee colonies all over the world and in our country.

It is worth to mention as a contribution the NGS-based metagenomic study of the traditional Bulgarian green cheese from the village of Tcherni Vit carried out by the doctoral student. This is the first scientific study of this unique dairy product due to the fact that until now no studies have been carried out on this exceptional Bulgarian product. Amazingly, the results show that Bulgarian green cheese has an extremely rich microbiome, as well as the only Bulgarian noble mold. The obtained data are of interest from a scientific and above all from a practical point of view, not only for the connection of lactic acid fermentation and the longevity of the Bulgarian

population from this region, but also since honeybees are an important biological resource of great economic importance for our country.

As a result of her studies, the doctoral student found that *Enterococcus durans* EDD2 has a strong inhibitory activity against *Paenibacillus larvae*, which is due to the production of bacteriocins. As the doctoral student herself notes, additional in vivo studies are needed against American foulbrood disease.

Certainly, the doctoral student, under the guidance of the two supervisors, Academician D. Toncheva and Associate Professor S. Dimov, has done a huge amount of work: For the first time in Bulgaria, an amplicon-based metagenomic analysis of a fermented milk food product - the green cheese from Tcherni Vit was carried out; for the first time in Bulgaria, the genomes of *Enterococcus* strains, probiotic for bees, were sequenced.

I have no objections to the credibility of the conclusions drawn, although some of them - 1, 2, 3, require more specifics.

I fully accept the scholarly contributions made and consider contributions #1, 3 and 4 to have their original character.

The content of the abstract fully corresponds to the dissertation work.

The most significant results of the dissertation are reflected in 5 scientific publications. In one of them, the doctoral student /1/ is the lead author.

**Conclusion:** Taking into account the overall assessment of the PhD thesis, the scientific significance of the obtained results, the originality of the topic, the modern methodical preparation of the studies, which are particularly important and useful for the genetics and the genomics, I definitely consider that Anita Bojidarova Gyurova fully meets the requirements of The Law on the Development of the Academic Staff in the Republic of Bulgaria. **Based on this, my assessment is positive, and recommends the honorable scientific jury to vote for awarding the educational and scientific degree in the professional field 4.3 "Biological Sciences (Genetics)".**

Sofia, March 20, 2023

Prof. Sevdalin Georgiev, D.Sc