

REVIEW

by Prof. DSc Ivan Iliev Atanassov, Agrobioinstitute /ABI/, Agricultural Academy /AA/

on the dissertation of Assoc. Prof. Penka Mladenova Petrova PhD, "Molecular-biological studies of new bacterial glycoside hydrolases with industrial applications" presented for the award of the scientific degree "Doctor of Science" in Higher Education Area 4. "Natural Sciences, Mathematics and Informatics", Professional Field 4.3. Biological Sciences, Specialty: Microbiology

Presentation of the procedure and the author

Following the order of the Director of the Institute of Microbiology /IMicB/, Bulgarian Academy of Sciences № I-128 from 23.12.2019 I have been appointed as an member of the academic board for evaluation of the dissertation "Molecular biology study of new bacterial glycoside hydrolases with industrial application" presented by Assoc. Prof. Penka Mladenova Petrova PhD for the award of the scientific degree "Doctor of Science". The dissertation and set of materials and documents, submitted by Assoc. Prof. Penka Petrova are fully in accordance with the procedure and requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria (ZRARB), the Rules for the implementation of the ZRARB and the corresponding Regulations of IMicB.

Assoc. Prof. Penka Petrova was born on 19.04.1971. In 1994 she received her Master's Degree from the Faculty of Biology at Sofia University "St. Kl. Ohridski ", majoring in Biotechnological Processes, Genetic and Cellular Engineering, and in 2003 defended his dissertation on "Creation of a gene cloning system for *Streptococcus thermophilus*" at IMicB, Bulgarian Academy of Sciences and obtained a PhD in Microbiology. Since 2003 she has been working as an assistant / head. Assistant Professor, and since 2011 as Assistant Professor at IMicB, BAS, where since 2013 she has been the Head of the Gene Expression Laboratory and since 2018 she is the Head of the Department of General Microbiology. Since 2020 she is the Director of IMicB, BAS. Speaks foreign languages, incl. excellent command of English and Russian, and very good in French. In her career, she has demonstrated excellent organizational skills including leadership and implementation of a large number of national and international projects, leadership of two successfully defended PhD students and a large number of graduates and postgraduates,

participation in the editorial board of Journal of Investigative Genomics, scientific juries and committees affiliated with scientific - research activity of IMicB, BAS

Assessment of dissertation work

Bacterial glycoside hydrolases are the subject of a great deal of scientific and applied research, both because of their wide industrial application and in relation to the study of the evolution and genetic adaptability of individual microbial groups. The rapid development in the 21st century of a wide range of different strands in microbiology related to medicine, nutrition, ecology and utilization of agro / bio-waste has stimulated a number of new studies aimed at characterizing new types of bacterial glycoside-hydrolases with different enzyme activity parameters, as well as isolation and characterization of new bacterial strains producing these enzymes outside the traditional microbial groups. Assoc. Prof. Petrova's dissertation includes complex studies on several groups of glycoside hydrolases, incl. isolation of lactic acid bacteria producing glycoside-hydrolase enzymes, characterization of enzyme activity, molecular biological study of relevant genes and gene expression and heterologous expression and characterization of recombinant enzymes. Given the high interest in the research and biotechnological applications of glycoside-hydrolases enzymes, as well as the isolation of lactic acid bacteria producing such enzymes, it is safe to say that the dissertation is up-to-date and the results and information with high potential for future applications in a number of scientific fields and applied fields of microbiology, biotechnology and the biological sciences in general.

The dissertation contains 358 pages, the main text includes 52 tables and 140 figures, with appropriately arranged separate parts. The bibliography of the dissertation includes an impressive number of 625 cited literary sources, demonstrating the high level of awareness and theoretical preparation of Assoc. Prof. Petrova on problems and scientific fields related to the topic of the dissertation. The dissertation is structured in the conventional way: literary review, purpose and tasks, materials and methods, results and discussion. The literature review presents intelligently and systematically a large body of information regarding: the structure of glycans, substrates of carbohydrate enzymes, bacterial producers of glycoside-hydrolases enzymes, and applications of lactic acid bacteria in the lactic acid fermentation of cereal-based foods and beverages. The purpose and tasks of the dissertation are clearly stated. The “Materials and Methods” and “Results and Discussion” sections demonstrate the excellent and versatile experimental preparation and in-depth

theoretical knowledge of Assoc. Prof. Petrova in a wide range of scientific fields and areas, including, microbiology - isolation and characterization of bacterial glycoside-hydrolases producers enzymes, molecular biology - cloning and characterization of genes and heterologous expression and biochemistry - purification and characterization of enzymes and recombinant proteins.

Results and contributions from the dissertation. The results, conclusions and contributions of the dissertation can be attributed to several main groups: isolation of ICD producers of glycoside-hydrolase enzymes (including the first reports of amyolytic representatives of the species *Lactobacillus sakei* and the genus *Enterococcus*) and identification, cloning and cloning responsible for the hydrolysis of α -glucans (including the first reports to identify such genes in *Lactococcus lactis* and *Lactobacillus paracasei* species); first reports on the characterization of cell-associated fructan- β -fructosidases of *Lactobacillus paracasei*; identification of a novel cyclodextrin glucanotransferase enzyme gene in *Bacillus pseudocalophilus*; successful heterologous expression of β -glucuronidase in diploid yeast strains of *Ogataea polymorpha*; sequenced genome of *Bacillus velezensis* 5RB and analysis of the results obtained. The scientific contributions of the dissertation include the expression of recombinant cyclodextrin glucanotransferase immobilized in magnetically modified carriers and the successful production of cyclodextrins, molecular biological characterization of neuraminidase from a non-toxicogenic strain of the *Vibrio cholerae* assay genes and a PCR-based method for the detection of short heat-shock genes in *Str. termophilus*, as well as original results regarding the presence of prebiotic carbohydrates galactooligosaccharides in Bulgarian yogurt.

The results of the studies are presented mainly in 33 scientific publications, 18 of which are in international refereed journals with impact factor, 2 chapters in books and 1 publication in SJR journal. Theses have been cited 268 times. The dissertation work is related to the participation and leadership of Assoc. Prof. Petrova in a number of international and national research projects at the National Science Fund, etc.

Abstract. The abstract presented is consistent with and reflects the structure and content of the dissertation.

Conclusion. Considering the high scientific and applied importance of the results and contributions of the dissertation presented, as well as their importance for the knowledge and development of

molecular biology of bacterial glycoside-hydrolases, microbiology of bacterial producers of glycoside-hydrolase enzymes, biotechnological related applications them, as a member of the Academic board, **I give my high positive assessment of the presented by the Assoc. Prof. Penka Mladenova Petrova PhD dissertation "Molecular-biological studies of new bacterial glycoside hydrolases with industrial applications". Accordingly, I would strongly recommend the respected Academic board to award the academic degree "Doctor of Science" to Assoc. Prof. Penka Mladenova Petrova, PhD, in Higher Education Area 4. "Natural Sciences, Mathematics and Informatics", Professional Field 4.3. Biological Sciences, Specialty: Microbiology.**

04.03. 2020

Prof. DSc Ivan Atanassov