

OPINION

By Prof. PhD Sonya Kostadinova Trifonova

Plovdiv University "Paisii Hilendarski"

of a dissertation thesis for awarding the educational and scientific degree "Doctor",

field of higher education: 4. Natural sciences, mathematics and informatics

professional direction: 4.3. Biologically sciences

Doctoral program: "**Biochemistry**"

Author: Stanimira Angelova Angelova

Topic: "STUDY OF THE PROPERTIES OF BIOENGINEERED ALPHA-D-GLUCANS SYNTHESIZED BY MUTANT GLUCANSUCHARASE URE 13-300"

Research supervisor: Prof. PhD Ilia Nikolov Iliev, Plovdiv University "Paisii Hilendarski"

1. General presentation of the procedure and the doctoral student

By order No. PD-21-2469 dated 18.12.2023 of the Rector of Plovdiv University "Paisii Hilendarski" (PU), I have been appointed as a member of the scientific jury to ensure a procedure for the defense of a dissertation work on the topic "**Researching the properties of bioengineered alpha-D- glucans synthesized by mutant glucansaccharase URE 13-300**" for the acquisition of the educational and scientific degree 'doctor' in the field of higher education 4. Natural sciences, mathematics and informatics, professional direction 4.3. Biological Sciences, PhD program Biochemistry. The author of the dissertation is Stanimira Angelova Angelova - full-time doctoral student at the Department of Biochemistry and Microbiology, supervised by Prof. Dr. Ilia Nikolov Iliev from the Department of Biochemistry and Microbiology, Faculty of Biology, PU "Paisii Hilendarski".

The set of materials presented by Stanimira Angelova in paper and electronic form is in accordance with Art. 36 (1) of the Rules for the Development of the Academic Staff of Plovdiv University "Paisii Hilendarski".

The PhD student has attached 3 publications in scientific journals.

Stanimira Angelova received her higher education at the Faculty of Biology of PU "Paisii Hilendarski". She graduated from the bachelor's degree in "Molecular Biology" in 2014 and the master's degree in "Biopharmaceutical Biochemistry" in 2018. She worked at Retail Direct Ltd. From May 2016 to January 2017, and from February 2017 to March 2020 at Biovet AD-Peshtera as a chemist-analyst. On 01.03.2020 she was enrolled as a full-time PhD student at the Department of Biochemistry and Microbiology, PhD program "Biochemistry" and at the same

time, since May 2020, she has been working as a researcher at the Center for Technology of PU "Paisii Hilendarski".

2. Actuality of the topic

Enzyme-catalyzed reactions are increasingly used in industrial processes, due to their undeniable advantages such as selectivity, economy, efficiency and a weaker environmental footprint. Therefore, the isolation and characterization of new enzymes and the improvement of the characteristics of existing ones is a major direction in modern biotechnology. Prospects in this direction are focused on microbial producers, which are preferred over plant and animal sources, since the production of microbial enzymes is cheaper, easier to control and reliable.

Glucansaccharases synthesize glucans of varying solubility, degree of branching, mass and conformation, which find application in the food, medical, pharmaceutical and cosmetic industries, as stabilizers, fillers, immunostimulants or prebiotic compounds. Given the diverse application possibilities, microbial glucansaccharases have been intensively investigated; the genes encoding these enzymes were sequenced and cloned; the structure-function relationship was analyzed. Rational design strategies are used to create molecules with improved enzyme functionalities.

In this aspect, the topic of the dissertation related to the preparation of mutant glucansaccharase by the methods of protein engineering and analyzing its properties is relevant and with potential for practical application.

3. Knowing the problem

The literature review is written analytically, and covers all aspects of the developed topic. In the first two sections of the review, information on microbial glucansaccharases, their structural organization, mechanisms of action and types of glucans synthesized by lactic acid bacteria are presented.

The main emphasis in the review is on site-directed mutagenesis in genes encoding GH70 glucansaccharases, which leads to changes in the structure and activity of enzymes, as well as in the properties of synthesized glucans. Logically, the review concludes with the applications of λ -glucans and oligosaccharides synthesized with microbial GH70 glucansaccharases, which argues the purpose of the dissertation.

The review, based on 175 literary sources in Latin (over 70% of which were published after 2000), shows very good theoretical knowledge of the doctoral student on the topic of the dissertation and her ability to analyze scientific information in view of the specific topic.

4. Research methodology

The aim of the dissertation and the resulting tasks are clearly and precisely formulated. An appropriate methodical approach has been chosen to achieve them. In solving them, Stanimira Angelova has applied a large number of modern biochemical, chemical, microbiological, molecular-biological methods, including HPLC chromatographic analysis, MRI structural analysis, methods for studying the kinetics of enzymatic reactions, site-directed mutagenesis and bioinformatics analysis.

A very good impression is made by the correct and comprehensive description of the methods, which allows them to be reproduced. The large number of diverse methods that are adequately planned and implemented clearly show that the doctoral student has acquired the necessary methodical experience to conduct a scientific experiment.

5. Characterization and evaluation of the dissertation work and contributions

The dissertation is written in 157 pages. The content is structured in sections between which optimal ratios are observed: Introduction – 2 pages, Literature review - 32 pages, Aim and objectives – 1 p., Materials and methods – 15 pages, Results and discussion – 50 pages, Conclusions – 2 pages, Contributions – 1 p., Literature – 11 pp.; 33 pages of annexes are included in the Results and discussion section.

The results of the experimental work are systematized in five parts. The properties of glucansaccharase (URE 13-300) synthesized by the recombinant strain *Escherichia coli* BL21 were analysed, including the influence of organic solvents on the activity, on the transferase reactions and degree of polymerization of the synthesized glucoligosaccharides (part 1 and 2). For the first time, the synthesis of oligosaccharides in water-organic medium with glucansaccharase URE 13-300 was carried out. An indisputable contribution of the work is the successfully conducted site-directed mutagenesis by replacing the amino acid glycine at the 449 position with lysine in the domain B of the catalytic domain 1 of the enzyme (part 3). The resulting mutant glucansaccharase U13M1 is expressed under different conditions and has altered biochemical and kinetic properties (part 4). The glucan synthesized with the mutant enzyme has reduced α -(1-3)-glycosidic bonds. Another significant contribution of the dissertation is the determination of the optimal conditions for the synthesis of oligosaccharides with glucansaccharase U13M1 (part 5). The experimental results are shown in 3 tables and 31 figures; in addition, 63 figures are presented in the annexes.

The results are competently and critically analyzed. Each part ends with a brief conclusion, in which the results are discussed in view of the aims and objectives set, and based on the available literature, and some perspectives for future work are given.

The conclusions are a logical consequence of the conducted experiments and accurately reflect the obtained results. Formulated contributions have scientific and applied significance.

6. Evaluation of the publications and personal contribution of the doctoral student

The results of the dissertation have been published in 3 scientific papers in refereed journals (Catalysts – Q2 ; Acta Microbiologica Bulgarica - Q4; Ecologia Balkanika - Q4). The articles are in English and in two of them Stanimira Angelova is the first author. With these publications, the doctoral student Stanimira Angelova fulfills the national minimum requirements for acquiring a PhD in the professional field 4.3 Biological Sciences: Indicator A – 50 points; Indicator D – 44 p.

During her studies, Stanimira Angelova has participated in 6 scientific forums, 5 research projects and has conducted a two-month specialization at the University of Ghent, Belgium.

I know personally the doctoral student Stanimira Angelova, as a working, dedicated, highly erudite young researcher and colleague who actively participates in the research and teaching activities of the Department of Biochemistry and Microbiology. She is also enthusiastically involved in administrative activities, as evidenced by her participation in procedures for program accreditation of doctoral programs. I can confidently state that Stanimira Angelova's dissertation is the result of her work under the guidance of Prof. Dr. Iliya Iliev.

7. Abstract

The abstract is formatted according to the requirements and correctly reflects the main results of the dissertation.

8. Critical remarks and recommendations

I recommend continuing work related to the use of the mutant glucansaccharase U13M1 for the synthesis of glucans with improved physico-chemical properties and applications.

CONCLUSION

The presented doctoral dissertation contains scientific and scientific-applied results that represent an original contribution to science and meet all the requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria (ZRASRB), the Regulations for the Implementation of ZRASRB and the relevant Regulations of PU "Paisiy Hilendarski"

The dissertation shows that the doctoral student Stanimira Angelova possesses in-depth theoretical knowledge and professional skills in the scientific specialty of **Biochemistry**, demonstrating qualities and skills for independent scientific research, presentation and interpretation of the results obtained.

Due to the above, I confidently give my positive evaluation of the conducted research, presented by the dissertation reviewed, autoreferate, achieved results and contributions, and **I recommend** to the honorable scientific jury to award the **educational and scientific degree "Doctor"** to **Stanimira Angelova Angelova** in the field of higher education: 4. Natural sciences, mathematics and informatics, professional field 4.3 Biological Sciences, PhD program **"Biochemistry"**.

19.02.2024

Prepared the opinion :.....

Prof. Sonya Kostadinova Trifonova