

# OPINION

by Prof. PhD Georgi Todorov Dobrev,  
University of Food Technology

of a dissertation thesis for acquiring of educational and scientific degree "doctor",

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

professional field **4.3. Biological Sciences,**

Doctoral Program "**Biochemistry**"

**Author:** Stanimira Angelova Angelova

**Dissertation topic:** "Investigation of the properties of bioengineered alpha-D -glucans synthesized by mutant glucansaccharase URE 13-300"

**Research supervisor:** Prof. PhD Ilia Nikolov Iliev, PU "Paisiy Hilendarski"

## 1. General performance of the procedure and the doctoral student

By order No. PD-21-2469 dated 18.12.2023 of the Rector of the Plovdiv University "Paisiy Hilendarski" (PU) I have been appointed as a member of the scientific jury in the procedure for the defense of a dissertation thesis on the topic "**Investigation of the properties of bioengineered alpha-D -glucans synthesized by mutant glucansaccharase URE 13-300**" for the acquisition of the educational and scientific degree "doctor of philosophy" by Stanimira Angelova Angelova - full-time doctoral student at the Department of "Biochemistry and Microbiology". The set of electronic materials presented by doctoral student Stanimira Angelova is in accordance with Article 36 (1) of the Regulations for the Development of the Academic Staff of the PU and includes all the required documents.

## 2. Actuality of the topic

Enzymes are a powerful tool to drive about the "green" transition of the modern economy. As "green" catalysts, they accomplish all modern requirements for safety and environmental protection. The specificity of their catalytic action and their high activity allow a multiple increase in the rate of desired chemical reactions in the absence of side products. Along with their advantages, the protein nature of enzymes also determines some limitations for their application - low operational stability and high cost. Significant efforts are being made to engineer enzymes to expand their applications. The main approaches to enzyme engineering are enzyme immobilization and protein engineering. A modern trend is also engineering enzymes to catalyze reactions that do not occur in nature, by using atypical reaction media.

The main research of the dissertation thesis is aimed to present the application of different protein engineering methods to obtain a mutant glucansaccharase, which to be used to obtain glucan with the potential for application in various fields of industry. The topic is current and correlates with modern trends in the development of enzymology. The PhD student applied the "rational design" approach to protein engineering to obtain a mutant glucansaccharase. Site-directed mutagenesis of glucansaccharase URE 13-300 consisting of a single point mutation (G449K) was performed and the properties of the resulting mutant enzyme as well as the enzymatically synthesized alpha-glucan were investigated. In the dissertation work, research on the influence of the reaction medium and the type of acceptor as a means of modeling the enzyme activity was also conducted. The topic, the methods used and the experimental work carried out are at a modern level.

### **3. Knowing of the problem**

The introduction substantiates the topicality and significance of the researched issues, where the doctoral student has presented and analyzed actual information on the classification, mechanism of action and structural organization of microbial glucansaccharases. Extensive information on the structural organization of glucansaccharases was critically analyzed and a strong connection between mechanism of action and specific biochemical properties of the enzymes has been made, as a successful prerequisite for the implementation of the chosen approach of "rational design" of protein engineering. The analyzed information confirms that site-directed mutagenesis allows to change significantly the biochemical characteristics of the enzyme, and based on this, the physicochemical properties of the synthesized polysaccharides. The material clearly shows the doctoral student's desire to make connection between the structure of protein molecules and their properties in order to determine the areas and domains in glucansaccharases that can be modified to obtain enzymes with new characteristics. The overview also includes information on the practical application of glucans with different structures and physicochemical properties, which allows the doctoral student to formulate reasonably the goal of obtaining glucans with specific properties through bioengineering of microbial glucansaccharase.

### **4. Research methodology**

In the development of the dissertation, the doctoral student used a large number of microbiological, chemical, enzymatic and instrumental methods of analysis. For me, mastering bioinformatics analysis and working with online bioinformatics tools, which are the basis of successful protein bioengineering tools, have a high added value. The doctoral student has also mastered methods for determining enzyme activity and the principles for studying the kinetics of enzyme reaction. NMR analysis was performed to determine the structure of the synthesized glucan. I believe that the necessary practical experience and knowledge for conducting scientific research at a modern level have been acquired. The methods used and the setup of the experiments correspond to the goals and tasks set in the dissertation work.

### **5. Characterization and evaluation of the dissertation work**

The dissertation is in a volume of 157 pages and consists of: Introduction (2 pages), Literature review (32 pages), Aim and objectives (1 page), Materials and methods (15 pages), Results and discussion (50 pages), Summary (5 pages), Conclusions (1 page), Contributions (1 page), References (11 pages).

The dissertation also includes 33 pages of appendices to the Results and Discussion section. The formulated goal is clear and achievable. Research is aimed at obtaining a mutant glucansaccharase with new biochemical properties, to be used for obtaining glucans with a specific structure and properties. Seven research tasks are presented. The Experimental work has been performed purposefully and correctly, and the experimental results are shown in 3 tables and 31 figures. Another 63 figures with experimental results directly related to the topic are presented as appendices. Experimental results are reasonably discussed and critically compared with results from the available literature.

As the main scientific contributions of the dissertation work, I could point out:

1. A successful single point mutation (G449K) was performed by site-directed mutagenesis in domain B of URE 13-300 glucansaccharase and an active mutant glucansaccharase U13M1 was obtained, which was characterized by altered biochemical and kinetic characteristics.

2. The optimal conditions for in-vitro synthesis of alpha-D-glucan with the obtained mutant glucansaccharase U13M1 were determined.

3. The mutation (G449K) in glucansaccharase URE 13-300 was found to significantly reduce  $\alpha$ -(1 $\rightarrow$ 3) linkages in the main chain and in the branches of the synthesized glucan.

4. The possibility of enzymatic synthesis of oligosaccharides with glucansaccharase URE 13-300 in an aqueous-organic medium.

## **6. Assessment of the PhD student's publications and personal contributions**

The results of the dissertation have been published in 3 scientific papers – one in quartile Q2 and two in quartile Q4. In two of the publications, the doctoral student is the first author. With the mentioned publications, the doctoral student fulfills the national minimum criteria for acquiring the doctoral degree in professional field 4.3. Biological Sciences (Indicator “A” – 50 p., Indicator “T” – 44 p.). The doctoral student participated in 6 scientific conferences and is part of the team of 5 research projects. He did a short-term specialization at the University of Ghent, Belgium.

## **7. Abstract**

The abstract is formatted according to the requirements and correctly reflects the structure and content of the dissertation work.

## **8. Recommendations for future use of dissertation contributions and results**

I have the following questions:

1. Why are alcohols used in the study of the influence of organic solvents on enzyme activity, they can be considered as substrates (acceptor with an -OH group) of the enzyme reaction?

2. How do you define the so-called of your "optimal substrate concentration" for enzyme action?

3. When determining  $K_m$  and  $V_{max}$ , why do you present the reaction rate in U/mg? Can protein content change the presented rate of an enzyme reaction?

## **CONCLUSION**

The dissertation contains scientific, scientific-applied and applied results, which represent an original contribution to science and meet all the requirements of DASRBA. The dissertation work shows that the doctoral student possesses in-depth theoretical knowledge and professional skills in the scientific specialty "Biochemistry", demonstrating qualities and skills for independent work of scientific research. I confidently give my positive assessment of the dissertation work and propose 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 direction 4.3. Biological Sciences, Doctoral Program in Biochemistry.

09.02.2024 г.

**Prepared the opinion:** .....

/Prof. dr. Georgi Dobrev/