



To the Chairman of the Scientific Jury  
IChE - BAS

## REPORT

by Prof. Dragomir Simeonov Yankov, PhD

Institute of Chemical Engineering - BAS

member of the scientific jury for the defence of a dissertation for obtaining the educational and scientific degree "Doctor"

Author of the dissertation: **Lidiya Plamenova Tsigoriyna**, doctoral student of independent study in specialty 4.2 Chemical Sciences at the Institute of Chemical Engineering - BAS

Dissertation topic: "Preparation of 2,3-butanediol from inulin by a modified non-pathogenic producer"

**Brief biographical data of the candidate:** MSc Lidia Plamenova Tsigoriyna graduated from higher education with a bachelor's degree at the Faculty of Biology of the University of St. Kliment Ohridski in 2020, where she obtained a master's degree (professional field "Industrial Biotechnologies") in 2021. Since 2020, L. Tsigoriyna has been working at IChE-BAS, initially as a technologist, and since 2021 she has been appointed to a position "assistant". In the same year, she was enrolled as a doctoral student of independent training. She was awarded with the right of defence in 2023, having met all the requirements of the law and the rules of the BAS.

**Brief description of the dissertation:** The dissertation is devoted to the microbial production of 2,3-butanediol (2,3-BD) from inulin, by a genetically modified strain of *Bacillus licheniformis* 24. 2,3-BD is a compound with a variety of applications, the increasing demand of which through recent decades has led to a continuous increase in the volume of its production. In recent years, there has been a growing interest in the microbiological production of 2,3-BD, with research aimed at finding new producers, yield increasing and using cheap and renewable substrates with a view to improving the profitability of the process. The topic of the dissertation is relevant in view of the increasing demand for 2,3-butanediol and optimization of the process of its fermentative production. The presented dissertation has a volume of 102 pages. The dissertation consists of an introduction, literature review (37 pages), aims and objectives, materials and methods (6 pages), results (26 pages), discussion of the results (6 pages), conclusions and contributions. The dissertation contains 19 figures and 16 tables. 206 literary sources are cited, 60 (29%) of which were published in the last ten years, and 29 (14%) after 2019. This shows that the doctoral student knows the literature on the issue very well. In the literature review, the properties of 2,3-BD, the metabolic pathways for its microbial production, the different producers, as well as the components of the nutrient medium and the main parameters influencing the process, are considered.

The doctoral student has set himself the goal of developing a biotechnology for the microbial production of 2,3-BD from inulin, through a genetically modified strain of *Bacillus licheniformis* 24.

To achieve the goal, the following tasks have been formulated:

1. Optimizing the composition of the nutrient medium for obtaining 2,3-BD;
2. Optimization of the process parameters for obtaining 2,3-BD;
3. Establishing the ability of the wild strain *B. licheniformis* 24 to convert inulin into 2,3-BD;
4. Cloning of a cell-bound inulinase gene from *Lactocaseibacillus paracasei* B41 into *B. licheniformis* 24;
5. Determination of the maximum ability of the modified strain *B. licheniformis* 24 to produce 2,3-BD from inulin-containing chicory flour.

Optimization of the composition of the culture medium and of the process parameters was carried out using planned experiments. After statistical analysis and selection of the significant parameters, equations describing the process were drawn up. After an optimization procedure, the optimal values of the parameters for maximum production of 2,3-BD were also determined.

Under the optimal conditions found, experiments were conducted to obtain 2,3-BD from different substrates - glucose, fructose in batch process and fed-batch process. Very good results have been achieved, comparable to the best described in the literature.

In order to improve the efficiency of the process, an attempt was made to replace the expensive sugars with a cheaper and more accessible substrate - chicory flour. The results show that *B. licheniformis* 24 does not have sufficient inulinase activity to degrade inulin. To overcome this problem, cloning of a cell-bound inulinase gene from *Lactocaseibacillus paracasei* B41 was performed.

For this purpose, the *inu* gene of *Lactocaseibacillus paracasei* DSM 23505 encoding a cell-bound fructan- $\beta$ -fructosidase was selected. All recombinant constructs were introduced into *B. licheniformis* 24 by electroporation. About 150 clones were analyzed, from which two were selected for further experiments. An eightfold increase in inulinase activity was achieved, and although fermentation of chicory flour (200 g/L) with the recombinant strain yielded only 18.5 g/L 2,3-BD, these results provide a good basis for further research.

The abstract fully and faithfully reflects the content of the dissertation work.

#### **Main contributions of the dissertation:**

1. The composition of the nutrient medium and the process parameters for obtaining 2,3-BD were optimized. Under the optimized conditions, strain *B. licheniformis* 24 produced 138.8 g/L 2,3-BD from glucose, and 156.1 g/L 2,3-BD from fructose.

2. The *inu* gene from *Lactocaseibacillus paracasei* DSM 23505 was successfully introduced into *B. licheniformis* 24 via the pBE-S vector in two variants – as a



pBES\_Inu construct, containing the entire gene and as a pBES\_Inu-tr construct containing the gene without its cell wall binding domains of the host.

3. By introducing the construct pBES\_Inu, the modified strain *B. licheniformis* 24 T26 was given the ability to produce 2,3-BD from inulin in a process of simultaneous saccharification and fermentation.



**Scientific publications and participation in scientific forums:** The dissertation is based on results published in 3 articles with impact factor and 2 participations in scientific conferences. One of the published articles is in a journal with quartile Q1 and is cited 6 times, the second is with Q2 and the third with Q4.

I have no particular remarks on the dissertation except for some spelling and grammatical inaccuracies.

#### **CONCLUSION:**

What has been stated so far gives me reason to assert that the presented results, in terms of volume and quality, fully meet the requirements of the Act on Development of the Academic Staff in the Republic of Bulgaria and the Regulations for its implementation, as well as the Regulations of the BAS. I recommend the esteemed jury to award MSc. Lidiya Plamenova Tsigoriyna the educational and scientific degree "Doctor" and I will vote "YES" with full conviction.

Sofia  
29.1.2024

  
  
(Prof. Dragomir Yankov, PhD)