



## CULTIVATION OF RAW MATERIAL BASED ON PICHIA PASTORIS FOR BIOSIMILAR GROWTH HORMONE MANUFACTURE IN REPUBLIC OF MOLDOVA

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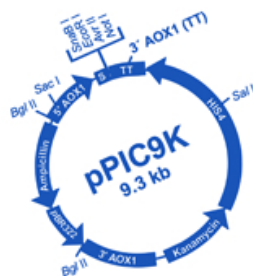


### INTRODUCTION

The Republic of Moldova currently only has two authorized medicinal products from the group of hormones of the anterior pituitary lobe and analogues. The recombinant human growth hormone rhGH therapy typically lasts several years and requires an individualized dosing regimen based on the patient's body weight. Patient adherence and compliance to treatment is a priority issue. The global rhGH market size was estimated at USD 2,840.70 million in 2018 and is expected to reach USD 5,563.60 million by 2026, with a CAGR of 8.6%. In Moldova, a centralized public procurement is held annually for the purchase of medicines for the treatment of patients with pituitary insufficiency/pituitary dwarfism. The domestic drug manufacturer Balkan Pharmaceuticals SRL aims to manufacture the first biosimilar in the Republic of Moldova, providing an exploration of the most promising segment of the global pharmaceutical industry with the fastest growth rate.

### AIM AND OBJECTIVES

In a collaboration between the Scientific Center of Drug Research of SUMPh "N. Testemițanu", the pharmaceutical company Balkan Pharmaceuticals, and ICGEB, a technology transfer for the production of recombinant growth hormone using *Pichia pastoris* was successfully carried out. *P. pastoris* was chosen due to its advantages in processing, protein folding, and post-translational modifications, as well as its easy manipulation. The GH1 gene was introduced into the *P. pastoris* genome using a plasmid vector and the AOX1 gene promoter, which can be induced by methanol. The obtained somatotropin was secreted into the culture medium. The team developed Technological Protocols for *Pichia pastoris* short-term and long-term storage, inoculum preparation, inoculation, and growth, as well as Standard Operating Procedures for creating a working cell bank and in-vitro biological activity assay of the recombinant human growth hormone.



### MATERIALS AND METHODS

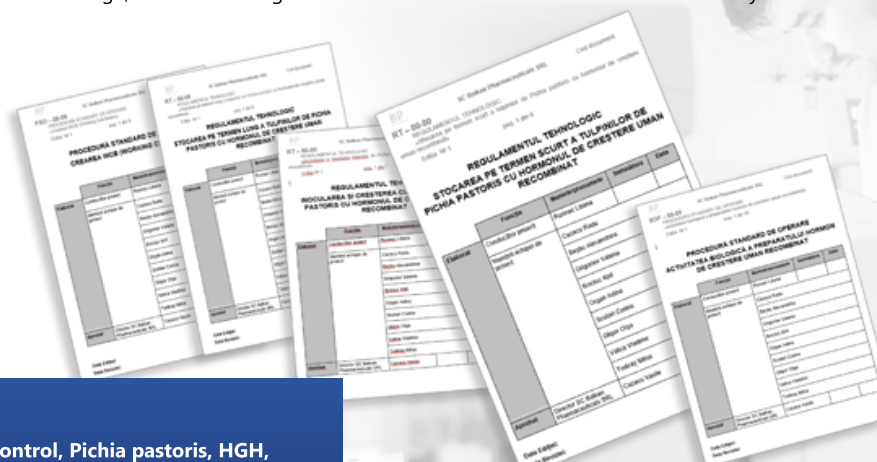
The use of working cell banks in cell line maintenance reduces the risk of contamination, loss of characteristics, genetic drift, and cross-contamination, while preserving cell line characteristics and reducing costs. *Pichia pastoris*, a methylotrophic yeast capable of metabolizing methanol as the sole carbon source, is commonly used for recombinant protein production using alcohol oxidase enzyme promoters. The AOX1 gene product accounts for most of the alcohol oxidase in the cell, and its expression is strictly regulated and induced by methanol. MutS strains, which have an active AOX2 gene and grow slowly, are advantageous for large-scale processes. *Pichia pastoris* can express the protein of interest intracellularly or extracellularly, with secretion requiring the presence of a signal sequence. For the cultivation of *Pichia pastoris* with growth hormone expression, the recombinant human somatotropin gene was introduced into the *Pichia pastoris* genome using the vector pPIC9K. The GH1 gene was modified to be inserted into the vector and activated by the AOX1 gene promoter using methanol induction.

### RESULT

This thesis highlights the collaboration between the International Center for Genetic Engineering and Biotechnology (ICGEB), Scientific Center of Drug Research of SUMPh "N. Testemițanu," and Balkan Pharmaceuticals for the industrial-scale manufacture of recombinant human growth hormone (rhGH) using *Pichia pastoris*.

The process of scale-up from laboratory to pilot phase was performed following Good Manufacturing Practices (GMP) rules, ensuring that the transition of the product created by the research service to the pilot phase at the manufacturing site was correctly executed. This stage enabled the final scale-up to the commercial batch manufacture of the biosimilar product.

A technological protocol was developed for the preparation and storage of *P. pastoris* colonies for short-term and long-term storage. YPg agar with added geneticin, G418 was used for the preparation of *P. pastoris* colonies intended for short-term and long-term storage. The optimal temperature for the short-term storage of *P. pastoris* strains was 30°C for 2-4 days or for several weeks or months at 4°C. Cryovials were frozen and stored in liquid nitrogen or at -80°C for long-term storage, and the working cell bank should be renewed after no more than 2 years.



### RESULT

The in-vitro assay of rhGH was performed using the Nb2-11 cell line. The testing methodology and requirements were established according to European Pharmacopoeia standards. The rhGH estimated potency should be >80% and <125% of the declared potency, with confidence limits (p=0.95) of the potency within 64-156% of the declared potency.

For the preparation, inoculation, and growth of *P. pastoris* strains with rhGH in the Erlenmeyer flask, a testing methodology was developed for each step, and the validity term of used medium was established. Technological protocols and Standard Operating Procedures were developed for each elaborated technological process during the industrial scale-up process. The impact of the elaborated technological processes on the obtained products was observed, and the results showed that the technological protocols were efficient in obtaining pharmaceutically active rhGH.

### CONCLUSION

The pharmaceutical company Balkan Pharmaceuticals Ltd, in collaboration with the Scientific Center for Drug Research of SUMPh "N. Testemițanu," has developed a working cell bank for the *Pichia pastoris* strain that expresses human growth hormone. This development is a crucial step towards the industrial manufacturing of biosimilar preparations. Standard operating procedures and technological protocols were established for the creation of the working cell bank and the short-term and long-term storage of *Pichia pastoris* strains. Additionally, a procedure for the biological activity assay of recombinant human growth hormone was established for quality control purposes. The creation of a biosimilar hormone working cell bank in Moldova is considered innovative and represents a significant advancement for the pharmaceutical industry in the country.

### KEY WORDS

Biosimilar development, Biologic medicines, Regulatory guidelines, Quality control, *Pichia pastoris*, HGH, Fermentation.

