

# The yeast *Pichia pastoris* as a cell factory for the production of recombinant proteins

## OBJECTIVES

- To have an overview of the fundamental aspects of the expression system.
- To know the characteristics that make it a distinctive system.
- To know the main expression tools
- To understand some of the problems of the recombinant protein secretion pathway and their corresponding solutions
- To be able to understand the mechanisms used for the high-level expression of Leghemoglobin.

## INTRODUCTION

Due to the impossibility of obtaining satisfactory yields from natural sources, proteins are synthesized in heterologous systems.

The methylotrophic yeast *Pichia pastoris* as a cellular host for the expression of recombinant proteins has become increasingly popular because of its characteristics:

- easy manipulation
- controlled glycosylation
- possibility to obtain intra- and extracellular proteins
- presence of the strong methanol-inducible promoter AOX1
- ability to perform post-translational modifications corresponding to higher eukaryotic organisms

Table 1. Examples of proteins expressed by *Pichia pastoris*.

Protein	Use	Reference
BACTERIA		
Azurin from <i>Pseudomonas aeruginosa</i>	Anticancer agent	(Unver et al., 2021)
Pectin methylesterase (PME) from <i>Pectobacterium chrysanthemi</i>	Catalyses the hydrolysis of the methyl ester of pectin to yield methanol and free carboxyl groups	(Acar & Unver, 2022)
FUNGI AND YEASTS		
Plectasin from <i>Pseudoplectanina nigrella</i>	Antimicrobial peptide	(Liang et al., 2022)
Fructosyltransferase from <i>Aspergillus oryzae</i>	Biocatalysis	(Alvarado-Obando et al., 2022)
PLANT		
Soy Leghemoglobin	Food additive	(Shao et al., 2022)
Potato patatin	Gelling agent	(Dai et al., 2022)
$\Delta 9$ -tetrahydrocannabinolic acid synthase (THCAS) from <i>Cannabis sativa</i>	Pharmaceutical product	(Zirpel et al., 2018)
INVERTEBRATES		
Neurotoxin TS8 from <i>Tityus serrulatus</i>	Antifungal	(Cordeiro et al., 2022)
Neurotoxin Tx4(6-1) from <i>Phoneutria nigriventer</i>	Insecticidal	(Li & Xia, 2019)
VERTEBRATES		
DesB30	Insulin Analogue	(Wu et al., 2019)
Lysozyme	Antimicrobial agent	(He et al., 2020)
Asprosin	Hormonal	(Zhang et al., 2021)
VIRUS		
ORF2 protein of the hepatitis E virus (HEV) capsid.	HEV antigen	(Gupta et al., 2022)

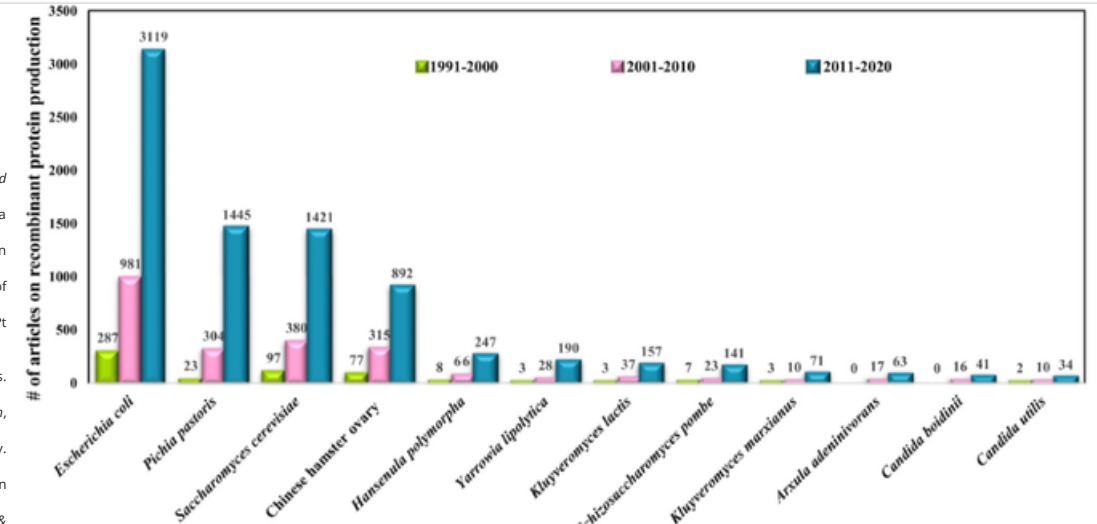


Figure 1. Number of published articles in Scopus related to recombinant protein production for 10 years of time periods between 1991 and 2020 (Burcu Gündüz et al., 2021).

## PROTEIN EXPRESSION SYSTEM

Points to be considered from the star:

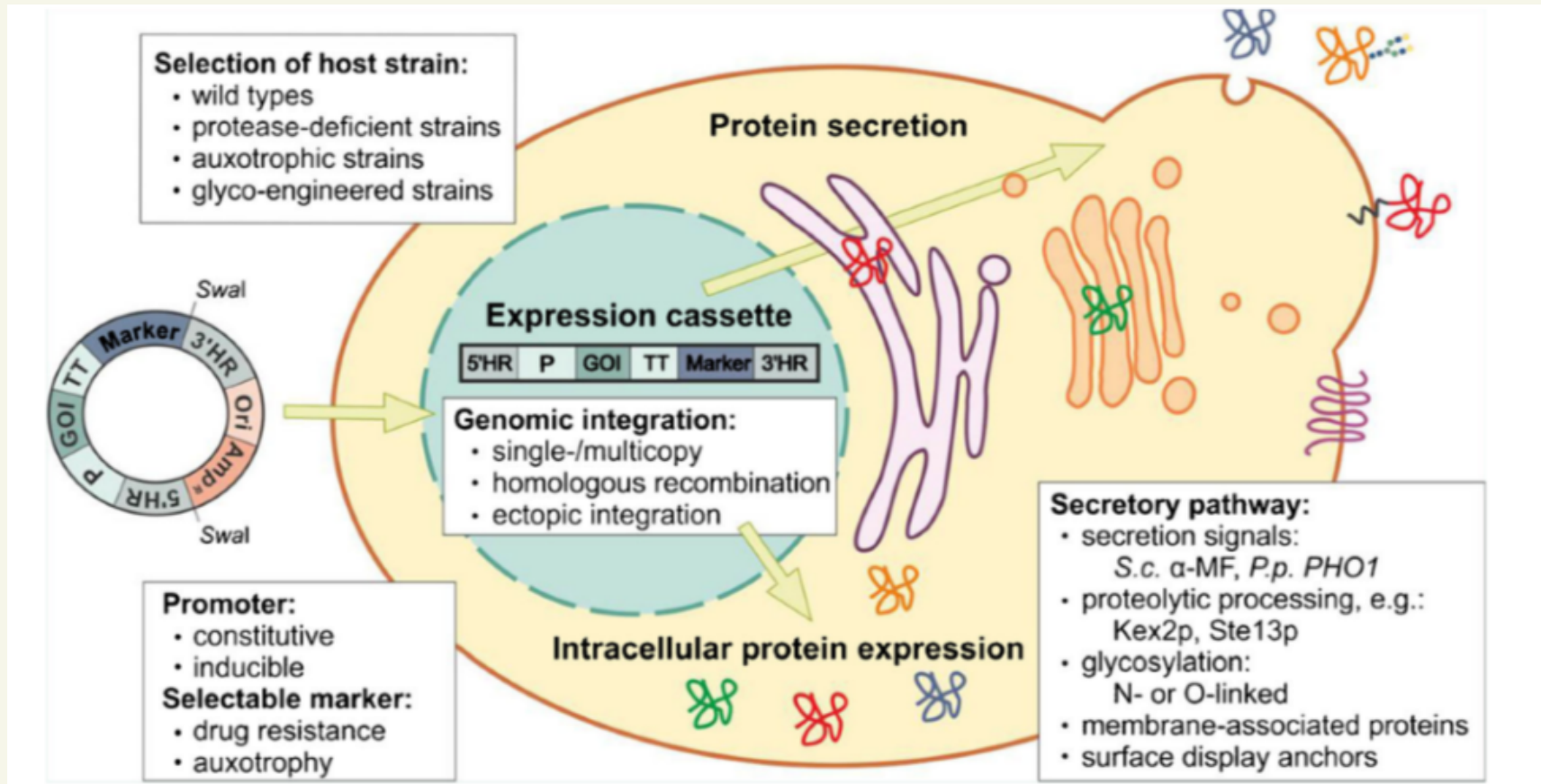


Figure 2. General considerations for heterologous gene expression in *P.pastoris* (Ahmad et al., 2014)

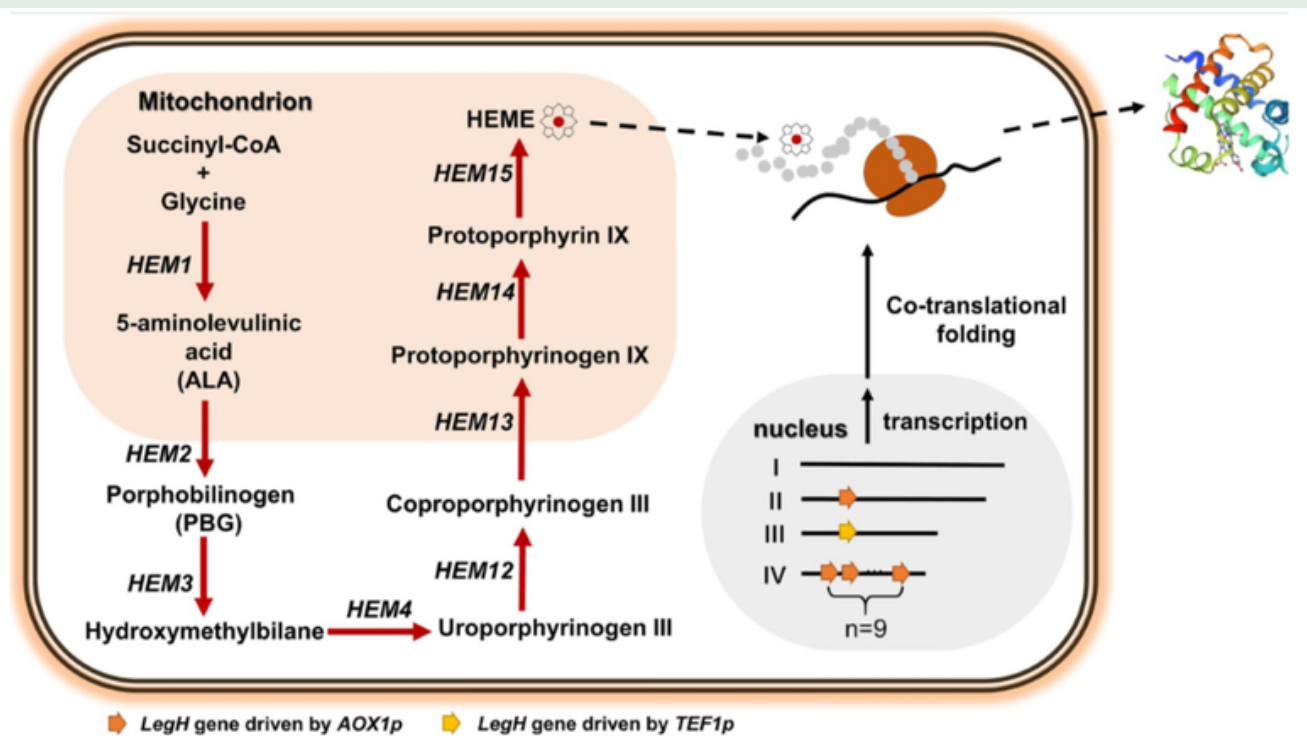


Figure 3. Schematic diagram describing the overall engineering strategy for LegH secretory production (Shao et al., 2022)

## HIGH-LEVEL SECRETORY PRODUCTION OF LEGHEMOGLOBIN

- Increasing LegH gene dosage
- consolidating the native heme biosynthesis pathway.

## CONCLUSIONS

- The choice of an expression strain, vector, promoter and signal sequence can have a crucial impact on the success of a project.
- Protein expression and secretion can be further optimized, which, in many cases, will depend on the desired product.