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

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Reference

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 methanolinducible promoter AOX1
- ability to perform post-translational modifications corresponding to higher eukaryotic organisms

Table 1. Examples of proteins expressed by Pichia pastoris.

BACTERIA		
Azurin from Pseudomonas	Anticancer agent	(Unver et
aeruginosa		al., 2021)
Pectin methylesterase (PME) from	Catalyses the hydrolysis of the methyl	(Acar &
Pectobacterium chrysanthemi	ester of pectin to yield methanol and	Unver,
-	free carboxyl groups	2022)
FUNGI AND YEASTS		
Plectasin from Pseudoplectania	Antimicrobial peptide	(Liang et
nigrella .		al., 2022)
Fructosyltransferase from	Biocatalysis	(Alvarado-
Aspergillus oryzae		Obando et
1 3		al., 2022)
PLANT		
Soy Leghemoglobin	Food additive	(Shao et
,8		al., 2022)
Potato patatin	Gelling agent	(Dai et al.,
r out o patient	Jenning agent	2022)
Δ9-tetrahydrocannabinolic acid	Pharmaceutical product	(Zirpel et
synthase (THCAS) from Cannabis	Thurmaceutical product	al., 2018)
sativa		al., 2010/
INVERTEBRATES		
Neurotoxin TS8 from Tityus	Antifungal	(Cordeiro
serrulatus		et al.,
		2022)
Neurotoxin Tx4(6-1)	Insecticidal	(Li & Xia,
from Phoneutria nigriventer		2019)
VERTEBRATES		
DesB30	Insulin Analogue	(Wu et
		al., 2019)
Lysozyme	Antimicrobial agent	(He et al.,
		2020)
Asprosin	Hormonal	(Zhang et
Tisprosiii	Tionnondi	al., 2021)
VIRUS		
ORF2 protein of the hepatitis E	HEV antigen	(Gupta et
virus (HEV) capsid.		al., 2022)
		,,

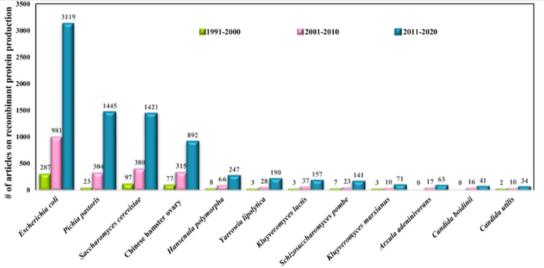


Figure 1. Number of published articles in Scopus related to recombinant protein production for 10 years of time periods between 1991 and 2020 (Burcu Gündüz et al., 2021).

PROTEIN EXPRESSION SYSTEM

Points to be considered from the star:

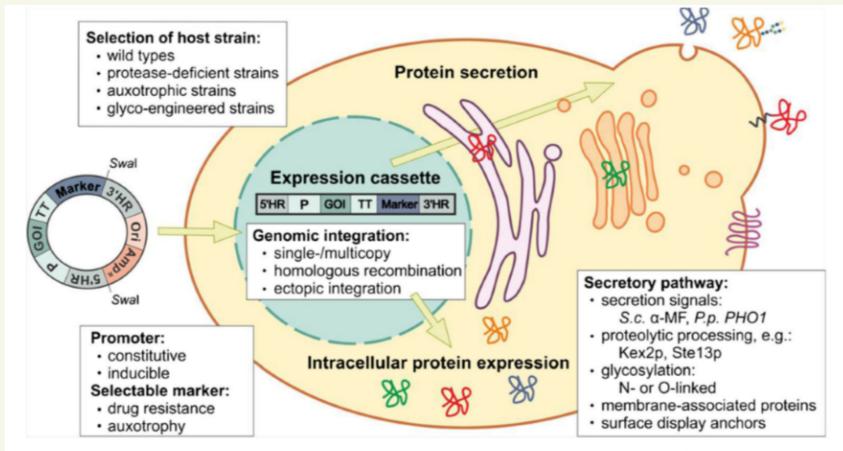


Figure 2. General considerations for heterologous gene expression in P.pastoris (Ahmad et al., 2014)

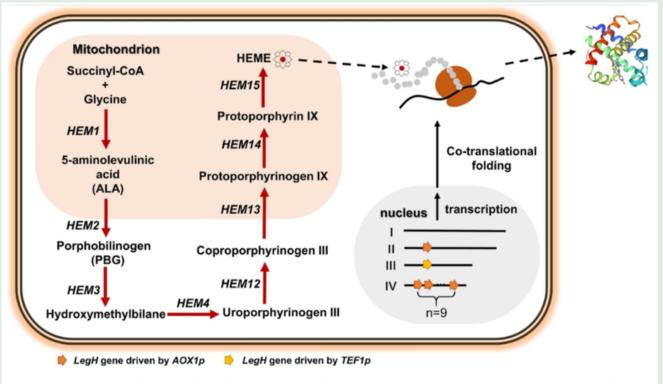


Figure 3. Schematic diagram describing the overall engineering strategy for LegH secretory production (Shao et al., 2022)

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.

• Acar M & Univer Y (202

- Acar, M., & Unver, Y. (2022). Constitutive and extracellular expression of pectin methylesterase from Pectobacterium chrysanthemi in Pichia pastoris. 3 Biotech, 12(9).

 Ahmad, M., Hirz, M., Pichler, H., & Schwab, H. (2014). Protein expression in Pichia pastoris: Recent achievements and perspectives for heterologous protein pro
- Microbiology and Biotechnology, 98(12), 5301–5317.
 Alvarado-Obando, M., Contreras, N., León, D., Botero, L., Beltran, L., Díaz, D., Rodríguez-López, A., Reyes, L. H., Alméciga-Díaz, C. J., & Sánchez, O. F. (2022). Engineering a heterologously expressed fructosyltransferase from Aspergillus oryzae N74 in Komagataella phaffii (Pichia pastoris) for kestose production. New Biotechnology, 69, 18–27.
 Burcu Gündüz, E., Berrios, J., Binay, B., & Fickers, P. (2021). Recombinant protein production in Pichia pastoris: from transcriptionally redesigned strains to bioprocess optimization
- and metabolic modelling. FEMS Yeast Research, 21(7).

 Cordeiro, F. A., Amorim, F. G., França, J. B., Pinheiro-Júnior, E. L., Cardoso, I. A., Zoccal, K. F., Peigneur, S., Faccioli, L. H., Tytgat, J., & Arantes, E. C. (2022). Heterologous expression of Ts8, a neurotoxin from Tityus serrulatus venom, evidences its antifungal activity. Toxicon: Official Journal of the International Society on Toxinology, 218, 47–56.
- Ts8, a neurotoxin from Tityus serrulatus venom, evidences its antifungal activity. Toxicon: Official Journal of the International Society on Toxinology, 218, 47–56.

 Dai, Z., Wu, X., Zeng, W., Rao, Y., & Zhou, J. (2022). Characterization of highly gelatinous patatin storage protein from Pichia pastoris. Food Research International (Ottawa, Ont.), 162(Pt A).
- Li, H., & Xia, Y. (2019). High-yield production of spider short-chain insecticidal neurotoxin Tx4(6-1) in Pichia pastoris and bioactivity assays in vivo. Protein Expression and Purification, 154, 66-73.
 Liang H. Gi, X. Via, O. Moor, D. Chan, D. & May Y. (2023). Proceeding expression level of plostasin in recombinant Pichia pastoris via 24 cells proceeding popular.
- Applied Microbiology and Biotechnology, 106(9–10), 3669–3678.

 Shao, Y., Xue, C., Liu, W., Zuo, S., Wei, P., Huang, L., Lian, J., & Xu, Z. (2022). High-level secretory production of leghemoglobin in Pichia pastoris through enhanced globin expression and heme biosynthesis. Bioresource Technology, 363, 127884.
- Biotechnology, 51(7), 723–730.

 Wu, J.; Gong, G.; Han, S.; Zhai, W.; Biu, Y.; Xie, L (2019). Expression, Purification, and Characterization of the Degludec Precursor DesB30. Protein Expr Purif 161, 28–39.

 Zhang, Y.; Zhu, Z.; Zhai, W.; Bi, Y.; Yin, Y.; Zhang, W. (2021). Expression and Purification of Asprosin in Pichia Pastoris and Investigation of its Increase Glucose Uptake Activity in the Company of the C
- Zhang, Y.; Zhu, Z.; Zhai, W.; Bi, Y.; Yin, Y.; Zhang, W. (2021). Expression and Purification of Asprosin in Pichia Pastoris and Investigation of Its Increase Glucose Uptake Activity Skeletal Muscle through Activation of AMPK. Enzyme Microb Technol.
 Zirpel, B., Degenhardt, F., Zammarelli, C., Wibberg, D., Kalinowski, J., Stehle, F., & Kayser, O. (2018). Optimization of Δ9-tetrahydrocannabinolic acid synthase production