EnPresso Y Defined and EnPump are useful tools for protein production in yeasts

Application Note

Summary of published results [1-7], by Dr. Antje Neubauer

Introduction

Komagataella phaffii (also known as *Pichia pastoris*) is a key host for recombinant protein production, renowned for its high cell density growth and strong protein secretion capabilities. *Saccharomyces cerevisiae*, commonly used in the food, beverage, and biofuel industries, is also utilized for producing enzymes and recombinant proteins. Both yeasts are essential in genetic engineering and synthetic biology. Advancements in strain engineering have enhanced their role as cell factories in industrial biotechnology, enabling efficient production of various biochemicals. Effective characterization, encompassing structural, functional, and biophysical analyses, depends on the availability of sufficient protein material. Consequently, optimizing expression systems to achieve high yield is a fundamental objective in protein science, influencing the efficiency and feasibility of subsequent studies.

This application note presents the results of several studies demonstrating the successful use of Enpresso Y Defined and EnPump for recombinant protein production in yeast. Enpresso Y Defined is a ready-to-use, enzyme-controlled glucose-releasing sterile culture medium for yeast cultivation, while EnPump can be combined with your own optimized cultivation medium. Both products create optimal conditions for growth, metabolism and protein expression in yeasts due to their glucose-limited cultivation mode.

Methods

a) EnPresso Y Defined Application

Komagataella phaffii was cultivated in 50 mL and 100 mL shake flask cultures [1-3] according to the manufacturer's instructions. The current name of the glucose-releasing enzyme "Reagent A" and its dosing during the cultivation steps differ from the previous EnPresso product sold by BioSilta Oy.

b) EnPump 200 Application

EnPump 200 was used in several studies with different yeast cultivation media and final concentrations for *Komagataella phaffii [5,7]* and *Saccharomyces cerevisiae* [4,6]:

[4]: Synthetic Complete medium with 60 g/L EnPump 200 substrate and 0.6% Reagent A
[5]: Synthetic screening medium ASMv6 with 50 g/L EnPump 200 substrate and 0.7% Reagent A
[6]: 1L fed-batch-like mineral medium consisting of 480 mL salt mix, 390 mL EnPump 200 substrate (100 g/L) in a phosphate buffer, 6 mL Reagent A, 9 mL $CaCl_2$, 10 mL vitamin mix, 10 mL microelements, 1 mL trace elements
[7]: Limiting glucose medium (1% yeast extract, 2% peptone, 100 mM phosphate buffer pH 6, 50 g/L EnPump 200 substrate and 5 mL/L Reagent A

Results

References for *Komagataella phaffii* Cultivated in EnPresso Y Defined

In Yuan's study [1], the Enpresso Y Defined medium was used to increase the production of **saporin L3, a cytotoxic ribosome-inactivating protein**. Both native and cysteine mutant forms of saporin L3 were produced, allowing for kinetic characterization of their distinct cytotoxic properties. This medium facilitated high-yield protein production and detailed functional analysis of the mutants.

Ashoor [2] and her team used cDNA sequences optimized for *Komagataella phaffii* to produce

Fc receptor variants with high yields. The EnPresso Y Defined culture medium in a fed-batch-like culture maintained stable intracellular conditions, avoiding excessive metabolism during initial cultivation. The study emphasized the importance of optimizing genetic constructs and cultivation conditions, including medium choice, to maximize protein production.

Bordas-Le Floch et al. [3] used Enpresso Y Defined medium to produce recombinant **dust mite allergens Der f 36 and Der p 36**. Yeast transformants were grown in this medium for a comprehensive characterization of the *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus* allergomes, expanding the known allergen repertoire and identifying two novel house dust mite allergens.

References for *EnPump applications*

Van der Hoek et al. [4] reported on the engineering of 16 *S. cerevisiae* strain variants carrying biosynthetic genes for ERG (L-(+)-Ergothioneine) production, aiming to select the best producer in defined media. They compared three different media, investigated the effect of amino acid supplementation, and generated knock-out variants. EnPump 200 was used to simulate the fed-batch medium for use in bioreactor cultivation. ERG concentrations peaked at 60 mg/L in the simulated fed-batch medium with EnPump, surpassing the maximum of 20 mg/L observed under batch conditions. In Figure 1, they displayed the outcomes of modifications in the nitrogen metabolism of *S. cerevisiae* using SC (A) and SC + EnPump 200 medium (B). The removal of Yih1 does not influence ergothioneine production, whereas the deletion of Tor1 appears to enhance ERG levels only in batch conditions. Given that future ERG production will likely occur under fed-batch conditions and neither genetic alteration showed beneficial effects in these conditions, they opted not to continue with these genetic modifications. Furthermore, they found that an additional copy of NcEgt1 and/or Egt2 affected the ERG titer depending on the batch

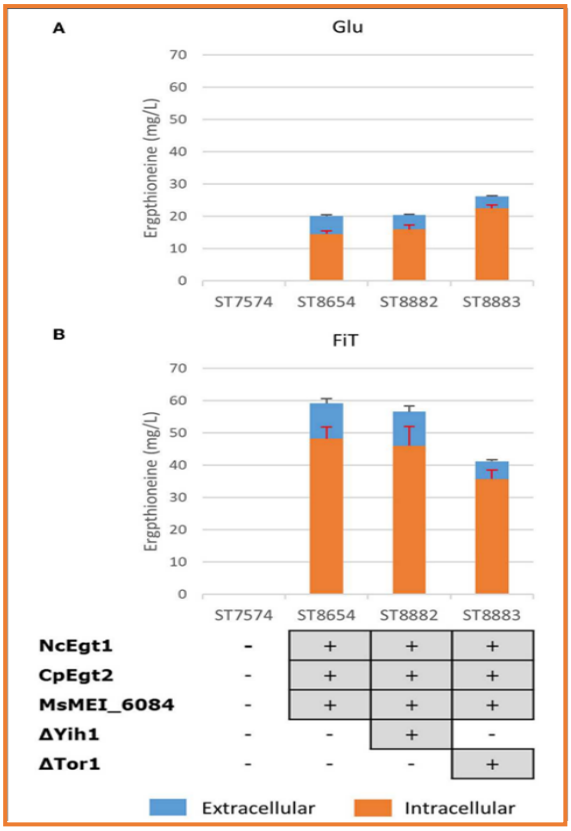

Figure 1: The effect of gene knock-outs (A) Glu: SC + 40 g/L glucose (B) FiT: SC + 60 g/L EnPump substrate, 0.6% Reagent A (van der Hoek et al., 2019)

or fed-batch cultivation mode.

Totaro et al [5] developed a microfluidic cultivation platform that accelerates the screening of rHSA (recombinant human serum albumin)-overproducing *K. phaffii* strains and achieves comparable titers to lab-scale cultures. This platform enabled precise substrate feeding control by using EnPump 200, impacting strain metabolism early in the fed-batch process development. The study showcased productivity linked to growth rate and significant strain differentiation just 12 hours post-inoculation.

Forman et al [6] investigated the primary steps in ginkgolide biosynthesis and produced diterpenoids in *S. cerevisiae* strains using a fed-batch-like mineral medium with EnPump 200.

Claes et al [7] analysed four *Komagataella phaffi* strains to find an equivalent open-access alternative to the industrial yeast, which is restricted by a licensing scheme. They developed their OPENPichia strain along with

a vector toolkit and expressed four protein types using BMY medium with the EnPump 200.

Conclusion

The application of EnPresso Y Defined medium in *Komagataella phaffii* expression systems has proven highly effective in enhancing protein production and facilitating detailed protein characterization. Across the reviewed studies, EnPresso® medium enabled the production of a diverse range of proteins, from highly cytotoxic ribosome-inactivating proteins to allergenic proteins and Fc receptor variants. These findings underscore the medium's versatility and efficacy, establishing it as a valuable tool for researchers aiming to optimize protein expression and gain insights into protein function.

The EnPump 200 approach can mimic bioreactor conditions, potentially expediting strain selection in biopharmaceutical development. This tool, combined with micro-cultivation platforms, demonstrates the feasibility of conducting complex experiments in a straightforward, cost-effective micro-scale setup. This capability could streamline bioprocess development and reduce reliance on larger bioreactors.

References

[1] Yuan, H., Du, Q., Sturm, M.B. and Schramm, V.L. Biochemistry 54.29 (2015): 4565-4574.
https://doi.org/10.1021/acs.biochem.5b00405

[2] Ashoor, D., Bourguiba-Hachemi,S., Marzouq, M.H.A. and Fathallah, M.D. Int Adv Res J Sci Eng Technol 3 (2016): 1-5.
DOI 10.17148/IARJSET.2016.3201

[3] Bordas-Le Floch, V., Le Mignon, M., Bussières, L, Jain, K., Martelet, A., Baron-Bodo, V., Nony, E., Mascarell, L. and Moingeon, P. PLOS ONE 2017, 12(10): e0185830.
https://doi.org/10.1371/journal.pone.0185830

[4] Van der Hoek, S. A., Darbani, B., Zugaj, K.E., Prabhala, B.K., Biron, M.,B., Randelovic, M., Medina, J.B., Kell, D.B. and Borodina, I. Frontiers in Bioengineering and Biotechnology 7 (2019): 262.
https://doi.org/10.3389/fbioe.2019.00262

[5] Totaro, D., Radoman, B., Schmelzer, B., Rothbauer, M., Steiger, M.G., Mayr, T., Sauer, M., Ertl, P. and Mattanovich, D. Biotechnology Journal 16.3 (2021): 2000215.
https://doi.org/10.1002/biot.202000215

[6] Forman,V., Luo, D., Geu-Flores, F., Lemcke, R., Nelson, D.R., Kampranis, S.C., Staerk, D., Møller, B.L and Pateraki, I. Nat Commun 13, 5143 (2022)
https://doi.org/10.1038/s41467-022-32879-9

[7] Claes, K., Van Herpe, D., Vanluchene, R., Roels, C., Van Moer, B., Wyseure, E., Vandewalle, K., Eeckhaut, H., Yilmaz, S., Vanmarcke, S., Çıtak, E., Fijalkowska, D., Grootaert, H., Lonigro, C., Meuris, L., Michielsen, G., Naessens, J., van Schie, L., De Rycke, R., De Bruyne, M., Borghgraef, P. and Callewaert, N. Nat Microbiol 9, 864–876 (2024)
https://doi.org/10.1038/s41564-023-01574-w