5TH APPLIED SYNTHETIC BIOLOGY IN EUROPE
2-4 November 2020

Construction of a novel and safe *S. cerevisiae* biocatalyst for lactulose production

Beatriz B. Cardoso[1,*], Sara C. Silvério[1], Joana L. Rodrigues[1], Lígia R. Rodrigues[1]

[1]Centre of Biological Engineering, Universidade do Minho, Campus de Gualtar, 4710-057 Braga, Portugal

** e-mail [beatriz.cardoso@ceb.uminho.pt]*

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Abstract

Prebiotics are defined as 'substrates that are utilized by host microorganisms conferring a health benefit'. These compounds have been incorporated in a wide variety of food products[1]. One of the most well-recognized prebiotics is lactulose. Lactulose is not found naturally so it has to be produced through different methods: chemical or enzymatic synthesis and electro-activation[2]. Recently, the production of lactulose through lactose isomerization catalyzed by cellobiose 2-epimerase from *Caldicellulosiruptor saccharolyticus* (*Cs*CE) was reported[3]. This strategy is gaining attention as a preferable methodology for industrial application due to its notable yields. *Saccharomyces cerevisiae*, one of the most well-characterized microorganisms, is widely used for the heterologous production of several enzymes, also due to the diverse genetic manipulating tools that are currently available. Here, we propose a new and promising *S. cerevisiae* biocatalyst. Taking advantage of its GRAS status and using lactose as a single substrate, we believe that it can be a more economic and attractive approach for the synthesis of lactulose. The *Cs*CE gene was cloned in the CEN.PK2-1C *S. cerevisiae* strain, under TEF or GAP promoters' control. Both biocatalysts were used in lactulose production, reaching a concentration of 1.26 g/L (TEF promoter) and 2.15 g/L (GAP promoter). The maximum prebiotic yield was 6.20%. These promising results represent the first use of a *S. cerevisiae* biocatalyst for lactulose production and demonstrated its potential as a sustainable and safe approach for food application.

1. R. Gibson, R. Hutkins, M. Sanders et al, Nature Reviews Gastroenterology and Hepatology (2017)
2. S. Silvério, E. Macedo, J. Teixeira, L. Rodrigues, Comprehensive Reviews in food Science and Food Safety (2016)
3. Y. Kim, D. Oh, Bioresource Technology, 104 (2012)

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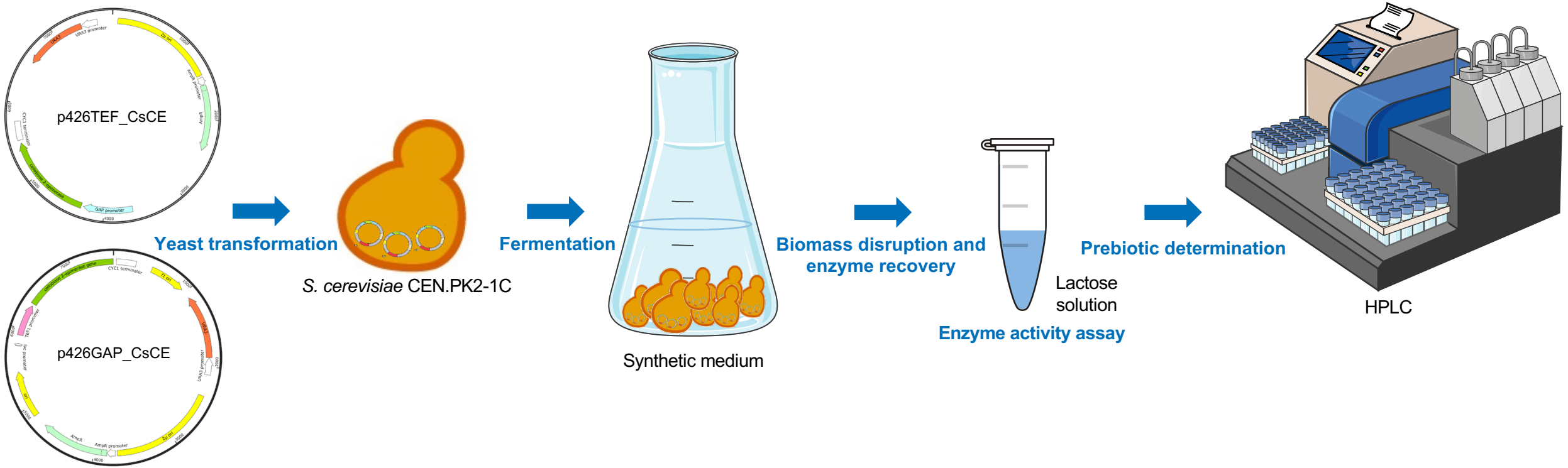
Aim: Development of a *S. cerevisiae* biocatalyst able to produce lactulose

Experimental



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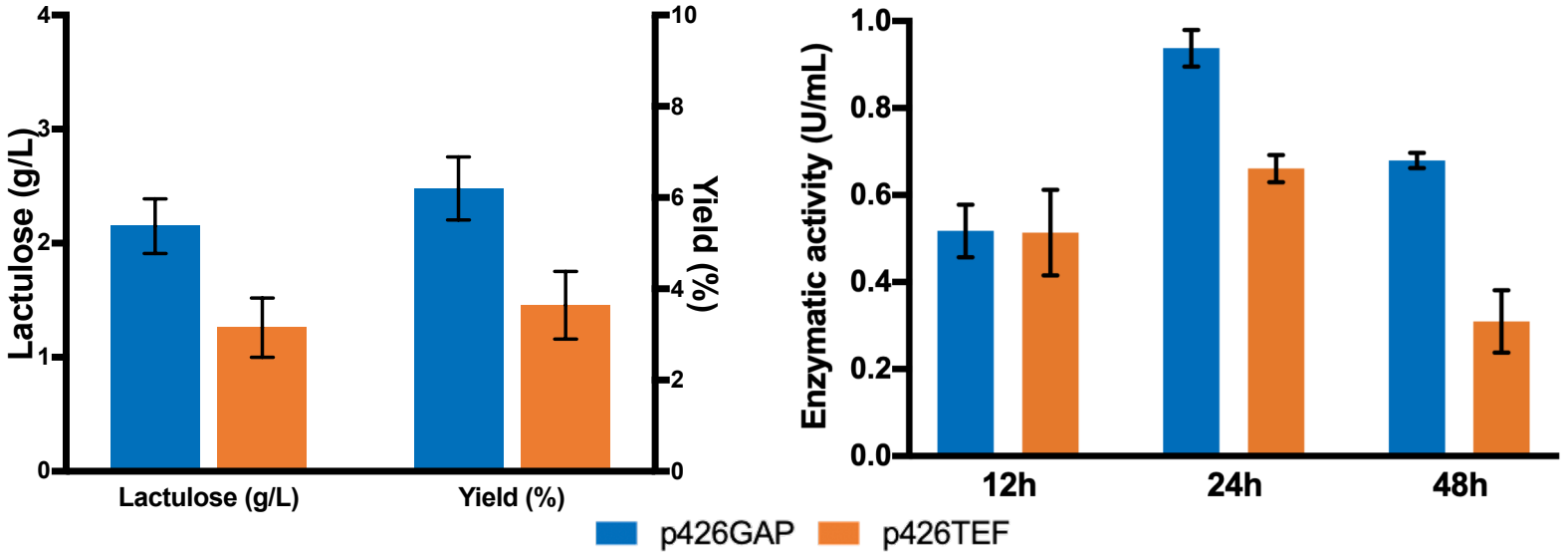
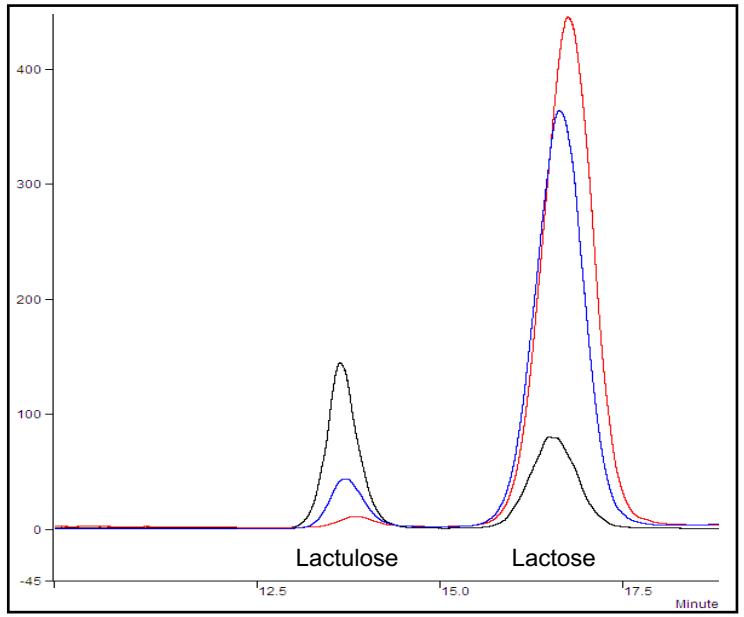
Results





HPLC profile for lactulose production. Blue chromatogram: *S. cerevisiae* carrying p426GAP; Red chromatograms: *S. cerevisiae* carrying p426TEF; Black chromatogram: standard solution containing lactulose and lactose (7.5 g/L each).

Conclusions

- First reported production of lactulose using *S. cerevisiae*
- Maximum yield reached at 24h using the GAP promoter
- Sustainable and safe approach
- Suitable for food application

Promising strategy for lactulose production

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