The 2nd International *BioDesign Research* Conference

December 6th - 17th, 2021, Virtual

Production of a lactose-based prebiotic mixture by engineered *Saccharomyces cerevisiae*

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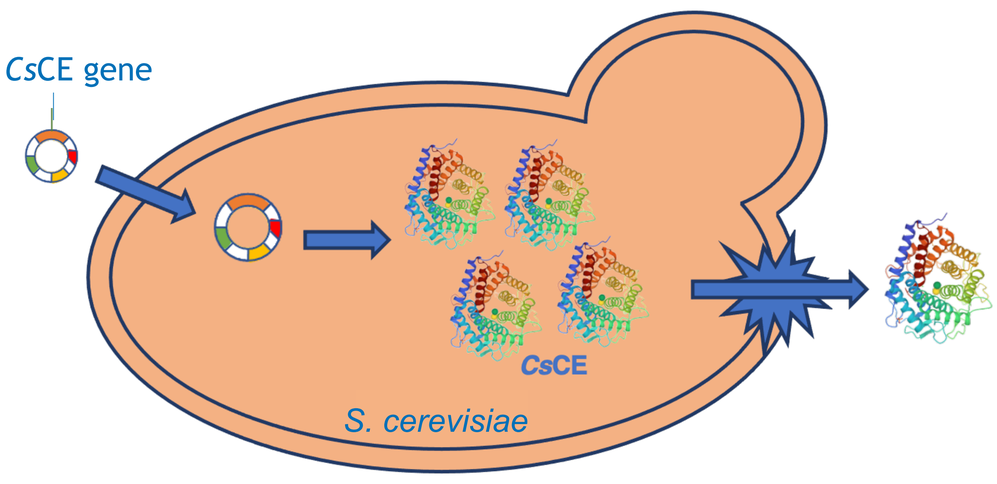
Introduction

Prebiotics are defined as 'substrates that are utilized by host microorganisms conferring a health benefit' and they have been incorporated in a wide variety of food products [1]. One of the most well-recognized prebiotics is lactulose. Recently, the production of lactulose through lactose isomerization catalyzed by cellobiose 2-epimerase (CE) was reported [2]. This strategy is gaining attention as a preferable methodology for industrial application due to its notable yields. Using lactose as a single substrate, CE can, under optimized conditions, lead to the formation of lactulose and epilactose [2]. Epilactose is a rare functional sugar that was shown to promote the proliferation of beneficial microorganisms, revealing its potential prebiotic effect [3]. *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 manipulation 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 and epilactose.

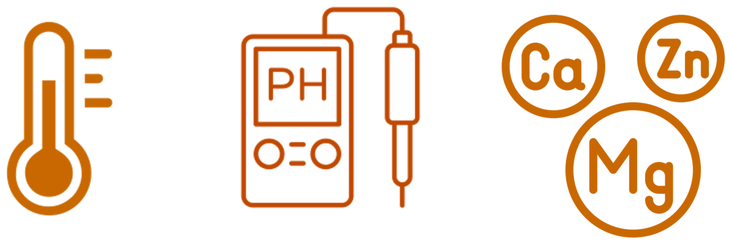
Aim: Production of prebiotics using a *S. cerevisiae* biocatalyst

Experimental

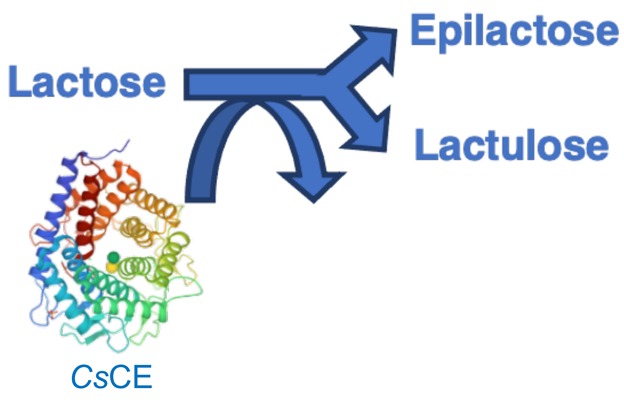
Strain construction



Enzyme characterization



Prebiotic production

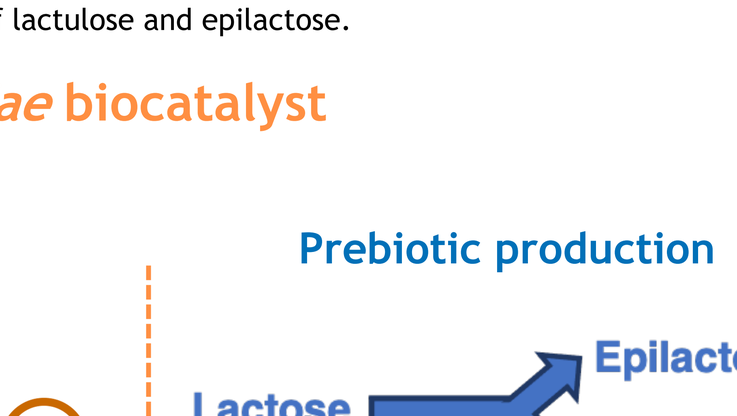


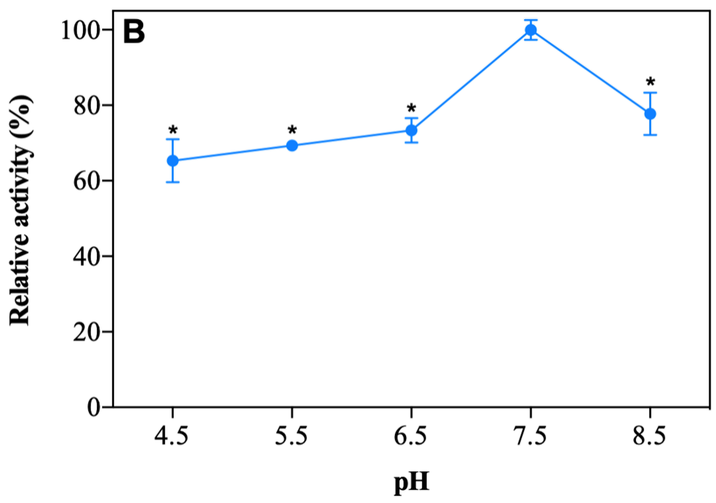
Results

Selection of the Most Promising *S. cerevisiae* Strain

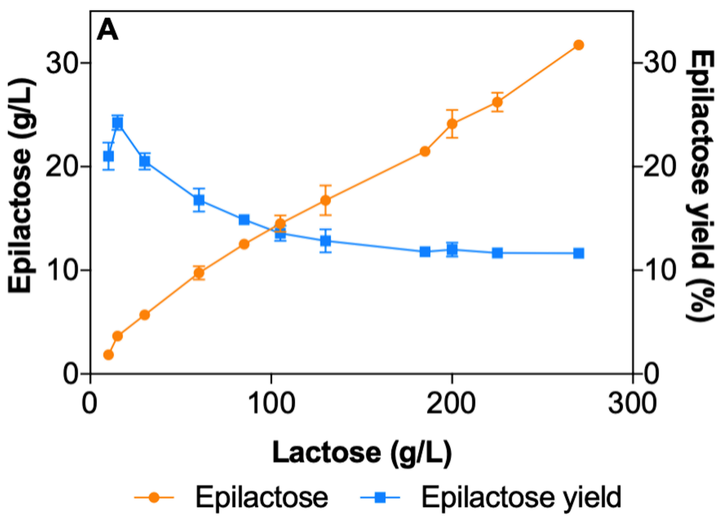
S. cerevisiae strains	plasmids		
	pSP-GM1_CsCE	p426TEF_CsCE	p426GAP_CsCE
BY4741	24.43 ± 1.34^{a,c}	22.66 ± 1.39^{a}	**34.47 ± 0.30^{b}**
CEN.PK2-1C	26.63 ± 1.22^{c}	27.42 ± 0.98^{c}	27.26 ± 2.34^{c}

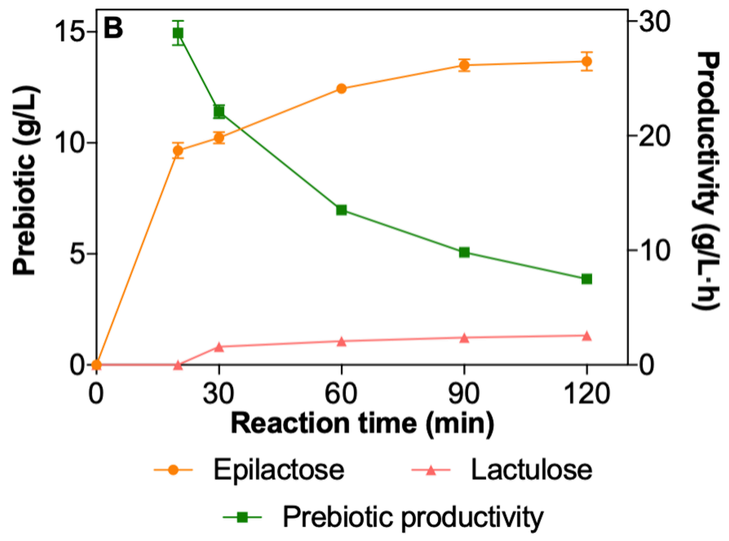
Characterization of Cellobiose 2-epimerase

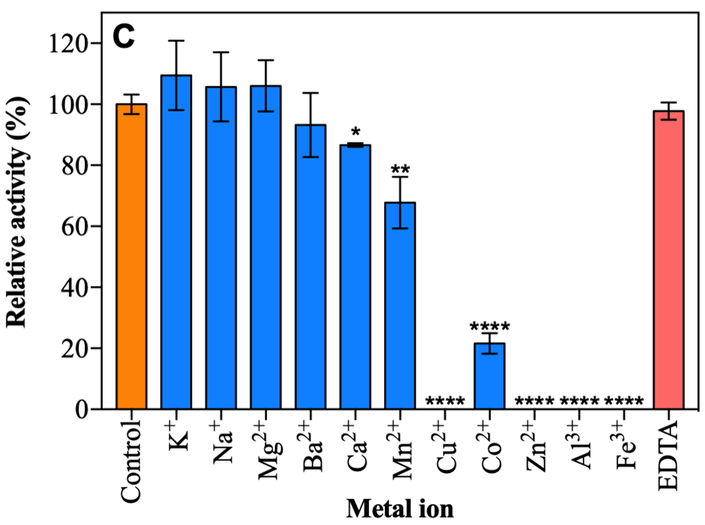




Production of prebiotics







Conclusions

S. cerevisiae was proven, for the first time, to be a suitable host to produce *Cs*CE towards further applications in prebiotics production. The maximum epilactose production (13.5 g/L) was achieved after 90 min, representing a yield of 27%. This is the highest yield reported so far using *Cs*CE and a GRAS microorganism as enzyme producer. Less than 3% yield was obtained for lactulose, which suggests that this *Cs*CE is more suitable to produce epilactose-enriched prebiotic mixtures. Despite the promising results, longer enzymatic reactions using lower temperatures and purified enzyme should be evaluated in the future towards an improved prebiotic production.

References

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2. Y. Kim, D. Oh, Bioresource Technology, 104 (2012)
3. Mu, W. Mu, Q. Li, C. Fan, C. Zhou, B. Jiang, Applied Microbiology Biotechnology (2013)

B.B.C. acknowledges her doctoral grant (SFRH/BD/132324/2017) from the Portuguese Foundation of Science and Technology (FCT). This study was supported by FCT under the scope of the strategic funding of the UID/BIO/ 04469/2020 unit and Project LIGNOZYMES (POCI-01- 0145-FEDER-029773).

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