DOI: 10.20396/revpibic2720192901

XXVII Congresso de Iniciação Científica Unicamp
16 a 18 de outubro de 2019 - Campinas | Brasil

Selection of wild-type S. cerevisiae strains tolerant to the presence of n-butanol from evolutionary engineering

Heitor P. Inoue*, Suéllen P. H. Azambuja, Rosana Goldbeck

Abstract

Butanol is a biofuel considered to be the best substitute for gasoline when compared to ethanol. The production of n-butanol from S. cerevisiae is still a challenge due to the low final concentration produced and low tolerance to this alcohol. The objective of this project was to select wild-type strains of S. cerevisiae tolerant to the presence of n-butanol, to carry out evolutionary engineering tests, aiming to increase the tolerance to n-butanol, and to characterize the selected colonies. Two strains were studied, and colonies more tolerant to n-butanol were obtained through evolutionary engineering.

Key words: *S.cerevisiae, n-butanol, tolerance.*

Introduction

With the increasing environmental concern caused by the massive exploitation of oil and the constant dependence of its derivatives[1], the growing awareness of these problems has increased the production of fuels from renewable sources[2]. Thus biofuels generated through the fermentation process has been a propitious choice for this substitution. Among biofuels, *n*-butanol stands out because it presents characteristics similar to gasoline, and can be produced from the yeast *S. cerevisiae*. However, this yeast is not yet able to tolerate high concentrations of butanol in the medium. Therefore, the objective of the project was to select wild-type strains of *S. cerevisiae* tolerant to the presence of *n*-butanol, to perform evolutionary engineering tests, aiming to increase the tolerance to *n*-butanol, and to characterize the selected colonies.

Results and Discussion

The strains studied in this project were *S. cerevisiae* CAT-1 and X2180-1B. The strains were submitted to successive passages in Verduyn[3] culture medium with the presence of 1% *n*-butanol at 30 °C and 200 rpm, at intervals of 24 h until reaching the hundredth generation (*n100*). In order to select the colonies that evolved a series of experiments were carried out as number of pixels (Fig. 1), maximum specific growth rate (μ_{max}, Fig. 2), and growth kinetics (Fig. 3).

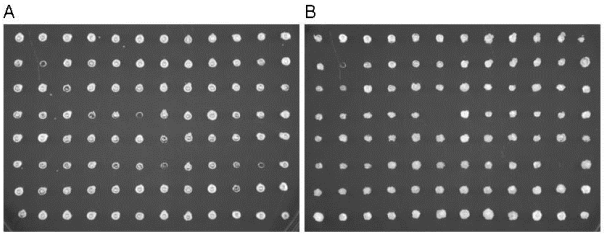

Figure 1. Colonies cultured in Petri dish containing Verduyn 1% *n*-butanol medium at 30 °C for 48 h. A) CAT-1_*n100* B) X2180-1B_*n100*

The evaluation of the growth through the number of pixels (Fig. 1) provided the evaluation of 87 colonies of each strain at the same time. From this, the 5 best colonies were selected and evaluated for growth in 96 well plates. From the results (Fig. 2), it was possible to observe that the isolated colonies were able to grow with μ_{max} larger than wild-type.

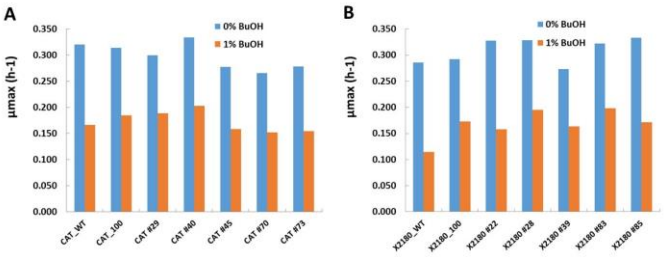

Figure 2. Maximum specific growth rate (μ_{max}) of the evolved colonies. A) CAT-1. B) X2180-1B

In this way, the 3 colonies with the highest growth rate were selected to evaluate their growth profile in shaker flasks (Fig 3.).

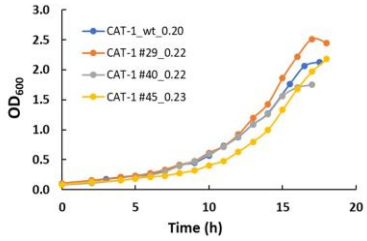

Figure 3. Growth kinetics of selected colonies. Legend: Strain #colonie_ μ_{max}

In the kinetics of the CAT-1 strain it was observed that the colonies showed even greater μ_{max} than the parental strain and that they were still able to reach a higher final OD_{600}. Characterization of X2180-1B strain has not yet been performed.

Conclusions

After the strains were submitted to the evolutionary engineering process in 1% of *n*-butanol, it was possible to identify and isolate colonies that possibly underwent the evolution process. From the obtained results, it was observed that possibly the strains have undergone an evolution and are more tolerant to the presence of *n*-butanol in the medium.

Acknowledgement

The authors are grateful to the Scientific and Technological Initiation Programs (PIBIC) of UNICAMP for the scholarship.

[1]CHEN, C. T.; LIAO, J. C. *FEMS Microbiology Letters*, **2016,** v. 363, n. 5, p. 1–13.
[2]STEEN, E. J. et al. *Microbial Cell Factories*, **2008,** v. 7, n. 1, p. 36.
[3]VERDUYN, C. et al. *Yeast*, **1992,** v. 8, n. 7, p. 501–517.