Evolutionary engineering as strategy to improve xylose utilization by *Saccharomyces cerevisiae*

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Background

The increasing demand for sustainable energy drives the development of biotechnological strategies for the production of fuels and chemicals from renewable resources, and the development of biorefineries (PAES; ALMEIDA, 2014). In this context, lignocellulosic biomass is an essential feedstock for the production of second generation biofuels, especially being considered for bioethanol production (INTERNATIONAL ENERGY AGENCY, 2015). Biomass utilization is especially advantageous because it does not compete with food crops and does not require the increase of farmlands. Biomass is mainly composed of three parts: cellulose, hemicellulose and lignin. The first two components are formed by polymers of pentose and hexose sugars (SAHA, 2004) which can be converted by microorganisms into bioethanol. The yeast *S. cerevisiae*, the main organism utilized in the industrial production of bioethanol, is unable to use pentoses, such as xylose (GONG et al., 1983), which is the second most abundant sugar in some biomasses. Thus, several strategies have been employed to genetically engineer this yeast for xylose utilization. In this work, new *S. cerevisiae* strains able to ferment xylose were obtained employing genetic and evolutionary engineering strategies. The strains obtained were characterized genetically and physiologically.

Methods

The *Piromyces sp.* xylose isomerase (XI) encoding gene was amplified by PCR using gene specific primers and the plasmid pRH218, which carries the xylA gene (HECTOR et al., 2011). The gene was cloned in the episomal plasmid p424 (MUMBERG et al., 1994), under the expression of GPD1 promoter. In addition, the endogenous gene coding for *S. cerevisiae* xilulokinase (XK) was cloned in the episomal plasmid p426 (MUMBERG et al., 1994). The laboratory strains of *S. cerevisiae* CEN.PK 113.14A *Δtrp1-289* (L2) and CEN.PK 113.3C *Δtrp1-289, Δura- 52* (L7) were transformed with the constructed plasmids. Thus, recombinant strains of *S. cerevisiae* expressing solely XI (L2XI), or in combination with XK (L7XIXK) were obtained. A third strain, L7XIΦ, expressing XI and carrying an empty plasmid

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was constructed as control. The obtained strains L2XI, L7XIΦ and L7XIXK underwent a conditioning process in selective medium with xylose as sole carbon source. The strains obtained after conditioning processes were then submitted to a curing process, in order to remove the plasmids initially introduced in rich medium supplemented with tryptophan. After curing, the strains were once again transformed with the same plasmids in order to investigate if changes observed in the conditioned strains were conserved or disappeared as a result of the removal of the episomal plasmids. Physiological characterization of the obtained strains was performed in minimal medium supplemented with xylose in flasks and in bioreactor fermentations.

Results and Conclusions

Three recombinant strains of *S. cerevisiae* expressing solely XI from *Piromyces sp.* (L2X and L7XIΦ) or in combination with endogenous XK (L7XIXK) from *S. cerevisiae* were obtained by genetic engineering. Among these three, the L7XIXK strain presented better fermentative rates of xylose aerobically than the other two, confirming the positive effect of XK overexpression. To improve fermentation capability of L2, L7XIΦ and L7XIXK strains, they were submitted to a conditioning process on minimal medium containing xylose. After conditioning, single adapted isolates were obtained and compared with the original strains. Results clearly demonstrated that the adapted strains presented shorter lag growth phase, increased growth rate, xylose consumption (1,8 to 18,5 fold) and ethanol yield (47% for L7XIXK). The performance of the strains was also investigated under anaerobic conditions, which showed significant improve in xylose fermentation for conditioned strains. These conditioned strains were submitted to the curing process for the plasmid removal. The strain LC7 was obtained from L7XIXK curing process and its incapability to grow in minimal medium supplemented with xylose or glucose was confirmed. This result is consistent with the removal of plasmids from the cytoplasm. However, still the XylA gene was identified through PCR when using yeast total DNA as template. The retransformation of this curated yeast with the originally constructed plasmids showed hat the pattern of improvements observed in the conditioned strains was conserved after re-transformation. The LC7 strain has a great potential for the development of a screening strain for new enzymes of the xylose catabolic pathway.

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References

GONG, C. S.; CLAYPOOL, T. A.; MCCRACKEN, L. D.; MAUN, C. M.; UENG, P. P.; TSAO, G. T. Conversion of pentoses by yeasts. **Biotechnology and bioengineering**, New York, v. 25, n. 1, p. 85-102, 1983.

HECTOR, R. E.; MERTENS, J. A.; BOWMAN, M. J.; NICHOLS, N. N.; COTTA, M. A.; HUGHES, S. R. *Saccharomyces cerevisiae* engineered for xylose metabolism requires gluconeogenesis and the oxidative branch of the pentose phosphate pathway for aerobic xylose assimilation. **Yeast**, Hoboken, v. 28, n. 9, p. 645-660, 2011.

INTERNATIONAL ENERGY AGENCY. **IEA Bioenergy Task 39:** commercializing liquid biofuels. Disponível em: <http://task39.org/about/definitions/>. Acesso em: 6 mar.2015.

MUMBERG, D.; MULIER, R.; FUNK, M. Regulatable promoters of *Saccharomyces cerevisiae*: comparison of transcriptional activity and their use for heterologous expression. **Nucleic Acids Research**, Oxford, v. 22, n. 25, p. 5767–5768, 1994.

PAES, B. G.; ALMEIDA, J. R. M. de. Genetic improvement of microorganisms for applications in biorefineries. **Chemical and Biological Technologies in Agriculture**, artigo 21, 2014.

SAHA, B. C. **Lignocellulose biodegradation and applications in biotechnology structure and composition of lignocellulosic biomass**. Washington, DC: American Chemical Society, 2004. (ACS Symposium Serie).