**Evolutionary engineering for development of a novel *S. cerevisiae* screening strain**

Bárbara G. Paes1,2 and João R. M. Almeida1,2

1Embrapa Agroenergy, Brasília, Brazil

2University of Brasilia, Brasília, Brazil

[joao.almeida@embrapa.br](mailto:joao.almeida@embrapa.br)

Growing interest in biofuel production has prompted engineering of *Saccharomyces cerevisiae* strains for the use of xylose, the second most abundant sugar in lignocellulose. In this work, three *S. cerevisiae* strains expressing *xylA* (encoding xylose isomerase - XI) from *Piromyces sp.* either alone or in combination with endogenous *XKS* (encoding xylulokinase -XK) were constructed using different episomal plasmids. The constructed strains underwent an adaptation process in minimal medium containing xylose as sole carbon source, which resulted in three adapted strains, with increased growth rate and shortened lag phase on xylose. In addition, xylose consumption and ethanol yield of improved strains increased up to 1758% and 47% respectively, whereas xylitol yield decreased up to 91.8%. Investigations of improved strains showed that no mutation occurred in xylA, suggesting that mutations probably occurred in the yeast’s genome. In addition, the adapted strains were cured of all plasmids and a new strain named *S. cerevisiae* LC7 was obtained. The LC7 strain lost the episomal plasmid, and consequently the ability to metabolize xylose. Absence of xylA on the yeast genome was also confirmed by PCR. When LC7 was re-transformed with plasmids containing XI and XK, its fermentation performance was similar to the adapted strain from which LC7 was derived, corroborating the hypothesis that mutations responsible for the adaptation occurred in the yeast genome. The new *S. cerevisiae* strain is being further characterized and employed in new studies on xylose metabolism.