ISOLATION OF XYLOSE ISOMERASES BY METAGENOMIC SCREENING TARGETING SACCHAROMYCES CEREVISIAE EXPRESSION FOR SECOND-GENERATION ETHANOL PRODUCTION

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The depletion of fossil fuels supplies and the continued use of these power sources pose a threat to the global economy and environment, therefore, the adoption of renewable resources to generate energy stands as a sustainable alternative. Many efforts have been concentrated on the production of second-generation ethanol (2G), derived from lignocellulosic feedstock, a largely unused source of carbohydrates. One of the main problems concerning the production of 2G ethanol is that the commonly used strains of the yeast Saccharomyces cerevisiae are unable to ferment xylose, the most abundant pentose in biomass. The bacterial enzyme xylose isomerase (XI) can bypass the metabolic feature responsible for this low ethanol production from xylose in yeast, but the expression of different orthologs in S. cerevisiae has been mostly unsuccessful. Our goal in this work was to identify new potentially functional XI genes. We selected xylose-consuming bacteria from an environmental sample of rotting sugarcane bagasse by cultivating them in a xylose-containing medium. Then next-generation sequencing technology was used to generate a metagenomic library. After an in silico analysis we identified the XI orthologs present in the library by searching for known sequence features. The genes were then amplified from the metagenomic DNA and cloned into yeast expression vectors. Currently their expression is being evaluated in industrial S. cerevisiae strains growing in xylose-rich medium. The metagenomics approach proved to be an efficient strategy in the pursuit of new gene sequences as we identified 16 distinct sequences of XI, most of them new.

We acknowledge the National Council for Scientific and Technological Development (CNPq) and BioCelere Agroindustrial for their financial support.

Key Words: Saccharomyces cerevisiae, second-generation ethanol, xylose isomerase