Development of an acetic acid tolerant Spathaspora passalidarum strain through evolutionary engineering with resistance to inhibitors compounds of autohydrolysate of Eucalyptus globulus

Evolutionary engineering strategy based on mutagenesis by UV irradiation and subsequent selection by continuous cultivation at increasing concentrations of acetic acid in synthetic medium with glucose and xylose mixtures was used to develop an evolved strain of the yeast Spathaspora passalidarum with improved resistance to acetic acid. After 380 generations, the yeast was able to produce 5.8 g/L ethanol in the presence of 3.5 g/L acetic acid in synthetic medium with mixture of 15 g L\(^{-1}\) glucose and 15 g L\(^{-1}\) xylose. To demonstrate the improved resistance to acetic acid of the evolved strain compared to the native strain, growth kinetics and bioethanol production of both strains in batch cultures under microaerobic condition were performed. The evolved strain reached an ethanol volumetric productivity of 0.23 g/L and ethanol yield of 0.48 g/g in the presence of 4.5 g/L acetic acid. These results were 7-fold and 2-fold higher than those obtained with the native strain, respectively. Inhibitors composition present in Euca\textit{lyptus} globulus autohydrolysate were (g L\(^{-1}\)): acetic acid, 4.7; furfural, 1.0; HMF, 0.36; formic acid, 0.6; syringaldehyde, 0.14; and vanillin, 0.017. When Euca\textit{lyptus} globulus autohydrolysate was used as culture medium, the evolved strain of \textit{S. passalidarum} showed complete consumption of glucose and cellobiose, and 56% of xylose. In contrast, the wild type strain was unable to completely consume any of these sugars and showed a lag phase of 46 h. In brief, evolutionary engineered strain of \textit{S. passalidarum} presented an improved resistance to inhibitors usually found in \textit{Euca\textit{lyptus} globulus} autohydrolysate and was able to co-ferment glucose, xylose and cellobiose under microaerobic condition without lag phase.
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