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INVESTIGATING D-XYLOSE SYMPORTERS FROM YEAST-LIKE *Aureobasidium leucospermi*

With the strong trend towards sustainable technologies, such as the gradual substitution of fossil fuel consumption, improvement in the utility of lignocellulosic sugars appears to be an alternative for bioenergy. However, from the large number of C_5 sugars, few are used in fermentative processes. This is mostly due to the inability of wild-type *S. cerevisiae* to efficiently co-utilize hexoses and pentoses via specific transporters for each type of sugar. Thus, a system of pentose uptake that is not modulated by D-glucose is required. Lignocellulosic material has been characterized as a renewable source of sugar capable of being bioconverted in byproducts, such as ethanol, xylitol, arabitol, and carboxylic acids. The use of this raw material indicates an opportunity to add value to agricultural waste, which does not compete against food supplies. The lignocellulosic material is capable of providing sugars, especially D-glucose, D-xylose, and L-arabinose. We investigate pentose transporters from *A. leucospermi* strain isolated from fruit samples, cultivated in D-xylose as sole sugar source (YPX 2% D-xylose), initial pH 4.5 and 6.5. H+/pentose symport activity was investigated in *A. leucospermi* cells collected at different growth stages (12, 24, 48 and 72 h). Kinetic studies were performed according to external alkalinisation of unbuffered cell suspensions after the addition of a pentose pulse at different concentrations of sugar to a concentrated and unbuffered suspension of cells. It was possible to determine the kinetic parameters K_M and V_{MAX} for the transport of D-xylose. We were able to identify a D-xylose symporter system, where uptake of D-glucose was not detected. The best D-xylose uptake route exhibited a K_M of 7.9 mM and V_{MAX} of 0.12 mmol $h^{-1}g^{-1}$ at 48 h, and K_M of 35 mM and V_{MAX} of 0.07 mmol $h^{-1}g^{-1}$ at 72 h. A 100% uptake of D-xylose was reached at 72 h in YPX medium, initial pH 4.5. In general, an alkaline medium limited the expression of symporters. Searching for natural pentose-utilizing species has been the main strategy in the identification of L- D-xylose transporters. However, the prospection of pentose transporter from *A. leucospermi* has not yet been reported. This reinforces the scientific merit of our investigation. The results obtained in this study will help in the further investigation of these symporters through their overexpression in engineered *S. cerevisiae*.

Keywords: Bioethanol, D-xylose, pentose, symporter

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