Many efforts have been made to hydrolyse the hemicellulosic material and to add value to biorefineries through the production of additional compound e.g. organic acids. To date, the fermentative yeast *Saccharomyces cerevisiae*, even modified for the assimilation of pentoses, has not presented satisfactory yield for ethanol production. Consequently, the search for pentose transporters from non-*Saccharomyces* yeasts for heterologous expression in *S. cerevisiae* has been recurrent. Thus, we investigate pentose transporters from a *Rhodotorula* sp. strain isolated from fruit samples. The strain was identified using sequences of the D1/D2 domains of the rDNA, maintained in 15% glycerol and stored at −80 °C. Posteriorly, the *Rhodotorula* sp. strain was cultivated in D-xylose (YPX 2% D-xylose) or D-glucose (YPD 2% D-glucose) as sole sugar source, initial pH 4.5 and 6.5. The consumption of D-xylose and D-glucose during fermentation was monitored, and the presence of H⁺ symporters for L-arabinose, D-xylose, and D-glucose was evaluated at different time-points during growth: 24 and 48 h. For transport kinetics, the influx of protons/sugar was calculated based on the rate of extracellular alkalization after the addition of a pulse at different concentrations of sugar to a concentrated and unbuffered suspension of cells. According to the results, kinetic characterization of pentose transport revealed that the highest affinity D-xylose symport was detected in cell cultivated in YPX media, pH 4.5 with $K_M = 15.7$ mM and $V_{Max} = 0.34$ mMol.h⁻¹.g⁻¹ (24 h), and $K_M = 7.7$ mM and $V_{Max} = 0.22$ mMol.h⁻¹.g⁻¹ (48 h).

As for L-arabinose, the highest affinity for H+/arabinose symporter was observed at 48 h with $K_M = 862$ mM and $V_{Max} = 1.28$ mMol.h⁻¹.g⁻¹. These transporters can be potentially used to overcome transport limitations imposed on the yields of metabolic pentose pathways and open an avenue for enabling *S. cerevisiae* and other yeasts for the better use of plant biomass.

**Keywords:** Fermentation, pentose, symporter, yeast

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