Regulation of lactose transport in *Kluyveromyces lactis* JA6

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*Kluyveromyces lactis* is able to ferment lactose and it is considered as an alternative model for biochemical, physiological and genetic studies in non-*Saccharomyces* yeasts. Understanding the mechanisms of lactose uptake and the regulation involved in the transport is an important step towards better biotechnological applications. The transport of lactose in *K. lactis* is mediated by a membrane permease encoded by the LAC12 gene. In this study we used the strain *K. lactis* JA6. Cells were harvested in mid-exponential growth phase, washed with cold distilled water, resuspended in 100 mM Tris/citrate buffer, pH 5.0 for [D-Glucose-1-14] lactose transport measurements. The kinetics of the initial rate of uptake [D-Glucose-1-14] lactose was investigated in the range of concentration of 0.025 to 10 mM. Galactose was tested as competitive inhibitor of lactose transport. To study the effect of glucose on lactose transport, cells were grown on lactose and divided in two aliquots (control and 100 mM glucose added). Cells were harvested, washed and the uptake was measured. The initial uptake rate of [D-Glucose-1-14] lactose was also investigated in cells grown on glucose and transferred to lactose in the presence/absence of 100 µM cycloheximide. The results indicate that the transport of lactose into cells grown on 2% lactose follows the kinetics of saturation. The half saturation constant (Ks) was 1.49 ± 0.38 mM and the maximum velocity (Vmax) was 953.47 ± 116.24 µmoles.h⁻¹.g⁻¹. The same transport system was present in cells grown on galactose and glycerol. Cells grown on 2% (w/v) glucose transport lactose at very low rates. However, when the cells grown on glucose were transferred to 2% (w/v) lactose, the transport exhibited a partial recovery. In addition, the lactose transport is not inactivated by glucose. These data allow us to infer that the lactose transport system in *Kluyveromyces lactis* JA6 is constitutive, and that glucose exerts a control not so clear in the lactose transport. From experiments with Lac12-GFPp, we conclude that glucose control involves transcriptional and posttranscriptional steps. In this strain, galactose in higher concentration is transported by LAC12p and based on the cell growth we hypothesize the existence of other galactose transport system.

Keywords: Kluyveromyces, lactose, transport

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