Molecular and biochemical characterization of glucose transport in *Torulaspora delbrueckii*

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Most of yeast biotechnological applications rely on their ability to efficiently ferment a great variety of sugars. This property is closely related to their sugar transport capacity, which has been widely considered a rate-limiting step of sugar metabolism.

In Saccharomyces cerevisiae, 34 genes encoding either putative or genuine transporters, the largest family of the major facilitator superfamily (MFS), have been identified (Niedner et al., 1997). *Torulaspora delbrueckii*, one of the yeast species most frequently found in homemade corn and rye bread dough (Almada and Pais, 1996), has been recognized as the most promising alternative to industrial strains of *S. cerevisiae*. Indeed, *T. delbrueckii* strains display flours/bran tolerance (Almada and Pais, 1996) and an exceptional resistance to osmotic and NaCl injury (Hernandez-Lopez et al., 2003). Nevertheless, there is a lack of knowledge on the physiology and molecular biology of this organism, an in-depth investigation being required to gain insights into the function and regulation of *T. delbrueckii* sugar transporters.

**Introduction**

Cloning of genes involved in glucose transport

A generic library of *T. delbrueckii* PYCC 5521 (Hernandez-Lopez et al., 2002) constructed into the vector pYES2/HisA was transformed into the *S. cerevisiae* strain ERVYF049 (Sac的主题, 1999), which is defective in glucose transport. Two transformants recovered from the selected transformant presented different restriction patterns. A representative of each group (referred to as YEpT1 and YEpT2) was used to reintroduce the *S. cerevisiae* mutant strain, confirming that all of them were able to restore the ability to grow on glucose as sole carbon source and maltose/gluconic transport, with Kd values in the range of 12-25 mM (Fig. 1).

**Characterization of L6T1 gene and of putative L6T1p**

L6T1 displays a high homology to other yeast glucose transporters (Fig. 2).

An analysis of the L6T1 amino acid sequences (550 bp upstream from the ATG) showed the presence of several Mig1- and Mig2p-binding sequences and of 4 potential TATA boxes at positions 117, 264, 480, and 675 from the ATG codon.

Some consensus Ahc1p sequences were found in the L6T1 5′-untranslated region (positions 1827 to 1832, and 1953 to 1958), and some initiator stop codons were present after the TGA codon.

The ORF codifies a potential 575-amino acid protein with the typical structure of a transport protein which has been predicted to contain 11 transmembrane domains. L6T1p amino acid sequence also shared the presence of 1 PHSR motif. L6T1p transport activity was determined in *L. elongatissima* (Hofstee plot).

**Induction of L6T1 expression**

Expression of L6T1 in *S. cerevisiae* was high in media containing 5% of glucose and almost undetected in galactose or raffinose media (Table I).

In the absence of glucose, expression of L6T1 repression, required the transcription factor Mig2p. However, a functional Mig2p does not appear to be required for a full induction of L6T1 at high glucose levels.

Deletion of the gene coding for the general repressor Mig2p had no effect on L6T1 expression, but additional disruption of SEG2, a mig2p target indicated that Mig2p both Mig2p and Mig3p is a repressor, as repressor of L6T1 expression at high glucose concentrations.

**Southern blot analysis**

Southern blot analysis revealed the presence of several genes with high homology to L6T1, *T. delbrueckii* genome (Fig. 3). These results are according to the kinetics of glucose transport showed by *T. delbrueckii*. These evidences suggest that like has been described for other yeasts *T. delbrueckii* contains several sugar transporters.

**Identification of L6T1 gene**

**Glucose transport in the L6T1 transformant of *S. cerevisiae* null strain versus *T. delbrueckii* PYCC 5521**

Glucose-grown cells of *T. delbrueckii* PYCC 5521 showed kinetics of glucose transport best fitted assuming a biphasic kinetics with a low and a high-affinity component (Fig. 4). A biphasic kinetics of glucose transport was also observed for fructose and maltose-grown cells (Table II).

Cells of *S. cerevisiae* null strain transformed with the L6T1 gene exhibited glucose transport in the range of the low-affinity component. L6T1 is also able to mediate significant fructose transport in the high-affinity component (Fig. 5).

In *T. delbrueckii* both the low and high-affinity components of the glucose transport were competitively inhibited by fructose and maltose. In *S. cerevisiae* cells transformed with the L6T1 gene the presence of fructose or maltose abolished the zero-affinity of glucose with fructose of competitive inhibition (Fig. 6). However, previous results showed that no measurable transport of fructose and maltose was detected in glucose-grown cells of *T. delbrueckii* (Alves-Araújo et al., 2004). The results obtained could be interpreted as the consequence of the binding of one glucose molecule to maltose residues of the extracellular binding site of the glucose transporters, impeding glucose transport, as previously suggested for *S. cerevisiae* and *T. bayanus*.

**Acknowledgments**

This work was supported by a POCI grant (BQ/61332/2004) from Fondo de Inversiones a Grupos e Inst. de Trabajo, Programa de Excelencia, FADU (Grant BQ/61332/2004) from Fundación para la Ciencia y la Tecnología, Portugal.

**References**

