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Exploring the Glucose Transport System of *Torulaspora delbrueckii*: Genomic and Functional Characterization

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INTRODUCTION & AIM

The **efficiency of transport of sugars** into the yeast cell is often considered a **rate-limiting step** in sugar metabolism [1]. While the **mechanisms underlying sugar uptake and the transporter system** are well characterized in *Saccharomyces cerevisiae*, they **remain poorly understood in non-conventional yeasts such as *Torulaspora delbrueckii***. Given the **increasing utilization of *T. delbrueckii* in bread and wine fermentations**, due to its contributions to the final product flavour and aroma, as well as its osmo- and cryotolerance [2], it is **essential to characterize its sugar transporters and explore strategies to optimize its performance** in these fermentative processes. In this context, we aim to characterize the glucose transporters of *T. delbrueckii* and determine their kinetic properties.

METHODS

Genome Analysis



1. BLAST search in the 41 strains genomes, using the sequence of the already characterized gene *LGT1* as query.
2. Assembly of the genome of the 41 *T. delbrueckii* strains in the CBMA collection.
3. Mapping of the previously identified genes onto the assembled genomes to determine their chromosomal locations.

Gene Expression Analysis



1. Fermentation in YPD medium for 24h with sample collection at 1h, 7h, 12h and 24h of growth.
2. RNA extraction, cDNA synthesis, for RT-qPCR analysis.

RESULTS & DISCUSSION

T. delbrueckii strains encode six or seven predicted *HXT*-family hexose transporters.

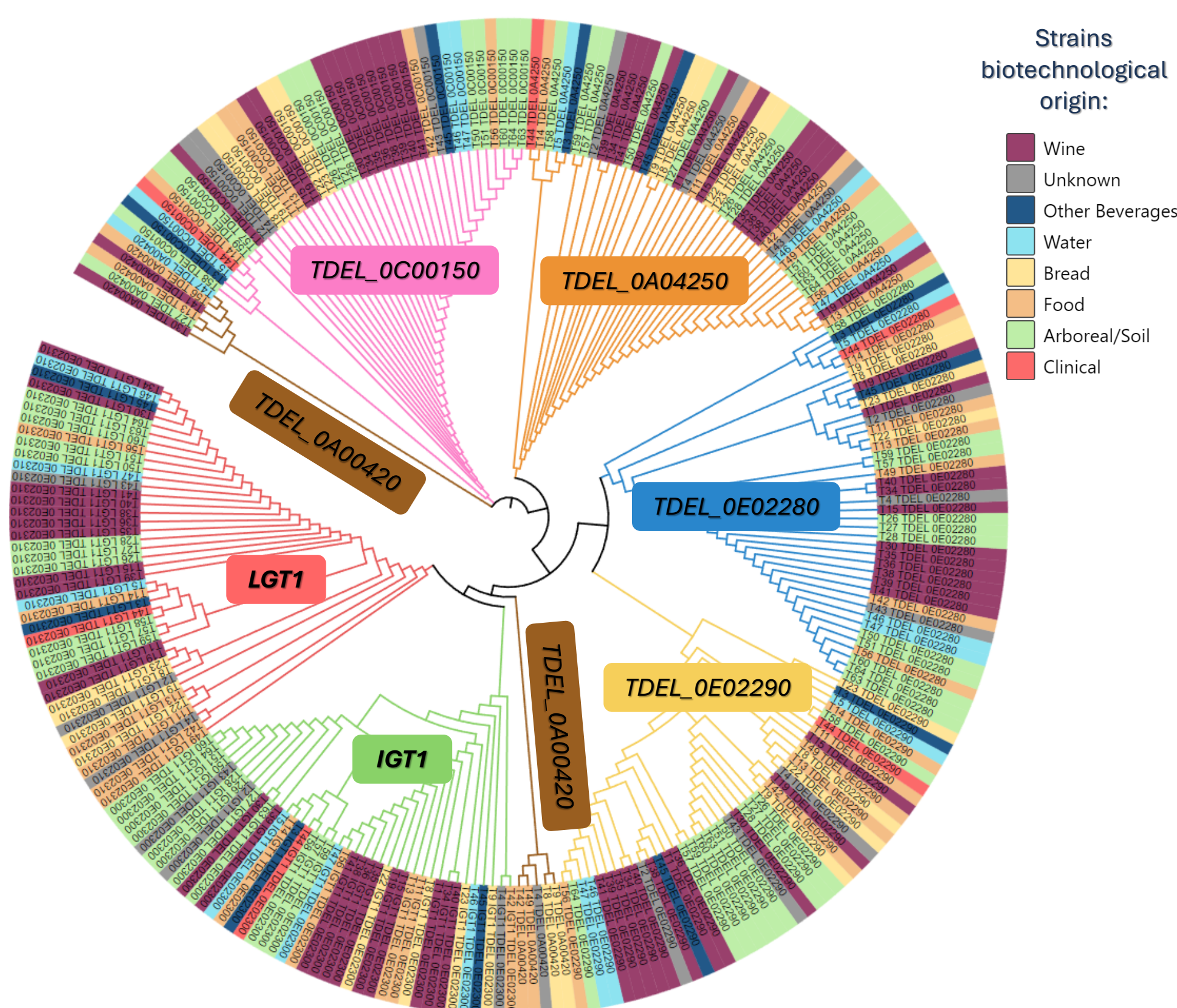


Figure 1. Genome analysis of 41 *T. delbrueckii* strains revealed that they encode six or seven putative hexose transporter genes. BLAST searches identified the transporters from the type strain (*T. delbrueckii* CBS 1146) with the highest similarity to each group: *LGT1* – TDEL_OE02310; *IGT1* – TDEL_OE02300; TDEL_OE02290; TDEL_OE02280; TDEL_OA04250; and TDEL_OC00150. The TDEL_OA00420 sequence was compared with the COFT1 strain instead of the type strain, as it appears to be located in the mitochondrial genome, which was not properly annotated in CBS 1146.

The four most conserved *T. delbrueckii* transporters are located within the same gene cluster on chromosome 5.

Chromosome 5



Figure 2. Schematic representation of the genomic locations of the four most conserved transporters among the 41 *T. delbrueckii* strains. These transporters are organized in a conserved cluster of four genes with identical arrangement on chromosome 5 in all strains.

Expression analysis of the four most conserved hexose transporters genes in *T. delbrueckii* PYCC 5321

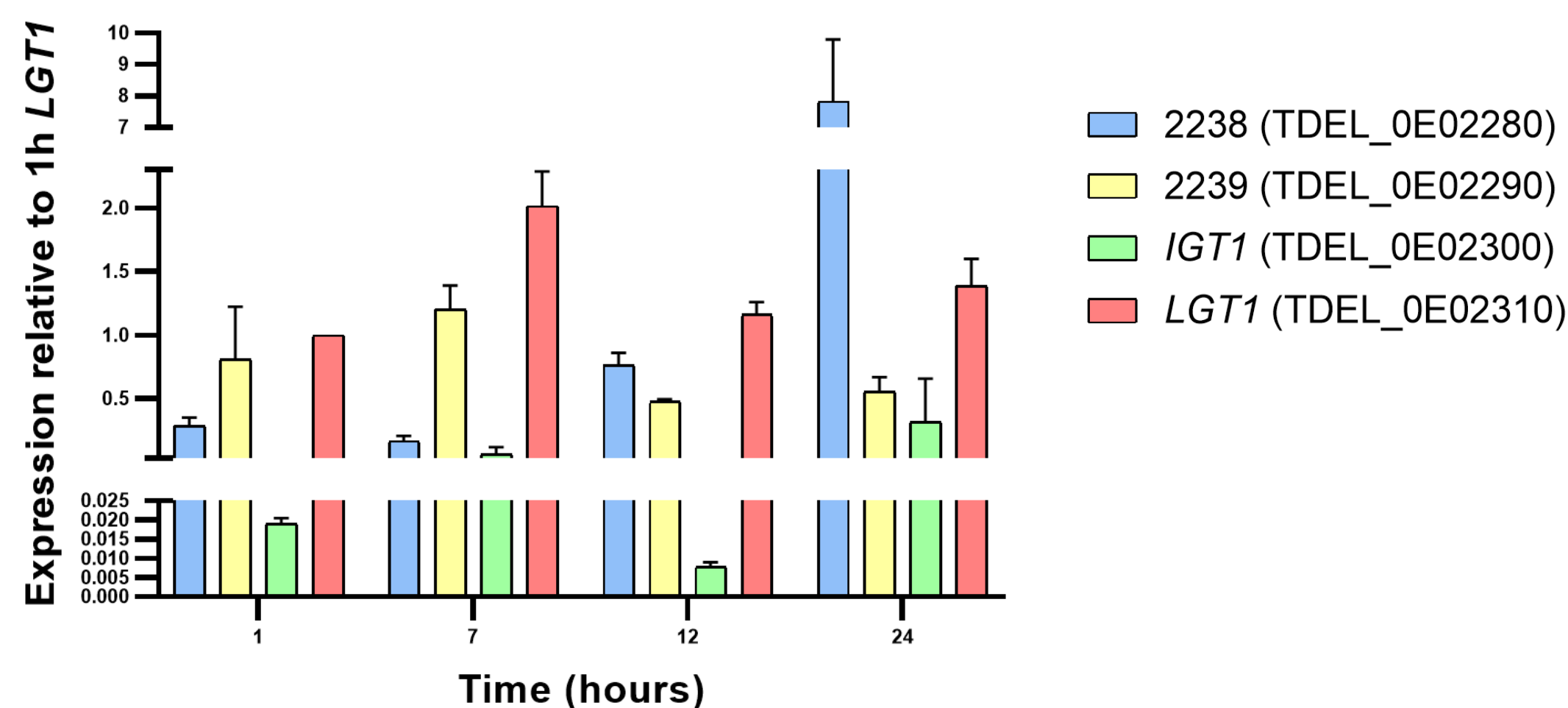


Figure 3. Expression of *LGT1*, *IGT1*, 2238 (*TDEL_OE02280*), and 2239 (*TDEL_OE02290*), the most conserved transporters in *T. delbrueckii* PYCC 5321, after 1, 7, 12 and 24 hours of growth.

CONCLUSIONS

Genome analysis of 41 *T. delbrueckii* strains revealed the **presence of six/seven putative hexose transporter genes**, with **no apparent relationship between gene number and strain origin**. The **four most conserved transporter genes** are organized in a **cluster on chromosome 5**. Among these, ***LGT1* is the most expressed** during the first 12 hours of growth and remains stable thereafter. ***TDEL_OE02280* expression increases progressively**, showing a **marked rise at 24 hours**, while ***TDEL_OE02290* remains stable** but consistently lower than *LGT1*. ***IGT1* has the lowest expression during fermentation** but shows a slight increase at 24 hours.

REFERENCES

- [1] Pacheco, A. et al. "Hexose transport in *Torulaspora delbrueckii*: identification of *Igt1*, a new dual-affinity transporter." *FEMS yeast research* vol. 20,1 (2020): foaa004. doi:10.1093/femsyr/foaa004. [2] Fernandes, T. et al. "Biotechnological Importance of *Torulaspora delbrueckii*: From the Obscurity to the Spotlight." *Journal of fungi (Basel, Switzerland)* vol. 7,9 712. 30 Aug. 2021. doi:10.3390/jof7090712. Bruna Oliveira thanks FCT for the fellowship 2023.00306.BD