Additional file 3.



VST Counts

0 5 10 15 20 25

Additional file 3. Phylogenetic tree of putative sugar transporters from C. intermedia and other pentose-assimilating yeasts. Putative sugar transporters in the C. intermedia CBS 141442 genome were tracked using protein sequences from characterized yeast transporters (S. cerevisiae Hxt1 and Stl1; S. stipitis Sut1 and Xut1; C. intermedia Gxs1 and Gxf1) as probes. Additionally, transporters reported as capable of xylose uptake from other yeast species were added to the dataset. A total of 45 sequences were aligned, and then used to construct a ML phylogenetic tree using the maximum-likelihood method with bootstrap numbers indicated at each branching point. The accession numbers are listed in the table below. Our analysis revealed a tree with 6 distinct branches of transporters. Branches 1 and 2 contain five transporters from C. intermedia, some of them closely related to several of the Xut transporters from S. stipitis that are known to transport xylose [1-4]. The previously identified Gxf1 and Gxs1 and novel homologs Gxf1 2 and Gxs1 2 from C. intermedia fall into branch 3, together with Hxt1, 4 and 7 and Gal2 from S. cerevisiae and Sut1 from S. stipitis, all of which are previously shown to transport xylose but having a much higher affinity for glucose over xylose [3, 5]. Branch 5 contains five C. intermedia genes for transporters that show similarities to either the arabinose/xylose transporters in M. guilliermondii and K. marxianus [6] or the putative arabinose-proton symporter in S. stipitis [2], and branch 6 contains 13 C. intermedia genes where the corresponding proteins show homology to permeases selective for disaccharides such as lactose and cellobiose as well as the monosaccharide galactose [7]. Normalized expression of C. intermedia protein-encoding genes is represented in all growth conditions using variance-stabilized counts (VST). Proteins are preceded by a prefix identifying the yeast species. Sc - Saccharomyces cerevisiae; Ss - Scheffersomyces stipitis; Sp - Spathaspora passalidarum; Ci – Candida intermedia; Km – Kluyveromyces marxianus; Mg - Meyerozyma guilliermondii; Dh – Debaryomyces hansenii; Df - Debaryomyces fabryi.

included.		
Gene	Accession Number	Reference
Sc Stl1	NP_010825.3	
Sc Hxt1	NP_011962.1	Hamacher et al. 2002
Sc Hxt4	NP_011960.2	Hamacher et al. 2002
Sc Hxt7	NP_010629.3	Hamacher et al. 2002; Young et al. 2011
Sc Gal2	NP_013182.1	Hamacher et al. 2002; Young et al. 2011
Ss Xut1	XP_001385583.1	Young et al. 2011
Ss Xut2	XP_001387242.2	Du et al. 2010
Ss Xut3	XP_001387138.1	Young et al. 2011
Ss Xut4 (Hgt3)	XP_001386715.1	Ma et al. 2012; Moon et al. 2013
Ss Xut5	XP_001385962.2	Moon at al. 2013
Ss Xut6 (Stl12)	XP_001386589.1	Ma et al. 2012; Moon et al. 2013
Ss Xut7 (Stl13)	XP_001387067.1	Moon et al. 2013
Ss Sut1	XP_001387898.1	Katahira et al. 2008
Ss Rgt2	XP_001386588.1	Young et al. 2014
Dh Xylhp	XP_458169.1	Young et al. 2011; Ferreira et al. 2013
Df Xylhp	AAR06925.2	Ferreira et al. 2013
Mg 05196	XP_001482176.1	Wang et al. 2015
Mg Axt1	XP_001482096.1	Knoshaug et al. 2015
Km Axt1	XP_022674058.1	Knoshaug et al. 2015
Ci Hgt1	SGZ50992.1	
Ci Hgt1_2	SGZ51695.1	
Ci Hgt1_3	SGZ49593.1	
Ci GXF1	SGZ57542.1	Leandro et a. 2006; Young et al. 2011
Ci GXF1_2	SGZ47691.1	
Ci GXS1	SGZ53008.1	Leandro et a. 2006; Young et al. 2011
Ci GXS1_2	SGZ50173.1	
Ci Stl1	SGZ58446.1	
Ci Stl1_2	SGZ47012.1	
Ci Stl1_3	SGZ46314.1	
Ci Stl1_4	SGZ57759.1	
Ci Lac12	SGZ47224.1	
Ci Lac12_2	SGZ49661.1	
Ci Lac12_3	SGZ48118.1	

Accession numbers for *C. intermedia* CBS 141442 putative MFS sugar transporters and known xylose-transporters from other yeast species. References for reported xylose uptake activity are included.

Ci Lac12_4	SGZ57765.1	
Ci Lac12_5	SGZ55027.1	
Ci Lac12_6	SGZ54820.1	
Ci Lac12_7	SGZ51011.1	
Ci Lac12_8	SGZ47318.1	
Ci Lac12_9	SGZ57769.1	
Ci Lac12_10	SGZ54727.1	
Ci Lac12_11	SGZ55091.1	
Ci Lac12_12	SGZ51023.1	
Ci Lac12_13	SGZ47317.1	
Ci Ybr241c_2	SGZ47420.1	
Ci Arae	SGZ57929.1	
Ci Arae_2	SGZ47799.1	

References

- 1. Moon J, Lewis Liu Z, Ma M, Slininger PJ: **New genotypes of industrial yeast** *Saccharomyces cerevisiae* engineered with YXI and heterologous xylose transporters improve xylose utilization and ethanol production. *Biocatal Agric Biotechnol* 2013, **2**(3):247-254.
- 2. Ma M, Liu ZL, Moon J: Genetic Engineering of Inhibitor-Tolerant *Saccharomyces cerevisiae* for Improved Xylose Utilization in Ethanol Production. *BioEnergy Res* 2012, **5**(2):459-469.
- Young E, Poucher A, Comer A, Bailey A, Alper H: Functional survey for heterologous sugar transport proteins, using Saccharomyces cerevisiae as a host. *Appl Environ Microbiol* 2011, 77(10):3311-3319.
- 4. Du J, Li S, Zhao H: Discovery and characterization of novel d-xylose-specific transporters from Neurospora crassa and Pichia stipitis. *Mol Biosyst* 2010, 6(11):2150-2156.
- Hamacher T, Becker J, Gardonyi M, Hahn-Hagerdal B, Boles E: Characterization of the xylose-transporting properties of yeast hexose transporters and their influence on xylose utilization. *Microbiology* 2002, 148(Pt 9):2783-2788.
- Knoshaug EP, Vidgren V, Magalhães F, Jarvis EE, Franden MA, Zhang M, Singh A: Novel transporters from *Kluyveromyces marxianus* and *Pichia guilliermondii* expressed in *Saccharomyces cerevisiae* enable growth on L-arabinose and D-xylose. *Yeast* 2015, 32(10):615-628.
- Rigamonte TA, Silveira WB, Fietto LG, Castro IM, Breunig KD, Passos FML: Restricted sugar uptake by sugar-induced internalization of the yeast lactose/galactose permease Lac12. FEMS yeast research 2011, 11(3):243-251.