



**HAL**  
open science

## Datamining and functional environmental genomics reassess the phylogenetics and functional diversity of fungal monosaccharide transporters

Florian Barbi, Laurent Vallon, Carmen Guerrero-Galán, Sabine D. Zimmermann, Delphine Melayah, Danis Abrouk, Jeanne Dore, Marc Lemaire, Laurence Fraissinet-Tachet, Patricia Luis, et al.

### ► To cite this version:

Florian Barbi, Laurent Vallon, Carmen Guerrero-Galán, Sabine D. Zimmermann, Delphine Melayah, et al.. Datamining and functional environmental genomics reassess the phylogenetics and functional diversity of fungal monosaccharide transporters. *Applied Microbiology and Biotechnology*, 2021, 105 (2), pp.647-660. 10.1007/s00253-020-11076-y . hal-03098260

**HAL Id: hal-03098260**

**<https://hal.science/hal-03098260v1>**

Submitted on 6 Jan 2021

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial - NoDerivatives 4.0 International License

[Click here to view linked References](#)

1 **Datamining and functional environmental genomics reassess the phylogenetics and**  
2 **functional diversity of fungal monosaccharide transporters**

3 Florian Barbi<sup>1,2\*</sup>, Laurent Vallon<sup>1\*</sup>, Carmen Guerrero-Galán<sup>3</sup>, Sabine D. Zimmermann<sup>3</sup>,  
4 Delphine Melayah<sup>1</sup>, Danis Abrouk<sup>1</sup>, Jeanne Doré<sup>1</sup>, Marc Lemaire<sup>4</sup>, Laurence Fraissinet-  
5 Tachet<sup>1</sup>, Patricia Luis<sup>1†</sup>, Roland Marmeisse<sup>1,5†</sup>.

6  
7 <sup>1</sup> Univ Lyon, Université Claude Bernard Lyon 1, CNRS, INRAE, VetAgro Sup, UMR Ecologie  
8 Microbienne, F-69622, Villeurbanne, France.

9 <sup>2</sup>Department of Soil and Environment, Swedish University of Agricultural Sciences, Box  
10 7014, SE-750 07, Uppsala, Sweden.

11 <sup>3</sup>BPMP, Univ Montpellier, CNRS, INRAE, Institut Agro, Montpellier, France

12 <sup>4</sup>Univ Lyon, Université Claude Bernard Lyon 1, CNRS, INSA de Lyon, UMR Microbiologie  
13 Adaptation et Pathogénie, F-69622, Villeurbanne, France.

14 <sup>5</sup> Institut de Systématique, Évolution, Biodiversité (ISYEB), Muséum national d'histoire  
15 naturelle, CNRS, Sorbonne Université, EPHE, Université des Antilles, CP39, 57 rue Cuvier,  
16 F-75005, Paris, France.

17  
18 \* These authors contributed equally to the study.

19 †These authors contributed equally as co-senior authors

20  
21 **Corresponding author:** Roland MARMEISSE; [roland.marmeisse@mnhn.fr](mailto:roland.marmeisse@mnhn.fr)

22 **Key words:** Fungi, sugar transporters, environmental genomics, metatranscriptomics

## 24 **Abstract**

25 Sugar transporters are essential components of carbon metabolism and have been extensively  
26 studied to control sugar uptake by yeasts and filamentous fungi used in fermentation  
27 processes. Based on published information on characterized fungal sugar porters, we show  
28 that this protein family encompasses phylogenetically distinct clades. While several clades  
29 encompass transporters that seemingly specialized on specific “sugar-related” molecules (e.g.  
30 *myo*-inositol, charged sugar analogs), others include mostly either mono- or  
31 di/oligosaccharide low-specificity transporters. To address the issue of substrate specificity of  
32 sugar transporters, that protein primary sequences do not fully reveal, we screened “multi-  
33 species” soil eukaryotic cDNA libraries for mannose transporters, a sugar that had never been  
34 used to select transporters. We obtained 19 environmental transporters, mostly from  
35 *Basidiomycota* and *Ascomycota*. Among them, one belonged to the unusual “fucose H<sup>+</sup>  
36 symporter” family, which is only known in *Fungi* for a rhamnose transporter in *Aspergillus*  
37 *niger*. Functional analysis of the 19 transporters by expression in yeast and for two of them in  
38 *Xenopus laevis* oocytes for electrophysiological measurements indicated that most of them  
39 showed a preference for D-mannose over other tested D-C6 (glucose, fructose, galactose) or  
40 D-C5 (xylose) sugars. For the several glucose and fructose-negative transporters, growth of  
41 the corresponding recombinant yeast strains was prevented on mannose in the presence of one  
42 of these sugars that may act by competition for the binding site. Our results highlight the  
43 potential of environmental genomics to figure out the functional diversity of key fungal  
44 protein families and that can be explored in a context of biotechnology.

## 51 **Key Points**

- 52 - Most fungal sugar transporters accept several sugars as substrates
- 53 - Transporters, belonging to 2 protein families, were isolated from soil cDNA libraries
- 54 - Environmental transporters featured novel substrate specificities

## 49 **Introduction**

1  
2 50 In osmotrophic microorganisms such as *Fungi*, sugar transporters inserted in the cell  
3  
4 51 membrane are at the frontline for the provision of external soluble sugars to fuel primary  
5  
6 52 carbon metabolism (Gonçalves et al. 2016). These transporters are confronted to, and may  
7  
8 53 cope with, rapid shifts in both external concentrations and chemical diversity of available  
9  
10 54 substrates. To adjust fluxes in responses to these shifts, fungi have evolved a series of  
11  
12 55 transcriptional and post-transcriptional mechanisms that modulate synthesis and turnover of  
13  
14 56 such membrane proteins (Özcan and Johnston 1999; Horák 2013). Additionally, these  
15  
16 57 transporters have evolved intrinsic characteristics, namely substrate ranges and affinities that  
17  
18 58 specialize them on a specific set of sugars in a specific range of concentrations.  
19  
20  
21  
22  
23

24 59 In nature, sugars are represented as soluble free mono or disaccharides that accumulate in  
25  
26 60 specific plant exudates such as flower nectar, plant sap, fruit pulp, rhizodeposits or milk  
27  
28 61 (Hutsch et al. 2002). However, most sugars are stored as polysaccharides constitutive of the  
29  
30 62 plant biomass (e.g. cellulose, hemicelluloses, starch) (Popper and Tuohy 2010; Schädel et al.  
31  
32 63 2010). Plant biomass degradation by extracellular hydrolytic enzymes continuously generates  
33  
34 64 a diversity of mostly C5 (e.g. xylose, arabinose) or C6 (e.g. glucose, galactose, mannose)  
35  
36 65 monosaccharides as well as oligosaccharides (e.g. maltose, xylobiose, cellobiose,  
37  
38 66 mannobiose, regarding di-saccharides) that are substrates for sugar transporters (Gunina and  
39  
40 67 Kuzyakov 2015). Consequently, sugar transporter-encoding gene families are among the most  
41  
42 68 diversified gene families in fungal genomes with up to 86 different members in the case of the  
43  
44 69 mold *Aspergillus niger* (Peng et al. 2018).  
45  
46  
47  
48  
49  
50

51 70 In yeasts and filamentous fungi, playing key roles in carbon metabolism, fermentation  
52  
53 71 processes, and biotechnology to achieve high conversion rates of complex substrates (e.g.  
54  
55 72 plant biomass, dairy effluents), sugar transporters have been the focus of numerous studies  
56  
57 73 and literature reviews (e.g. Bisson et al. 2016; Hara et al. 2017). Most noticeably, is the  
58  
59  
60  
61  
62  
63  
64  
65

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

74 characterization of the substrate specificity of most of the sugar transporters in the model and  
75 industrial yeast *Saccharomyces cerevisiae* (e.g. Bisson et al. 2016). For this species, mutant  
76 and recombinant strains unable to use either mono- or disaccharides (Wieczorke et al. 1999;  
77 Marques et al. 2017) have been selected and extensively used to characterize transporters  
78 from other fungal species by functional complementation. This has also been the case for  
79 several degradation products from cellulose, hemicellulose or pectin that *S. cerevisiae* cannot  
80 naturally metabolize, such as xylose, cellobiose, xylobiose, cellobionic acid or galacturonic  
81 acid (e.g. Galazka et al. 2010; Li et al. 2015; Sloothaak et al. 2016; Zhang et al. 2017; Protzko  
82 et al. 2018).

83 From a structural point of view, sugar transporters are described within the Major  
84 Facilitator Superfamily (MFS) referenced in the Transported Classification Database (TCDB,  
85 Saier et al. 2016) as TC 2.A.1. Almost all fungal sugar transporters described so far belong to  
86 the so-called Sugar Porter (SP) family (TC 2.A.1.1). More marginally, sugar acquisition  
87 through specific members of the Drug:H<sup>+</sup> Antiporter-1 family (DHA1, TC 2.A.1.2) has been  
88 reported (essentially for fructose) in *Saccharomycotina* yeasts (Leandro et al. 2011;  
89 Gonçalves et al. 2016), as well as through a “Fucose: H<sup>+</sup> Symporter” (FHS) family member  
90 (TC 2.A.1.7) in the case of L-rhamnose in *A. niger* (Sloothaak et al. 2016). Putative SWEET  
91 transporters (TC 2.A.123; Chen et al. 2010; Takanaga and Frommer, 2010) have also been  
92 identified in the genome of chytrid fungi (Hu et al. 2016). In what concerns the SP family, it  
93 is found in all domains of life and virtually in all eukaryotic species examined. The 3-  
94 dimensional structures of several bacterial, plant and animal members of this family have  
95 been resolved (e.g. Deng et al. 2014; Wisedchaisri et al. 2014; Paulsen et al. 2019) along with  
96 the identification of key amino acid residues interacting with the transported sugars (e.g.  
97 Young et al. 2014; Zhang et al. 2015). Furthermore, it was shown that very limited changes in  
98 the fungal SP primary sequences, obtained through directed or random mutagenesis, can alter

99 transport features in terms of substrate range, affinity or transport inhibition by non-  
100 transported competitive substrates (e.g. Reznicek et al. 2015; Li et al. 2016; Trichez et al.  
101 2019). Despite this apparent wealth of knowledge regarding fungal sugar transporters, their  
102 true phylogenetic and functional diversity within the kingdom Fungi remains underexplored.  
103 Indeed, functional data available for fungal sugar transporters suffer from several potential  
104 biases. On the one hand, (i) these data have been acquired from a limited number of fungal  
105 species not necessarily representative of the phylogenetic and functional diversity of this  
106 taxonomic group. On the other hand, (ii) a large number of the characterized transporters had  
107 been selected either based on their phylogenetic proximity to already characterized  
108 transporters or based on their capacity to transport a restricted number of sugars (often  
109 glucose, xylose or fructose in the case of monosaccharide transporters).

110 To overcome the taxonomic bias, in the present study, we selected transporters by the  
111 functional complementation of a *S. cerevisiae* yeast mutant defective in monosaccharide  
112 transport using metatranscriptomic libraries made from polyadenylated mRNA extracted from  
113 a broadleaf (*Fagus sylvatica*) and an evergreen (*Picea abies*) forest soils. Fungal communities  
114 present in these soils encompass several hundreds of taxa distributed among several main  
115 orders belonging to *Basidiomycota*, *Ascomycota* and *Mucoromycota* (Damon et al. 2010).  
116 These taxa encompass nutritional strategies featuring either soil or litter saprotrophs, white or  
117 brown rots, plant or animal pathogens and mycorrhizal mutualists. Regarding the sugars used  
118 as "baits" to select sugar transporters, we concentrated our efforts on D-mannose. In the  
119 literature, this monosaccharide had never been specifically used to select transporters  
120 although it is widespread and abundant in natura as a component of plant biomass (mannans)  
121 or as a component of glycoproteins as for example in the fungal cell wall mannoproteins  
122 (including yeasts; see Lipke and Ovalle, 1998). Noticeably, gymnosperm cell walls are  
123 specifically enriched in mannans that represent on average 31% of the needle's hemicellulose

124 biomass (Schädel et al. 2010). It could therefore be hypothesized that fungal species that  
125 specifically thrive in gymnosperm (e.g. *P. abies*) forest may express mannose-specific  
126 transporters. Furthermore, in a context of biotechnology, the characterization of transporters  
127 that efficiently transport mannose but no other monosaccharides could be used to engineer  
128 yeast strains for an efficient use of this plant-derived sugar in a context where other sugars are  
129 present.

130 Our objectives were thus (i) to update our current knowledge on fungal sugar  
131 transporter functional diversification through the establishment of an exhaustive database of  
132 characterized members of this fungal protein family and subsequent phylogenetic analysis of  
133 the corresponding proteins, and (ii) to search for novel members possibly defining novel  
134 substrate preferences (such as mannose) through an environmental genomics approach.

135 **Materials and methods**

136 *Soil sampling, RNA extraction and cDNA library construction*

137 Two organic matter-rich composite soil samples were collected in July 2007 in a beech (*F.*  
138 *sylvatica*) and in a spruce (*P. abies*) forest (Damon et al. 2010; 2011; 2012) located in central  
139 France (Breuil-Chenue site, 47°18'10''N, 4°4'44''E). More details about forests, soil  
140 characteristics and sampling strategy are given in Damon et al. (2010). Total RNA was  
141 extracted from 104 g of each frozen soil samples as previously described (Damon et al. 2010)  
142 and eukaryotic cDNAs were synthesized from 3 µg of total soil RNA using the Mint-2 cDNA  
143 synthesis kit (Evrogen, Moscow, Russia). After size-fractionation by 2-dimensional agarose  
144 gel electrophoresis (Yadav et al. 2014), the recovered 1-3 kb cDNA fractions were PCR-  
145 amplified (26 cycles using the Mint-2 cDNA synthesis kit) and cloned downstream of the *S.*  
146 *cerevisiae* PMA1 promoter in the pDR196 yeast–*Escherichia coli* shuttle vector (Rentsch et  
147 al. 1995) into which a kanamycin resistance gene and *SfiIA* and *SfiIB* cloning sites had been  
148 introduced. For each soil cDNA library, recombinant plasmids were introduced into electro-  
149 competent *E. coli* cells (MegaX DH10B™ T1R Electrocomp™ cells, Invitrogen, Thermo  
150 Fisher Scientific, Illkirch, France) and at least 2 10<sup>6</sup> kanamycin resistant bacterial colonies  
151 growing on agar medium were collected and pooled to constitute two soil cDNA plasmid  
152 libraries after alkaline lysis extraction (Sambrook and Russell 2001).

153 *Yeast strains, media, and culture methods*

154 Environmental cDNAs encoding sugar transporters were selected for their capacity to restore  
155 growth on mannose or xylose of yeast mutant strains deleted of their monosaccharide  
156 transporters. For mannose, we used the EBY.VW4000 *S. cerevisiae* mutant strain (*MATa*  
157 *leu2-3, 112 ura3-52 trp1-289 his3-Δ1 MAL2-8c SUC2 Δhxt1-17 Δgal2 stlΔ::loxP agt1Δ::loxP*  
158 *mph2Δ::loxP mph3Δ::loxP*) unable to use mannose, but also glucose, fructose and galactose  
159 as carbon sources (Wieczorke et al. 1999). For xylose, we used the EBY.X1-2 *S. cerevisiae*



160 strain which is the EBY.VW4000 strain in which had been introduced plasmids p414-TEF.X1  
161 and p25G.X2 containing the *XYL1* and *XYL2* genes from *Scheffersomyces stipitis*, respectively  
162 (Young et al. 2011). These two genes code for a xylose reductase (*XYL1*) and a xylitol  
163 dehydrogenase (*XYL2*), which are the two enzymes activities, absent from *S. cerevisiae*,  
164 needed for the use of xylose as a carbon source. The EBY.VW4000 strain was grown at 30°C  
165 in liquid or on solid yeast nitrogen base medium (YNB, 6.7 g.l<sup>-1</sup> without amino acids, pH 5.9)  
166 supplemented with (in g.l<sup>-1</sup>) adenine, 0.01; arginine, histidine and methionine, 0.02; lysine,  
167 tryptophan and uracil, 0.04; threonine and tyrosine, 0.05; isoleucine, leucine and  
168 phenylalanine, 0.06; valine, 0.15 and maltose, 20. Selection of EBY.VW4000 transformants  
169 expressing monosaccharide transporters was performed on solid YNB medium lacking uracil  
170 and supplemented with amino acids and mannose as sole carbon source. Concerning the  
171 EBY.X1-2 strain, it was grown at 30°C in liquid or on solid YNB medium lacking leucine and  
172 tryptophan and supplemented with 20 g.l<sup>-1</sup> maltose. Selection of EBY.X1-2 transformants  
173 expressing xylose transporters was performed on solid YNB medium lacking uracil, leucine  
174 and tryptophan and supplemented with xylose as sole carbon source. The wild-type *S.*  
175 *cerevisiae* CEN.PK2-1C and its mutant derivatives (EBY.VW4000 and EBY.X1-2) were  
176 complemented with the empty pDR196-*SfiIA/B* plasmid to use them as positive and negative  
177 controls during growth rate measurements on different monosaccharides, respectively.

#### 178 *Selection of fungal sugar transporters by functional yeast complementation*

179 Both soil cDNA plasmid libraries (from spruce and beech forest soils) were used to transform  
180 *S. cerevisiae* using a standard lithium acetate protocol (Rose and Broach 1990). Concerning  
181 the functional screening on mannose, EBY.VW4000 transformed yeast cells were selected  
182 directly on a solid yeast nitrogen base (YNB) minimal medium supplemented with mannose  
183 (0.2 or 0.5%) and amino acids but lacking uracil. In the case of xylose-transporter screening,  
184 EBY.X1-2 transformed yeast cells were first plated on a solid YNB minimal medium

185 supplemented with 2% maltose and amino acids but lacking uracil, leucine and tryptophan.  
1  
2 186 Then, after 3 days of growth, uracil prototrophic yeast colonies were collected, washed by  
3  
4 187 centrifugation and resuspended in sterile water before being plated on solid YNB minimal  
5  
6 188 medium supplemented with 2% xylose and amino acids but lacking uracil, leucine and  
7  
8 189 tryptophan. Plasmids from the yeast transformants were extracted using the Zymoprep Yeast  
9  
10 190 Plasmid Miniprep I (Zymo Research, Irvine, CA, U.S.A.) and used to transform One Shot  
11  
12 191 TOP10 chemically competent *E. coli* (Invitrogen) to kanamycin resistance. After plasmid  
13  
14 192 extraction (NucleoSpin Plasmid kit; Macherey Nagel, Hoerd, France), inserts were Sanger  
15  
16 193 sequenced using primer PMA (5'-CTCTCTTTTATACACACATTC-3') and additional  
17  
18 194 internal primers. The different fungal sugar transporters retrieved from spruce and beech soil  
19  
20 195 cDNA libraries were annotated spS1 to spS12 and spB1 to spB7, respectively. Both yeast  
21  
22 196 mutant strains (EBY.VW4000 and EBY.X1-2) were further transformed with all of these  
23  
24 197 different environmental fungal sugar porters to study their capacity to transport different C6  
25  
26 198 and C5 monosaccharides. Sequences of environmental transporters that support the findings  
27  
28 199 of this study have been submitted to the EMBL database with accession codes LR862110-  
29  
30 200 LR862128.

#### 201 *Phenotypic characterization of yeast transformants and growth rate measurements*

202 The monosaccharide utilization profile of EBY.VW4000 transformants was performed using  
203 drop-test assays on solid YNB minimal medium (Rose and Broach 1990) and  
204 electrophysiology assays (Damon et al. 2011). Only mannose, fructose and glucose could be  
205 tested using drop-test assays as other sugar sources are either not assimilated or naturally  
206 transported by the EBY.VW4000 mutant strain. Due to the complexity of the methodological  
207 approach, electrophysiological analyses were only performed with potential mannose specific  
208 transporters (spS1 and spS2), found by screening of EBY.VW4000 cells, to test the transport  
209 of sugar constitutive of cellulose and hemicelluloses (cellobiose, glucuronic acid, glucose,

210 fucose, galactose, rhamnose, arabinose, mannose, fructose and xylose). Additionally, maltose  
211 and two disaccharides abundantly found in plant tissues (mannobiose and sucrose) were also  
212 tested using these electrophysiological assays. Procedures used to characterize by voltage-  
213 clamp recordings the sugar transporters spS1 and spS2 expressed in *Xenopus laevis* oocytes  
214 are detailed in Online Resource 1.

215 Growth rates of the transformed EBY.VW4000 yeast strains were measured in triplicate using  
216 a Bioscreen C system (Growth Curves USA, Piscataway, NJ) equipped with a wide-band  
217 filter (420 to 580 nm) recommended for optical density (OD) measurements (Young et al.  
218 2011). A 1  $\mu\text{l}$  inoculum of fully-grown cultures diluted to  $5 \cdot 10^6$  cells. $\text{ml}^{-1}$ , was added to each  
219 well filled with 250  $\mu\text{l}$  of YNB minimal medium supplemented with amino acids but lacking  
220 uracil and with 0.1%, 0.5% or 1% (w/v) of carbon source (either maltose, D-glucose, D-  
221 fructose, D-mannose or D-galactose). The experiment was run 4 days at 30°C using a medium  
222 amplitude and normal intermittent shaking (10s) and OD measurement every 10 min.

223 Inhibition of mannose transport by other non-assimilated carbon sources (either glucose or  
224 fructose) was assessed in media containing 0, 0.1, 0.5 or 1% mannose and supplemented with  
225 either 0 or 1% of either glucose or fructose. Growth rates of EBY.X1-2 transformants in  
226 presence of xylose as carbon source were tested in liquid YNB minimal medium  
227 supplemented with 1% xylose as described by Colabardini et al. (2014). Yeast transformants  
228 were first grown on plates containing solid YNB minimal medium supplemented with 2%  
229 xylose. After 21 days of incubation at 30°C, colonies were transferred in 3 ml of YNB  
230 minimal medium supplemented with 1% maltose. After 24 h of culture at 180 rpm and 30°C,  
231  $1.5 \cdot 10^6$  yeast cells were washed with water and transferred in 3 ml of YNB minimal medium  
232 supplemented with 1% xylose. Yeast-cell growth was estimated once every 2-3 days during a  
233 period of 21 days by measuring the absorption at 600 nm.

234 Exponential growth rates were analyzed using R (version 3.5.3) and the package  
1  
2 235 Growthcurver (Sprouffs et al. 2016). Growth data were fitted to a standard logistic equation  
3  
4  
5 236 and the Area Under the Curve (AUC) parameter was estimated with a 95% confidence  
6  
7 237 interval to be able to detect any significant change between each condition ( $P$  value < 0.05).  
8

9  
10 238 *Phylogenetic analyses*

11  
12 239 Environmental transporter sequences were compared to closest reference sequences retrieved  
13  
14 240 from public databases as well as to all sequences of fungal sugar transporters that have been  
15  
16  
17 241 functionally characterized. Sequences highly similar to the selected environmental sugar  
18  
19 242 transporters were identified by BLASTp searches in the GenBank database. For the  
20  
21  
22 243 taxonomic assignment of environmental sequences, the ten best BLAST hits were selected.  
23

24 244 Amino acid sequences were aligned using MUSCLE (Edgar 2004). Maximum-likelihood  
25  
26 245 (ML) phylogenetic trees were generated with PhyML v.3.0 (Guindon et al. 2010) using the  
27  
28  
29 246 LG substitution model (Le and Gascuel 2008) as implemented in SeaView v.4 (Gouy et al.  
30  
31 247 2010). Robustness of tree topologies was assessed by bootstrap analysis (1000 replicates).  
32  
33  
34 248 Tree was drawn using FigTree v.1.4 (<http://tree.bio.ed.ac.uk/software/figtree/>).  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

## 249 **Results**

### 250 *Phylogenetic and functional diversity of characterized fungal sugar porters (SPs)*

251 From more than 70 literature references, we identified 123 fungal sugar transporters,  
252 belonging to the SP family (TC 2.A.1.1) that had been functionally characterized (Online  
253 Resource 2). A majority (109) of these genes originated from 30 *Ascomycota* species with an  
254 overrepresentation of *Saccharomycotina* yeasts (15 species), among which *S. cerevisiae* is  
255 represented by 22 genes. The database included nevertheless two transporters from the  
256 *Glomeromycotina* (2 species) and 12 from the *Basidiomycota* (7 species).

257 In a majority of cases functional characterization consisted in the expression of the  
258 corresponding fungal genes in a *S. cerevisiae* mutant deleted of its endogenous transporter  
259 genes followed by growth tests of the corresponding recombinant strain on one or several  
260 sugars that could no longer be assimilated by the yeast mutant (Young et al. 2011; Online  
261 Resource 2). Based on this often-fragmentary functional information in terms of number and  
262 diversity of tested substrates, we classified the transporters as monosaccharide (M) or  
263 oligosaccharide (O) transporters. Monosaccharide transporters encompassed those capable of  
264 transporting C5 or C6 monosaccharides but also structurally related molecules such as polyols  
265 (e.g. glycerol, mannitol, *myo*-inositol or sorbitol), gluconic, galacturonic or quinic acids.  
266 Oligosaccharides tested as potential substrates were mostly disaccharides (e.g. sucrose,  
267 cellobiose, xylobiose, maltose or lactose) and more rarely tri- (e.g. cello- or maltotriose) or  
268 tetraoses (cellotetraose). In five cases, transporters had been shown to transport both mono-  
269 and oligosaccharides (MO transporters). This is the case of the *Kluyveromyces lactis* *LAC12*  
270 that transports both lactose and galactose Riley et al. 1987; Baruffini et al. 2006), but also of  
271 the *AGT1*, *MPH2* and *MPH3* “maltose transporters” of *S. cerevisiae* whose deletions were  
272 needed to suppress monosaccharide transport in the EBY.VW4000 mutant strain (Wieczorke  
273 et al. 1999). Besides their substrate (sugars) specificities, fungal SPs have also been shown to

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

274 function as either facilitators (e.g. Jordan et al. 2016) or H<sup>+</sup> symporters (e.g. Dos Reis et al.  
275 2013) co-transporting a proton along with a sugar molecule. Since the transport mode was  
276 very rarely assessed for published transporters, this information was not analyzed further.

277         Despite the fact that almost all "M" transporters had not been tested on  
278 oligosaccharides and inversely regarding "O" transporters on monosaccharides (Online  
279 Resource 2), this simple classification was well supported in a global phylogenetic analysis of  
280 the characterized transporter sequences that grouped almost all "O" and "M" transporters in  
281 distinct and well-supported clades (bootstrap values >80%; Fig. 1). The very few exceptions  
282 to this rule may require additional functional data to further refine their true substrate  
283 specificity prior to speculate about occasional reversal of "O" transporters to transport  
284 monosaccharides. At least in the case of monosaccharide "M" transporters, sequences  
285 originating from a single species, belonging to *Ascomycota* (e.g. the *Saccharomycotina* yeast  
286 *Yarrowia lipolytica* or the *Pezizomycotina A. nidulans*) or to *Basidiomycota* (e.g. the  
287 *Agaricomycotina Laccaria bicolor*) were often distributed in different clades suggesting that  
288 divergences between these sequences occurred at least prior to the divergence between  
289 *Ascomycota* and *Basidiomycota*. Besides specialization towards mono or oligosaccharides,  
290 functional data available for most transporters (Online Resource 2) are however insufficient to  
291 attribute a more specific specialization in terms of transported substrates to most clades  
292 identified in the global phylogenetic tree. Nevertheless, we observed that six of the seven  
293 *myo*-inositol transporters included in the tree, originating from three *Ascomycota* and one  
294 *Basidiomycota* species respectively, grouped together to form a homogeneous clade (clade  
295 VII, Fig. 1). This is also the case for (i) four transporters, from four filamentous *Ascomycota*  
296 species, known to transport charged, acidic molecules, either quinic acid, galacturonic acid or  
297 4-deoxy-L-erythro-5-hexoseulose uronate, a constituent of alginate (clade IX) or (ii) two  
298 glycerol transporters from the yeasts *S. cerevisiae* and *Debaryomyces hansenii* (clade XI).

299           Regarding monosaccharide transporters, 52 of them had been tested on at least the four  
1  
2 300 C6 sugars D-glucose, D-galactose, D-mannose and D-fructose that share the same molecular  
3  
4 301 formula (C<sub>6</sub>H<sub>12</sub>O<sub>6</sub>). While 25 of them (48%) transported all four sugars and 42 of them (81%)  
5  
6  
7 302 glucose, only five of them were apparently specific for one of these four sugars, three for  
8  
9 303 fructose, one for mannose, one for galactose and none for glucose (Online Resource 2).  
10  
11 304 Regarding xylose transporters, widely studied in the context of plant biomass utilization, of  
12  
13 305 the 24 xylose transporters studied for their capacity to transport at least one C6  
14  
15 306 monosaccharide, only one of them (*XltB* from *A. niger* tested on mannose, glucose, galactose  
16  
17 307 and fructose) was seemingly specific for xylose (Sloothaak et al. 2016). This analysis suggests  
18  
19 308 that strict specificity towards one of the most common sugar constitutive of plant biomass is a  
20  
21 309 rare, if not inexistent, feature among fungal SPs.  
22  
23  
24  
25

26 310           Focusing on the transport of mannose, we observed a close association between  
27  
28 311 mannose and glucose transport (Online Resource 2). Indeed, of the 47 published mannose  
29  
30 312 transporters tested on glucose, only two (both from the yeast *Y. lipolytica*) could not transport  
31  
32 313 glucose. Conversely, of the 49 published glucose transporters tested on mannose, only five  
33  
34 314 (from *S. cerevisiae*, *Neurospora crassa* and *Piriformospora indica*) could not transport  
35  
36 315 mannose.  
37  
38  
39  
40

#### 41 316 *Phylogenetic diversity of sugar transporters isolated from soil cDNA libraries*

42

43 317 The screening of two forest soil metatranscriptomic cDNA libraries for the functional  
44  
45 318 complementation of the growth defect of the EBYVW4000 yeast mutant on mannose-  
46  
47 319 containing media led to the isolation of 15 different cDNAs; 11 from the spruce (*P. abies*, spS  
48  
49 320 genes and proteins) library and 4 from the beech (*F. sylvatica*, spB genes and proteins) one. A  
50  
51 321 similar screening on xylose-containing media using the same yeast strain expressing the *S.*  
52  
53 322 *stipitis* xylose catabolic pathway led to the isolation of 4 cDNAs; 1 from the spruce library  
54  
55 323 and 4 from the beech one. Eighteen of these 19 transporters were unambiguously attributed to  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

324 the SP family (TCDB #2.A.1.1). In blast searches against the curated SwissProt or TCDB  
325 databases, their best hits all belonged to this family and they were all predicted to have 12  
326 transmembrane domains that displayed characteristic signature sequences for this SP family  
327 (Online Resource 3). Conversely, the spB4 transporter, that also possessed 12 putative  
328 transmembrane domains, displayed high similarity values to members of the so-called  
329 “Fucose: H<sup>+</sup> Symporter” (FHS) family (TC 2.A.1.7). Members of this FHS protein family  
330 have been experimentally shown to transport different monosaccharides in *Bacteria*, including  
331 L-fucose in the case of the *E. coli FucP* transporter (Gunn et al. 1994). Although members of  
332 this family have been predicted in the proteomes of many (but not all) fully sequenced fungal  
333 species, only the *RhtA* gene from *A. niger* has been demonstrated to encode a L-rhamnose  
334 transporter (Sloothaak et al. 2016).

335 A phylogenetic analysis that incorporated all 19 transporters and their 10 most similar  
336 sequences identified in blast searches against the generalist GenBank nr database suggested  
337 that all these 19 environmental sequences most likely originated from *Fungi*. Ten of them  
338 could originate from *Ascomycota* and eight from *Basidiomycota* (Fig. 2). Regarding the  
339 divergent spB4, although most likely of fungal origin, its exact taxonomic placement could  
340 not be precisely ascertained as similar sequences originated from *Basidiomycota* but also  
341 *Chytridiomycota* (namely *Gonapodya prolifera*, belonging to the *Monoblepharidomycetes*;  
342 Fig. 2). BLASTp searches for spB4 homologs in fully sequenced fungal genomes showed that  
343 homologous sequences were absent in numerous fungal species and extremely rare in specific  
344 taxonomic groups such as the *Saccharomycotina* yeasts where a single sequence was  
345 identified in the genome of *S. stipitis*. A preliminary phylogenetic analysis using sequences  
346 from different fungal phyla, as well as from *Bacteria*, placed the spB4 environmental  
347 sequence, with other sequences from *Chytridiomycota*, *Ascomycota* and *Basidiomycota* in a  
348 clade (Online Resource 4) associated to a second clade encompassing bacterial sequences, as



349 well as a sequence from the *Mucoromycotina Mucor circinelloides*. Sequences identified in  
1  
2 350 the genomes of fungal species extensively studied for sugar transport (*Aspergillus* sp., *N.*  
3  
4 351 *crassa*, *Trichoderma reesei*, *S. stipitis*, but also *Tuber melanosporum*; Online Resource 1),  
5  
6  
7 352 including the *RhtA* rhamnose transporter from *A. niger*, grouped together to form another  
8  
9  
10 353 distinct clade (Online Resource 4).

11  
12 354 When all environmental sequences were incorporated in the phylogenetic tree that  
13  
14 355 encompassed nearly all characterized fungal SPs, we observed that all of them (except the  
15  
16 356 divergent spB4 sequence used to root the tree) fell in clades of monosaccharide "M"  
17  
18  
19 357 transporters (Fig. 1). Furthermore, more than half (11) of the environmental sequences fell in  
20  
21  
22 358 a single clade (clade III, Fig. 1) that encompassed exclusively characterized proteins from  
23  
24 359 *Ascomycota* species although five of the environmental sequences attributed to this clade were  
25  
26 360 predicted to be from *Basidiomycota* (spS1, S2, S7, B2and B7; Fig. 2).

27  
28  
29 361 *Monosaccharide utilization profiles of sugar transporters isolated from soil cDNA libraries*

30  
31 362 Substrate range and preferences for D-monosaccharides was assessed by measuring the  
32  
33  
34 363 growth of the recombinant EBYVW4000 yeast strain constitutively expressing each of the  
35  
36 364 environmental transporters on different concentrations of either fructose, galactose, glucose  
37  
38  
39 365 and mannose as the sole C sources in both liquid (Fig. 3) and solid (Online Resource 5)  
40  
41 366 media. Additionally, growth of the recombinant EBY.X1-2 yeast strain expressing each of the  
42  
43  
44 367 environmental transporters in combination with the *S. stipitis* xylose assimilation pathway  
45  
46 368 was assessed on a medium containing 10 g.l<sup>-1</sup> of xylose as sole carbon source (Online  
47  
48  
49 369 Resource 6). Regarding the use of C6 sugars a great variability was observed among  
50  
51 370 environmental transporters. While for each transporter, mannose was always the best sugar, or  
52  
53 371 among the best, in terms of final biomass yield at the end of the culture period (4 days), this  
54  
55  
56 372 yield was particularly low for several transporters (namely spB4, spS2 and spS7) on all of the  
57  
58 373 tested concentrations of the different sugars. This may be the result of improper expression of  
59  
60  
61  
62  
63  
64  
65

374 these proteins in yeast (as already reported for sugar transporters by e.g. Dreyer et al. 1999;  
375 Wieczorke et al. 2003) or may suggest that in vivo, in their respective fungal species, these  
376 transporters may preferentially transport other sugars.

377 While several transporters could be categorized as relatively generalists (e.g. spB2,  
378 spS4, spS8, spS9 or spS11), leading to comparable growth rates on all four C6 sugars tested,  
379 others had a more restricted substrate range. Noticeably, five environmental transporters  
380 (spB3, spB4, spS1, spS2 and spS7) did not allow growth of the recombinant yeasts on all  
381 three tested concentrations of glucose (particularly well visible in drop tests on solid media,  
382 Online Resource 5). This proportion of "glucose negative" environmental transporters among  
383 "mannose positive" ones (26%) is significantly higher (Chi2 test,  $P < 0.01$ ) than the proportion  
384 observed among published transporters (two in 47; 4%). Among these five SPs, the  
385 phylogenetically related spS2 and spS7 ones (Fig. 2) were seemingly mannose specific in  
386 both liquid and solid cultures. However, both spS2 and spS7 were among the transporters for  
387 which the final biomass yield was always low irrespective of the monosaccharide added to the  
388 culture medium. Altogether, clustering of environmental transporters according to their  
389 substrate range, deduced from the growth of the recombinant EBYVW4000 strain on different  
390 concentrations of the four C6 monosaccharides (Fig. 3) demonstrated an absence of  
391 correspondence between substrate preferences and phylogenetic proximity of the primary  
392 sequences (Fig. 1 and 2).

393 As already reported in the literature, growth on xylose of recombinant yeast strains  
394 expressing a heterologous xylose catabolic pathway is intrinsically low (Tran Nguyen Hoang  
395 et al. 2018). We could nevertheless show that 18 out of the 19 environmental transporters  
396 supported growth of this strain on xylose (Online Resource 6). Furthermore, all of these  
397 proteins harbored the amino-acid motif "G-G/F-XXX-G" (where X represents a variable, but  
398 usually non-polar, amino acid residue) in the first predicted transmembrane domain (Online

399 Resource 3). According to Young et al. (2014), this motif represents a signature for xylose  
1  
2 400 transport in SPs. Only the transporter spB4 belonging to FHS family did not support xylose  
3  
4 401 assimilation at all and did not present this xylose specific amino-acid motif (Online Resource  
5  
6  
7 402 3).

9 403 To further analyze substrate range and specificity of the environmental transporters,  
10  
11 404 we hypothesized that they could function as symporters and two of them (spS1 and spS2  
12  
13 405 featuring different substrate specificities in growth tests) were expressed in *X. laevis* oocytes  
14  
15 406 to measure electrophysiological responses in presence of mono- or disaccharides including  
16  
17 407 several sources (e.g. L-C5 or L-C6 monosaccharides, cellobiose or mannobiose) that are not  
18  
19 408 assimilated by the wild-type *S. cerevisiae*. The selected transporters were spS1, which  
20  
21 409 supported good growth on mannose, moderate growth on fructose, galactose and xylose and  
22  
23 410 no growth on glucose and spS2 supporting poor growth on all tested C6 sugars (Fig. 3).  
24  
25 411 Transport of mannose was confirmed for both selected transporters as expected from the yeast  
26  
27 412 complementation assay (spS1, n=24, N=7; spS2 n=16, N=7; where n is the number of studied  
28  
29 413 oocytes from N different batches) in test solutions complemented with 10 mM mannose at pH  
30  
31 414 5.5 (Fig. 4). When applying hyperpolarizing voltage pulses, negative currents of up to several  
32  
33 415 100 nA were induced dependent on the applied potential, thus indicating that both spS1 and  
34  
35 416 spS2 indeed functioned as sugar/ion co-transporters (Fig. 4A,B). Current induction by  
36  
37 417 mannose was not observed in water-injected control oocytes (n=4, N=3) and was reversible in  
38  
39 418 transporter-expressing oocytes, as perfusion with control solution reduced the substrate  
40  
41 419 specific currents (reversibility was tested for all recorded currents). These sugar-specific  
42  
43 420 negative currents would correspond to cation influx (or anion efflux). The observed currents  
44  
45 421 could be shown to be dependent on external pH (i.e. H<sup>+</sup> concentration) as no currents were  
46  
47 422 induced with mannose at pH 7.5 (n=3, N=2; Fig. 4C) indicating influx of protons and thus H<sup>+</sup>-  
48  
49 423 mannose symport. Several sugar transporters have been described to function as H<sup>+</sup>-sugar  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

424 symporters (Bush 1990; 1993; Wolf et al. 1991; Boorer et al. 1996; Lemoine et al. 1996; Zhou  
1  
2 425 et al. 1997; Rottmann et al. 2016). Amplitudes of current induction varied between oocytes  
3  
4 426 probably because of different expression levels in oocytes and oocyte batches. Interestingly,  
5  
6  
7 427 both transporters differed clearly with respect to transport of glucose (Fig. 4C). In agreement  
8  
9  
10 428 with growth measurements, the transporter spS1 did not transport glucose (n=10, N=4;  
11  
12 429 Fig. 4C) whereas spS2 mediated undoubtedly glucose transport (n=6, N=5; Fig. 4B,C). In  
13  
14 430 contrast, fructose was never transported by spS2 (n=6, N=5; Fig. 4C) but partly by spS1 (n=5,  
15  
16 431 N=3; Fig. 4A,C). However, first experiments suggested that spS1 did not transport fructose  
17  
18  
19 432 (n=4, N=3) most probably by an inhibitory effect of the non-transported glucose due to the  
20  
21  
22 433 sequential arrangement of the tests. This result suggests that glucose might bind to the spS1  
23  
24 434 transporter without inducing currents, thus hindering transport of other substrates. The  
25  
26  
27 435 observed differences for the transport of glucose and fructose for the two tested mannose  
28  
29 436 transporters spS1 and spS2 underline the specificity of such transport systems. Interestingly,  
30  
31  
32 437 both mannose transporters mediated currents in oocytes only for D-mannose but not for L-  
33  
34 438 mannose (Fig. 4C; spS1 n=3, spS2 n=4) pointing to highly specific structural requirements  
35  
36 439 needed for binding or transport. Further tests of substrates with either D- or L-configuration  
37  
38  
39 440 were pooled and confirmed the structural substrate specificity. The “L-pool” with L-  
40  
41 441 arabinose, L-rhamnose, and L-fucose did not induce any current (spS1 n=5, spS2 n=1). The  
42  
43  
44 442 “D-pool” with D-galactose, D-xylose, and D-glucuronic acid provoked unfortunately  
45  
46 443 endogenous currents also in control oocytes, so that these three substrates had to be tested  
47  
48  
49 444 separately. In fact, D-glucuronic acid destabilized oocytes, even control ones, and could not  
50  
51 445 be tested. However, in agreement with growth measurements, D-galactose and D-xylose  
52  
53  
54 446 induced currents for both transporters (n=3, each; Fig. 4C). In agreement with the  
55  
56 447 phylogenetic placement of the spS1 and S1 transporters among monosaccharide (M)  
57  
58 448 transporters (Fig. 1), four tested disaccharides (maltose (n=5, each), sucrose (spS1 n=3, spS2  
59  
60  
61  
62  
63  
64  
65

1  
2 449 n=4), mannobiose (n=4 each), and cellobiose (n=4 each)) did not activate any currents in  
3 450 oocytes expressing the two transporters.

4  
5 451           Glucose inhibition of transport of other monosaccharides by SPs that do not transport  
6  
7 452 glucose, as suggested here in the oocyte experiment for spS1, has been repeatedly reported in  
8  
9 453 the literature (Subtil and Boles 2012; Farwick et al. 2014). We evaluated this phenomenon, as  
10  
11 454 well as potential inhibition by fructose, for five environmental transporters (spS1, spS2, spS7,  
12  
13 455 spB3 and spB6) that did not support significant growth on any concentration of both glucose  
14  
15 456 and fructose. For all five cases, we observed that in the presence of 10 g.l<sup>-1</sup> of either glucose  
16  
17 457 or fructose in the culture medium, the EBYVW4000 strain expressing the corresponding  
18  
19 458 transporters failed to grow on either low (1 g.l<sup>-1</sup>), medium (5 g.l<sup>-1</sup>) or high (10 g.l<sup>-1</sup>)  
20  
21 459 concentrations of mannose (Online Resource 7).  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

## 460 Discussion

1  
2 461 The compilation of possibly all fungal SPs characterized thus far and their inclusion in a  
3  
4 462 phylogenetic context support earlier observations, made on more fragmentary datasets, of a  
5  
6  
7 463 clear separation between mono- and oligosaccharide SPs in the Fungi (e.g. Peng et al. 2018)  
8  
9  
10 464 However, the present analysis did not address the issue of potentially multiple origins of  
11  
12 465 either mono- or oligosaccharide clades of SPs in the *Fungi*. The presence, in several of the  
13  
14 466 clades, of transporters from both the *Basidiomycota* and *Ascomycota* and even from the  
15  
16  
17 467 *Glomeromycotina* suggests that the corresponding clades originated early during the  
18  
19 468 diversification of the *Fungi*. Alternatively, this distribution of transporters across the  
20  
21  
22 469 phylogenetic tree could also be the results of horizontal gene transfers between species  
23  
24 470 belonging to different clades as demonstrated for other transporter genes (Slot et al. 2007;  
25  
26  
27 471 Coelho et al. 2013).

28  
29 472 Confrontation of the functional database to the SP phylogenetic tree suggested that  
30  
31 473 few clades of "monosaccharide transporters" may feature functional specialization on specific  
32  
33  
34 474 substrates structurally related to monosaccharides, such as *myo*-inositol, acidic sugar  
35  
36 475 derivatives or glycerol. This phylogenetic information could thus be used for the functional  
37  
38  
39 476 annotation of similar transporters from sequenced fungal genomes. As for the other clades of  
40  
41 477 monosaccharide transporters, they encompass sequences that transport bona fide  
42  
43  
44 478 monosaccharides (either C5 or C6 sugars), but also sugar alcohols without any clear  
45  
46 479 correspondence between clade identity and the exact nature of the transported molecules.  
47  
48  
49 480 While for several yeast species (e.g. *S. cerevisiae* or *Schizosaccharomyces pombe*), most of  
50  
51 481 their monosaccharide SP genes seemed to originate from recent duplication events and belong  
52  
53 482 to the same clade, other species (e.g. *A. nidulans*, *N. crassa*, or *Y. lipolytica*) seem to rely on  
54  
55  
56 483 different highly divergent SPs, belonging to different clades for their supply of  
57  
58 484 monosaccharides.  
59  
60  
61  
62  
63  
64  
65

485 A comprehensive analysis of the substrate range of monosaccharide transporters  
1  
2 486 indicates that a large majority of the studied transporters accepts several sugars as substrates  
3  
4 487 and that specificity towards one or two molecules seems to be an exception (Online Resource  
5  
6  
7 488 1). In this respect, occasional claims of a substrate-specific transporter often reflect the fact  
8  
9 489 that a very limited number of potential substrates have been studied (e.g. Wang et al. 2016). It  
10  
11 490 could however be argued that overexpression of a sugar transporter in the yeast *S. cerevisiae*,  
12  
13 491 the most widely used approach to characterize such transporters from fungi (Young et al.  
14  
15 492 2011), may overestimate the substrate range of the corresponding transporter. Overexpression  
16  
17 493 may indeed allow significant influx of otherwise poorly transported substrates (Dreyer et al.  
18  
19 494 1999) that would not normally support cell multiplication. These experimental artifacts can be  
20  
21 495 partly solved using the more labor-intensive expression of transporters in *X. laevis* oocytes for  
22  
23 496 electrophysiological measurements that also allows the evaluation of molecules that are not  
24  
25 497 normally assimilated by *S. cerevisiae* (e.g. C5 or L-sugars) as potential substrates. Using this  
26  
27 498 approach with two environmental SPs originally selected on mannose, we confirmed yeast  
28  
29 499 growth assay observations by showing that both spS1 and spS2 transport mannose, xylose and  
30  
31 500 galactose, and that they differ in their specificity towards fructose and glucose. Corresponding  
32  
33 501 to the yeast assays, we also observed that one of them accepted fructose, the other one glucose  
34  
35 502 as a substrate. Furthermore, we demonstrated that both transporters did not accept L-  
36  
37 503 monosaccharides and di-saccharides as substrates.  
38  
39  
40  
41  
42  
43  
44  
45

46 504 Although not being specific towards one or two potential substrates, many transporters  
47  
48 505 are neither truly generalists and clearly do not transport several "common" monosaccharides.  
49  
50  
51 506 Overrepresentation of glucose-transporting monosaccharide transporters may reflect the  
52  
53 507 widespread occurrence of this sugar in nature (albeit often as monomer of polysaccharides)  
54  
55 508 and its centrality in carbon metabolism (Gunina and Kuzyakov 2015). Alternatively, it could  
56  
57 509 also be hypothesized that overrepresentation of glucose-transporting transporters in databases  
58  
59  
60  
61  
62  
63  
64  
65

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

510 may reflect a scientific bias as numerous fungal transporters were precisely selected for their  
511 capacity to transport glucose. For the specific case of mannose, the high prevalence of  
512 glucose-transporting porters that can also transport mannose could be explained by the  
513 observation that, at least in yeast, genes encoding enzymes necessary for its use as a carbon  
514 source (hexokinases and mannose-6-phosphate isomerases) are expressed in both glucose and  
515 mannose-containing media. This suggests that these two sugars can be assimilated  
516 simultaneously, with however a preference for glucose over mannose.

517           By selecting transporters from metatranscriptomes based on their capacity to transport  
518 mannose, we tested the hypothesis that "mannose specific" transporters, or at least  
519 transporters that preferentially transport mannose over glucose may be present in soil  
520 organisms occasionally confronted to carbon sources with particularly high mannose content  
521 (e.g. gymnosperm plant biomass or fungal necromass) (Lipke and Ovalle 1998; Schädel et al.  
522 2010)). As previously observed in the case of oligopeptides (Damon et al. 2011), the  
523 screening of environmental cDNA libraries for sugar transporters yielded genes from only  
524 *Fungi*, although genes from other eukaryotic organisms were present among the studied  
525 metatranscriptomes (Damon et al. 2012). Furthermore, all but one of the selected genes  
526 encoded proteins that belonged to the SP family, suggesting that other sugar transporters  
527 reported in fungal genomes may play a minor role in sugar assimilation by fungi. From a  
528 taxonomic perspective, eight of the environmental transporters likely originated from  
529 *Basidiomycota*, thus doubling the number of characterized sugar transporters from this fungal  
530 phylum published thus far (Online Resource 1). From a phylogenetic perspective,  
531 environmental SPs were distributed in several of the distinct clades that form this protein  
532 family. However, more than half of them (11), from both *Basidiomycota* and *Ascomycota*, fell  
533 in a single clade (clade III in Fig. 1) that, thus far, encompassed only published *Ascomycota*  
534 sequences. This uneven distribution of environmental SPs across the global phylogenetic tree



1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

535 of this fungal protein family could originate from a preferential functional expression of these  
536 structurally related proteins in yeast. It could also suggest that *Fungi* preferentially use SPs  
537 from a limited number of clades for their supply in monosaccharides. This latter hypothesis  
538 could be addressed in model organisms by measuring the relative expression levels and  
539 contribution to monosaccharide utilization of endogenous sugar porter encoding genes  
540 belonging to different evolutionary lineages. Finally, screening of soil metatranscriptomes  
541 also yielded a highly divergent fungal sugar transporter sequence (spB4) that belonged to the  
542 poorly studied “Fucose: H<sup>+</sup> Symporter” (FHS) family (TC 2.A.1.7). In *Fungi*, only one  
543 member of this FHS family able to transport monosaccharides was reported so far. In *A.*  
544 *niger*, the related *RhtA* transports L-rhamnose and marginally D-fructose (Sloothaak et al.  
545 2016). Since both rhamnose and fucose are deoxy sugars that occur in nature in the unusual  
546 levorotary (L-) conformation, it could be hypothesized that fungal FHS family members are  
547 specialized on these substrates and marginally accept other sugars as in the case of spB4 that  
548 confers poor growth of recombinant *S. cerevisiae* on mannose and all other tested sugars. This  
549 hypothesis could be tested by expression of this protein, as well as other homologous ones  
550 found in fungal genomes, in *X. laevis* oocytes as performed for the spS1 and spS2 SPs.  
551 Overall, our observations that result from the analysis of "environmental" sequences further  
552 illustrate the power of functional genomics for quickly accessing the true diversity of specific  
553 eukaryotic protein families (Damon et al. 2011; Marmeisse et al. 2017).

554 From a functional perspective, the analysis of environmental fungal sequences  
555 reevaluates the known functional diversity of fungal SPs. It revealed the existence of  
556 phylogenetically distinct transporters that allow mannose, but not glucose transport. This  
557 lends support to the hypothesis that the presence of sugars other than glucose in the  
558 environment may have led to the independent evolution of sugar transporters that  
559 preferentially use mannose (and eventually other monosaccharides) over glucose.

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

560 Furthermore, as an excess of glucose seemingly inhibits these transporters, this observation  
561 further suggests that indeed they may only be operational in environments depleted of this  
562 sugar.

563 "Other than glucose" (e.g. xylose) monosaccharide transporters have been searched for  
564 the independent, simultaneous and non-competitive entry of glucose and other sugars in  
565 fungal (e.g. yeast) cells for their simultaneous co-fermentation. Alternatively, these  
566 transporters can also be used for the specific utilization of the "other than glucose"  
567 monosaccharide as carbon source in a context where glucose is also present. One limitation to  
568 this strategy is that transporters that do not transport glucose are often inhibited by this sugar  
569 (Farwick et al. 2014; Li et al. 2016) which, possibly, clogs the entry pore of the protein. We  
570 showed that this phenomenon may also affect mannose transport by mannose porters that do  
571 not support glucose transport. Furthermore, we also show that this phenomenon is not  
572 glucose-specific but can also be generated by other non-transported sugars such as fructose.  
573 Several of the selected environmental transporters could thus be used for the co-fermentation  
574 of mannose and other sugars or the specific use of mannose in the presence of other sugars,  
575 providing that their inhibition by competing substrates could be relieved (Farwick et al. 2014).  
576 In the case of xylose transporters, mutagenesis and directed evolution, which resulted in a  
577 limited number of changes in the primary sequence, have been successfully employed to  
578 render glucose-insensitive the corresponding transporters (Farwick et al. 2014; Li et al. 2016).

579 **Acknowledgements.**

1  
2 580 The EBY.VW4000 *S. cerevisiae* mutant strain used to select environmental transporters was  
3  
4  
5 581 provided by Dr. Eckhard Boles. Part of this work was financed by the CNRS-INSU ECCO  
6  
7 582 Microbien program and the INRA métaprogramme M2E (project Metascreen).  
8

9  
10 583

11  
12 584 **Author contribution**

13  
14 585 FB, PL and RM conceived and designed research. FB, LV, CG-G, SDZ, DM, JD and PL  
15  
16  
17 586 conducted experiments. LF-T co-lead with PL the first part of the experiments performed by  
18  
19 587 FB. CG-G and SDZ performed and interpreted electrophysiological measurements. ML  
20  
21  
22 588 contributed new reagents and analytical tools. FB, LV, SDZ, DM, DA, PL and RM analyzed  
23  
24 589 data. SDZ, PL and RM wrote the manuscript. All authors read and approved the manuscript.  
25

26 590 **Conflict of interest**

27  
28  
29 591 The authors declare that they have no conflict of interest  
30

31 592 **Ethical approval**

32  
33  
34 593 Experiments using oocytes from *Xenopus* frogs have been performed accordingly to the  
35  
36 594 French national rules for animal maintenance and experiments (agreement A34172044).  
37

38  
39 595  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

596 **References**

- 1  
2  
3 597 Bailly J, Fraissinet-Tachet L, Verner MC, Debaud JC, Lemaire M, Wésolowski-Louvel M,  
4  
5 598 Marmeisse R (2007) Soil eukaryotic functional diversity, a metatranscriptomic approach.  
6  
7 599 ISME J 1:632-642.  
8  
9  
10 600 Baruffini E, Goffrini P, Donnini C, Lodi T (2006) Galactose transport in *Kluyveromyces*  
11  
12 601 *lactis*: major role of the glucose permease *Hgt1*. FEMS Yeast Res 6:1235-1242.  
13  
14 602 doi:10.1111/j.1567-1364.2006.00107.x  
15  
16  
17  
18 603 Bisson LF, Fan Q, Walker GA (2016) Sugar and glycerol transport in *Saccharomyces*  
19  
20 604 *cerevisiae*. Adv Exp Med Biol 892:125-168. doi:10.1007/978-3-319-25304-6\_6  
21  
22  
23  
24 605 Boorer KJ, Loo DDF, Frommer WB, Wright EM (1996) Transport mechanism of the cloned  
25  
26 606 potato H<sup>+</sup>/sucrose transporter StSUT1. J Biol Chem 271:25139–25144.  
27  
28  
29 607 Bush DR (1990) Electrogenicity, pH-dependence, and stoichiometry of the proton-sucrose  
30  
31 608 symport. Plant Physiol 93:1590–1596.  
32  
33  
34 609 Bush DR (1993) Proton-coupled sugar and amino acid transporters in plants. Annu Rev Plant  
35  
36  
37 610 Physiol Plant Mol Biol 44:513–542.  
38  
39  
40 611 Chen LQ, Hou BH, Lalonde S, Takanaga H, Hartung ML, Qu XQ, Guo WJ, Kim JG,  
41  
42 612 Underwood W, Chaudhuri B, Chermak D, Antony G, White FF, Somerville SC, Mudgett MB,  
43  
44 613 Frommer WB (2010). Sugar transporters for intercellular exchange and nutrition of  
45  
46 614 pathogens. Nature 468:527-532.  
47  
48  
49  
50 615 Coelho MA, Gonçalves C, Sampaio JP, Gonçalves P (2013) Extensive intra-kingdom  
51  
52 616 horizontal gene transfer converging on a fungal fructose transporter gene. PLoS Genet  
53  
54 617 9:e1003587. doi:10.1371/journal.pgen.1003587  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

618 Colabardini AC, Ries LN, Brown NA, Dos Reis TF, Savoldi M, Goldman MH, Menino JF,  
1  
2 619 Rodrigues F, Goldman GH (2014) Functional characterization of a xylose transporter in  
3  
4 620 *Aspergillus nidulans*. Biotechnol Biofuels 7:46. doi:10.1186/1754-6834-7-46  
5  
6  
7 621 Damon C, Barroso G, Férandon C, Ranger J, Fraissinet-Tachet L, Marmeisse R (2010)  
8  
9 622 Performance of *COXI* gene as a marker for the study of metabolically active Pezizomycotina  
10  
11 623 and Agaricomycetes fungal communities from the analysis of soil RNA. FEMS Microbiol  
12  
13 624 Ecol 74:693-705.  
14  
15  
16  
17 625 Damon C, Lehembre F, Oger-Desfeux C, Luis P, Ranger J, Fraissinet-Tachet L, Marmeisse R  
18  
19 626 (2012) Metatranscriptomics reveals the diversity of genes expressed by eukaryotes in forest  
20  
21 627 soils. PLoS One 7:e28967.  
22  
23  
24  
25 628 Damon C, Vallon L, Zimmermann S, Haider MZ, Galeote V, Dequin S, Luis P, Fraissinet-  
26  
27 629 Tachet L, Marmeisse R (2011) A novel fungal family of oligopeptide transporters identified  
28  
29 630 by functional metatranscriptomics of soil eukaryotes. ISME J 5:1871–1880.  
30  
31  
32  
33 631 Deng D, Xu C, Sun P, Wu J, Yan C, Hu M, Yan N (2014) Crystal structure of the human  
34  
35 632 glucose transporter GLUT1. Nature 510:121-5. doi:10.1038/nature13306.  
36  
37  
38  
39 633 Dos Reis TF, Menino JF, Bom VL, Brown NA, Colabardini AC, Savoldi M, Goldman MH,  
40  
41 634 Rodrigues F, Goldman GH (2013) Identification of glucose transporters in *Aspergillus*  
42  
43 635 *nidulans*. PLoS One 8(11):e81412. doi: 10.1371/journal.pone.0081412.  
44  
45  
46  
47 636 Dreyer I, Horeau C, Lemaillet G, Zimmermann S, Bush DR, Rodríguez-Navarro A,  
48  
49 637 Schachtman DP, Spalding EP, Sentenac H, Gaber RF (1999). Identification and  
50  
51 638 characterization of plant transporters using heterologous expression systems. J Exp Bot 50  
52  
53 639 (SPEC. ISS.):1073-1087.  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

640 Farwick A, Bruder S, Schadeweg V, Oreb M, Boles E (2014) Engineering of yeast hexose  
1  
2 641 transporters to transport d-xylose without inhibition by d-glucose. Proc Natl Acad Sci U S A.  
3  
4 642 111: 5159–5164. doi: 10.1073/pnas.1323464111  
5  
6  
7 643 Galazka JM, Tian C, Beeson WT, Martinez B, Glass NL, Cate JH (2010) Cellodextrin  
8  
9 644 transport in yeast for improved biofuel production. Science 330:84-86.  
10  
11  
12 645 doi:10.1126/science.1192838  
13  
14  
15 646 Gonçalves C, Coelho MA, Salema-Oom M, Gonçalves P, (2016) Stepwise functional  
16  
17 647 evolution in a fungal sugar transporter family. Mol Biol Evol 33:352–366.  
18  
19  
20  
21 648 Gouy M, Guindon S, Gascuel O (2010) SeaView version 4: A multiplatform graphical user  
22  
23 649 interface for sequence alignment and phylogenetic tree building. Mol Biol Evol 27:221-224.  
24  
25  
26 650 doi:10.1093/molbev/msp259  
27  
28  
29 651 Guindon S, Dufayard JF, Lefort V, Anisimova M, Hordijk W, Gascuel O (2010) New  
30  
31 652 algorithms and methods to estimate maximum-likelihood phylogenies: assessing the  
32  
33 653 performance of PhyML 3.0. Syst Biol 59:307-321. doi:10.1093/sysbio/syq010  
34  
35  
36 654 Gunina A, Kuzyakov Y (2015) Sugars in soil and sweets for microorganisms: Review of  
37  
38 655 origin, content, composition and fate. Soil Biol Biochem 90:87-100.  
39  
40  
41  
42 656 Gunn FJ, Tate CG, Henderson PJ (1994) Identification of a novel sugar-H<sup>+</sup> symport protein,  
43  
44 657 *FucP*, for transport of L-fucose into *Escherichia coli*. Mol Microbiol 12:799-809.  
45  
46 658 doi:10.1111/j.1365-2958.1994.tb01066.x  
47  
48  
49  
50 659 Hara KY, Kobayashi J, Yamada R, Sasaki D, Kuriya Y, Hirono-Hara Y, Ishii J, Araki M,  
51  
52 660 Kondo A (2017) Transporter engineering in biomass utilization by yeast. FEMS Yeast Res  
53  
54 661 17:fox061. doi.org/10.1093/femsyr/fox061  
55  
56  
57 662 Horák J (2013) Regulations of sugar transporters: insights from yeast. Curr Genet 59:1-31.  
58  
59  
60  
61  
62  
63  
64  
65

663 Hu Y-B, Sosso D, Qu X-Q, Chen L-Q, Ma L, Chermak D, Zhang D-C, Frommer WB (2016)  
1  
2 664 Phylogenetic evidence for a fusion of archaeal and bacterial SemiSWEETs to form eukaryotic  
3  
4 665 SWEETs and identification of SWEET hexose transporters in the amphibian chytrid pathogen  
5  
6  
7 666 *Batrachochytrium dendrobatidis*. FASEB J 30:3644-3654.  
8  
9  
10 667 Hutsch BW, Augustin J, Merbach W (2002) Plant rhizodeposition - an important source of  
11  
12 668 carbon turnover in soils J. Plant Nutr Soil Sci 165:397-407.  
13  
14  
15 669 Jordan P, Choe JY, Boles E, Oreb M (2016) Hxt13, Hxt15, Hxt16 and Hxt17 from  
16  
17 670 *Saccharomyces cerevisiae* represent a novel type of polyol transporters. Sci Rep. 6:23502.  
18  
19  
20 671 doi: 10.1038/srep23502.  
21  
22 672 Julius BT, Leach KA, Tran TM, Mertz RA, Braun DM (2017) Sugar transporters in plants:  
23  
24  
25 673 New insights and discoveries. Plant Cell Physiol 58:1442–1460.  
26  
27 674 <https://doi.org/10.1093/pcp/pcx090>  
28  
29  
30 675 Kang A, Lee TS (2015) Converting sugars to biofuels: ethanol and beyond. Bioengineering.  
31  
32 676 2:184-203.  
33  
34  
35 677 Lazar Z, Neuvéglise C, Rossignol T, Devillers H, Morin N, Robak M, Nicaud JM, Crutz-Le  
36  
37 678 Coq AM (2017) Characterization of hexose transporters in *Yarrowia lipolytica* reveals new  
38  
39 679 groups of sugar porters involved in yeast growth. Fungal Genet Biol 100:1-12.  
40  
41 680 doi:10.1016/j.fgb.2017.01.001  
42  
43  
44 681 Le SQ, Gascuel O (2008) An improved general amino acid replacement matrix. *Mol Biol Evol*  
45  
46 682 25:1307-1320. doi:10.1093/molbev/msn067  
47  
48  
49  
50  
51 683 Leandro MJ, Sychrová H, Prista C, Loureiro-Dias MC (2011) The osmotolerant fructophilic  
52  
53 684 yeast *Zygosaccharomyces rouxii* employs two plasma-membrane fructose uptake systems  
54  
55 685 belonging to a new family of yeast sugar transporters. Microbiology 157:601-608.  
56  
57 686 doi:10.1099/mic.0.044446-0  
58  
59  
60  
61  
62  
63  
64  
65

687 Lemoine R, Kühn C, Thiele N, Delrot S, Frommer WB (1996) Antisense inhibition of the  
1  
2 688 sucrose transporter: effects on amount of carrier and sucrose transport activity. *Plant Cell*  
3  
4  
5 689 *Environ* 19:1124–1131.  
6  
7  
8 690 Li X, Chomvong K, Yu VY, Liang JM, Lin Y, Cate JHD (2015) Cellobionic acid utilization:  
9  
10 691 from *Neurospora crassa* to *Saccharomyces cerevisiae*. *Biotechnol Biofuels*. 8:120.  
11  
12 692 doi:10.1186/s13068-015-0303-2  
13  
14  
15 693 Li H, Schmitz O, Alper HS (2016) Enabling glucose/xylose co-transport in yeast through the  
16  
17  
18 694 directed evolution of a sugar transporter. *Appl Microbiol Biotechnol* 100:10215-10223. doi:  
19  
20 695 10.1007/s00253-016-7879-8.  
21  
22  
23 696 Liman ER, Tytgat J, Hess P (1992) Subunit stoichiometry of a mammalian K<sup>+</sup> channel  
24  
25  
26 697 determined by construction of multimeric cDNAs. *Neuron* 9:861-871.  
27  
28  
29 698 Lipke PN, Ovalle R (1998) Cell wall architecture in yeast: new structure and new  
30  
31 699 challenges. *J Bacteriol* 180:3735-3740. doi:10.1128/JB.180.15.3735-3740.1998  
32  
33  
34 700 Marmeisse R, Kellner H, Fraissinet-Tachet L, Luis P (2017) Discovering protein-coding  
35  
36  
37 701 genes from the environment: Time for the eukaryotes? *Trends Biotechnol* 35:824-835.  
38  
39  
40 702 Marques WL, Mans R, Marella ER, Cordeiro RL, van den Broek M, Daran J-MG, Pronk JT,  
41  
42 703 Gombert AK, van Maris AJA (2017) Elimination of sucrose transport and hydrolysis in  
43  
44 704 *Saccharomyces cerevisiae*: a platform strain for engineering sucrose metabolism. *FEMS*  
45  
46 705 *Yeast Res* 17:fox006. doi.org/10.1093/femsyr/fox006  
47  
48  
49  
50 706 Nogueira K, de Paula RG, Antoniêto A, Dos Reis TF, Carraro CB, Silva AC, Almeida F,  
51  
52 707 Rechia C, Goldman GH, Silva RN (2018) Characterization of a novel sugar transporter  
53  
54  
55 708 involved in sugarcane bagasse degradation in *Trichoderma reesei*. *Biotechnol Biofuels* 11:84.  
56  
57  
58 709 Özcan S. Johnston M. (1999) Function and regulation of yeast hexose transporters. *Microbiol*  
59  
60 710 *Mol Biol Rev* 63:554-569.  
61  
62  
63  
64  
65



1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

711 Paulsen PA, Custódio TF, Pedersen BP (2019) Crystal structure of the plant symporter STP10  
712 illuminates sugar uptake mechanism in monosaccharide transporter superfamily. Nat  
713 Commun. 10:407. doi: 10.1038/s41467-018-08176-9.

714 Peng M, Aguilar-Pontes MV, de Vries RP, Mäkelä MR (2018) In silico analysis of putative  
715 sugar transporter genes in *Aspergillus niger* using phylogeny and comparative  
716 transcriptomics. Front Microbiol 9:1045.

717 Popper ZA, Tuohy MG (2010) Beyond the green: understanding the evolutionary puzzle of  
718 plant and algal cell walls. Plant Physiol 153:373-83. doi:10.1104/pp.110.158055.

719 Protzko RJ, Latimer LN, Martinho Z, de Reus E, Seibert T, Benz JP, Dueber JE (2018).  
720 Engineering *Saccharomyces cerevisiae* for co-utilization of D-galacturonic acid and D-  
721 glucose from citrus peel waste. Nat Commun 9:5059. [https://doi.org/10.1038/s41467-018-](https://doi.org/10.1038/s41467-018-07589-w)  
722 [07589-w](https://doi.org/10.1038/s41467-018-07589-w)

723 Rentsch D, Laloi M, Rouhara I, Schmelzer E, Delrot S, Frommer WB (1995) NTR1 encodes a  
724 high affinity oligopeptide transporter in *Arabidopsis*. FEBS Lett 370:264-268.  
725 doi:10.1016/0014-5793(95)00853-2

726 Reznicek O, Facey SJ, de Waal PP, Teunissen AW, de Bont JA, Nijland JG, Driessen AJ,  
727 Hauer B (2015) Improved xylose uptake in *Saccharomyces cerevisiae* due to directed  
728 evolution of galactose permease *Gal2* for sugar co-consumption. J Appl Microbiol 119:99-  
729 111. doi: 10.1111/jam.12825. Epub 2015 May 19.

730 Riley MI, Sreekrishna K, Bhairi S, Dickson RC (1987) Isolation and characterization of  
731 mutants of *Kluyveromyces lactis* defective in lactose transport. Mol Gen Genet 208:145-151.  
732 doi:10.1007/BF00330435

733 Rose AB, Broach JR (1990) Propagation and expression of cloned genes in yeast: 2-microns  
734 circle-based vectors. Methods Enzymol 185:234-279. doi:10.1016/0076-6879(90)85024-i

735 Rottmann T, Zierer W, Subert C, Sauer N, Stadler R (2016) STP10 encodes a high-affinity  
1  
2 736 monosaccharide transporter and is induced under low-glucose conditions in pollen tubes of  
3  
4  
5 737 *Arabidopsis*. J Exp Bot 67:2387–2399. doi: 10.1093/jxb/erw048  
6  
7  
8 738 Saier MH, Reddy VS, Tsu BV, Ahmed MS, Li C, Moreno-Hagelsieb G (2016) The  
9  
10 739 Transporter Classification Database (TCDB): recent advances. Nucleic Acids Res 44:D372–  
11  
12 740 379.  
13  
14  
15 741 Sambrook JF, Russell D (2001). Molecular cloning: a laboratory manual. Cold Spring Harbor  
16  
17  
18 742 Laboratory Press, Cold Spring Harbor, NY, U.S.A.  
19  
20  
21 743 Schädel C, Blöchl A, Richter A, Hoch G (2010) Quantification and monosaccharide  
22  
23 744 composition of hemicelluloses from different plant functional types. Plant Physiol Biochem.  
24  
25 745 48:1-8.  
26  
27  
28 746 Sloothaak J, Odoni DI, Martins Dos Santos VA, Schaap PJ, Tamayo-Ramos JA (2016)  
29  
30  
31 747 Identification of a novel L-rhamnose uptake transporter in the filamentous fungus *Aspergillus*  
32  
33 748 *niger*. PLoS Genet 12:e1006468. doi:10.1371/journal.pgen.1006468  
34  
35  
36 749 Sloothaak, J., Tamayo-Ramos, J. A., Odoni, D. I., Laothanachareon, T., Derntl, C., Mach-  
37  
38  
39 750 Aigner, A. R., Martins Dos Santos, V., Schaap, P. J. (2016). Identification and functional  
40  
41 751 characterization of novel xylose transporters from the cell factories *Aspergillus niger* and  
42  
43 752 *Trichoderma reesei*. Biotechnol Biofuels 9:148. <https://doi.org/10.1186/s13068-016-0564-4>  
44  
45  
46  
47 753 Slot JC, Hallstrom KN, Matheny PB, Hibbett DS (2007) Diversification of NRT2 and the  
48  
49 754 origin of its fungal homolog. Mol Biol Evol 24:1731-1743. doi:10.1093/molbev/msm098  
50  
51  
52 755 Sprouffske K, Wagner A (2016) Growthcurver: A R package for obtaining interpretable  
53  
54 756 metrics from microbial growth curves. BMC Bioinformatics. 17:172. doi:10.1186/s12859-  
55  
56  
57 757 016-1016-7  
58  
59  
60  
61  
62  
63  
64  
65

758 Subtil T, Boles E (2012) Competition between pentoses and glucose during uptake and  
1  
2 759 catabolism in recombinant *Saccharomyces cerevisiae*. *Biotechnol Biofuels* 5:14.  
3  
4  
5 760 doi:10.1186/1754-6834-5-14  
6  
7  
8 761 Takanaga H, Frommer WB (2010) Facilitative plasma membrane transporters function during  
9  
10 762 ER transit. *FASEB J* 24:2849-2858.  
11  
12  
13 763 Tran Nguyen Hoang P, Ko JK, Gong G, Um Y, Lee SM (2018) Genomic and phenotypic  
14  
15 764 characterization of a refactored xylose-utilizing *Saccharomyces cerevisiae* strain for  
16  
17 765 lignocellulosic biofuel production. *Biotechnol Biofuels* 11:268. doi:10.1186/s13068-018-  
18  
19 766 1269-7  
20  
21  
22  
23 767 Trichez D, Knychala MM, Figueiredo CM, Alves SL Jr, da Silva MA, Miletti LC, de Araujo  
24  
25 768 PS, Stambuk BU (2019) Key amino acid residues of the AGT1 permease required for  
26  
27 769 maltotriose consumption and fermentation by *Saccharomyces cerevisiae*. *J Appl Microbiol*  
28  
29 770 126:580-594. doi: 10.1111/jam.14161  
30  
31  
32  
33  
34 771 Wang M, Yu C, Zhao H (2016) Directed evolution of xylose specific transporters to facilitate  
35  
36 772 glucose-xylose co-utilization. *Biotechnol Bioeng* 113:484-491. doi:10.1002/bit.25724  
37  
38  
39 773 Wieczorke R, Krampe S, Weierstall T, Freidel K, Hollenberg CP, Boles E (1999) Concurrent  
40  
41 774 knock-out of at least 20 transporter genes is required to block uptake of hexoses in  
42  
43 775 *Saccharomyces cerevisiae*. *FEBS Lett* 464:123-128. doi:10.1016/s0014-5793(99)01698-1  
44  
45  
46  
47 776 Wieczorke R, Dlugai S, Krampe S, Boles E (2003) Characterisation of mammalian GLUT glucose  
48  
49 777 transporters in a heterologous yeast expression system. *Cell Physiol Biochem*. 13:123-134. doi:  
50  
51 778 10.1159/000071863  
52  
53  
54 779 Wisedchaisri G, Park MS, Iadanza MG, Zheng H, Gonen T (2014) Proton-coupled sugar  
55  
56 780 transport in the prototypical major facilitator superfamily protein Xyle. *Nat Commun* 5:4521.  
57  
58 781 doi: 10.1038/ncomms5521.  
59  
60  
61  
62  
63  
64  
65

782 Wolf K, Sauer N, Tanner W (1991) The *Chlorella* H<sup>+</sup>/hexose co-transporter gene. *Curr Genet*  
1  
2 783 19:215–219.  
3  
4  
5 784 Yadav RK, Barbi F, Ziller A, Luis P, Marmeisse R, Reddy MS, Fraissinet-Tachet L (2014)  
6  
7 785 Construction of sized eukaryotic cDNA libraries using low input of total environmental  
8  
9 786 metatranscriptomic RNA. *BMC Biotechnol* 14:80. doi:10.1186/1472-6750-14-80  
10  
11  
12  
13 787 Young E, Poucher A, Comer A, Bailey A, Alper H (2011) Functional survey for heterologous  
14  
15 788 sugar transport proteins, using *Saccharomyces cerevisiae* as a host. *Appl Environ Microbiol*  
16  
17 789 77:3311–3319. doi: 10.1128/AEM.02651-10  
18  
19  
20  
21 790 Young EM, Tong A, Bui H, Spofford C, Alper HS (2014) Rewiring yeast sugar transporter  
22  
23 791 preference through modifying a conserved protein motif. *Proc Natl Acad Sci U S A.* 111:131-  
24  
25 792 136.  
26  
27  
28  
29 793 Zhang C, Acosta-Sampson L, Yu VY, Cate JHD (2017) Screening of transporters to improve  
30  
31 794 xylohextratin utilization in the yeast *Saccharomyces cerevisiae*. *PLoS ONE* 12:e0184730.  
32  
33 795 <https://doi.org/10.1371/journal.pone.0184730>  
34  
35  
36  
37 796 Zhang W, Cao Y, Gong J, Bao X, Chen G, Liu W (2015) Identification of residues important  
38  
39 797 for substrate uptake in a glucose transporter from the filamentous fungus *Trichoderma reesei*.  
40  
41 798 *Sci Rep* 5:13829.  
42  
43  
44  
45 799 Zhou JJ, Theodoulou F, Sauer N, Sanders D, Miller AJ (1997) A kinetic model with ordered  
46  
47 800 cytoplasmic dissociation for SUC1, an *Arabidopsis* H<sup>+</sup>/sucrose cotransporter expressed in  
48  
49 801 *Xenopus* oocytes. *J Membr Biol* 159:113–125.  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

## 802 **Legends of the Figures**

1  
2 803 **Fig. 1** Phylogenetic diversity of 104 functionally characterized fungal sugar transporters  
3  
4 804 belonging to the Sugar Porter (SP) family. The maximum likelihood (PhyML) tree was rooted  
5  
6  
7 805 using the environmental spB4 protein that belongs to the “Fucose H<sup>+</sup> symporter” family.  
8  
9  
10 806 Only bootstrap values (1000 replicates) above 80% are indicated and proteins were grouped in  
11  
12 807 13 clades (I to XIII). Colors refer to the taxonomic origin of the proteins, red, *Ascomycota*;  
13  
14 808 blue, *Basidiomycota* and black, *Glomeromycotina*. Proteins were named according to the  
15  
16 809 species they come from and the category of molecules they transport; M, monosaccharides  
17  
18  
19 810 and related molecules; I, *myo*-inositol; O, di and oligosaccharides; S, sucrose; MO, both mono  
20  
21  
22 811 and disaccharides. Full correspondence between protein names, species names, amino acid  
23  
24 812 sequences and accession numbers are given in Online Resource 3. Asterisks highlight  
25  
26 813 environmental sequences selected from soil cDNA libraries. For legibility, we excluded from  
27  
28  
29 814 the final phylogenetic analysis several characterized sequences from the yeasts  
30  
31  
32 815 *Saccharomyces* sp. that in preliminary analyses were all closely related to the sequences from  
33  
34 816 these species retained for the final analysis.

35  
36 817 **Fig. 2** Putative taxonomic origins of the 19 environmental sugar transporter proteins retrieved  
37  
38  
39 818 from soil metatranscriptomic cDNA libraries. The phylogenetic (PhyML) tree encompasses  
40  
41 819 all 19 environmental sequences (asterisks) and their 10 best hits following BLASTp searches  
42  
43  
44 820 in the GenBank database (144 reference sequences in total). Colors refer to the taxonomic  
45  
46 821 origin of the proteins, red, *Ascomycota*; blue, *Basidiomycota* and black, *Chytridiomycota*  
47  
48 822 (*Monoblepharidomycetes*). Only bootstrap values (1000 replicates) above 80% are indicated.

49  
50  
51 823 **Fig. 3** Growth of recombinant *S. cerevisiae* EBYVW4000 strain expressing each of the 19  
52  
53 824 environmental transporter proteins (spS1-12; spB1-7) in liquid media containing 3  
54  
55  
56 825 concentrations (1; 0.5 or 0.1%) of 4 D(+)-monosaccharides (glu, glucose; gal, galactose, fru,  
57  
58 826 fructose and man, mannose). “Area under the curves” were measured after four days’ growth  
59  
60  
61  
62  
63  
64  
65

1 827 in microplates and used to draw a heatmap where sugar transporters clustered according to the  
2 828 growth profile of the recombinant yeast strains. Panels on the right illustrate the growth  
3  
4 829 profiles on the different sugar concentrations (as well as on 1% maltose used as a positive  
5  
6  
7 830 growth control) for recombinant yeasts expressing 3 genes, each representative of one of the  
8  
9 831 three main clusters observed in the heat map (spS1 for cluster 1, spS2 for cluster 2 and spS11  
10  
11 832 for cluster 3).

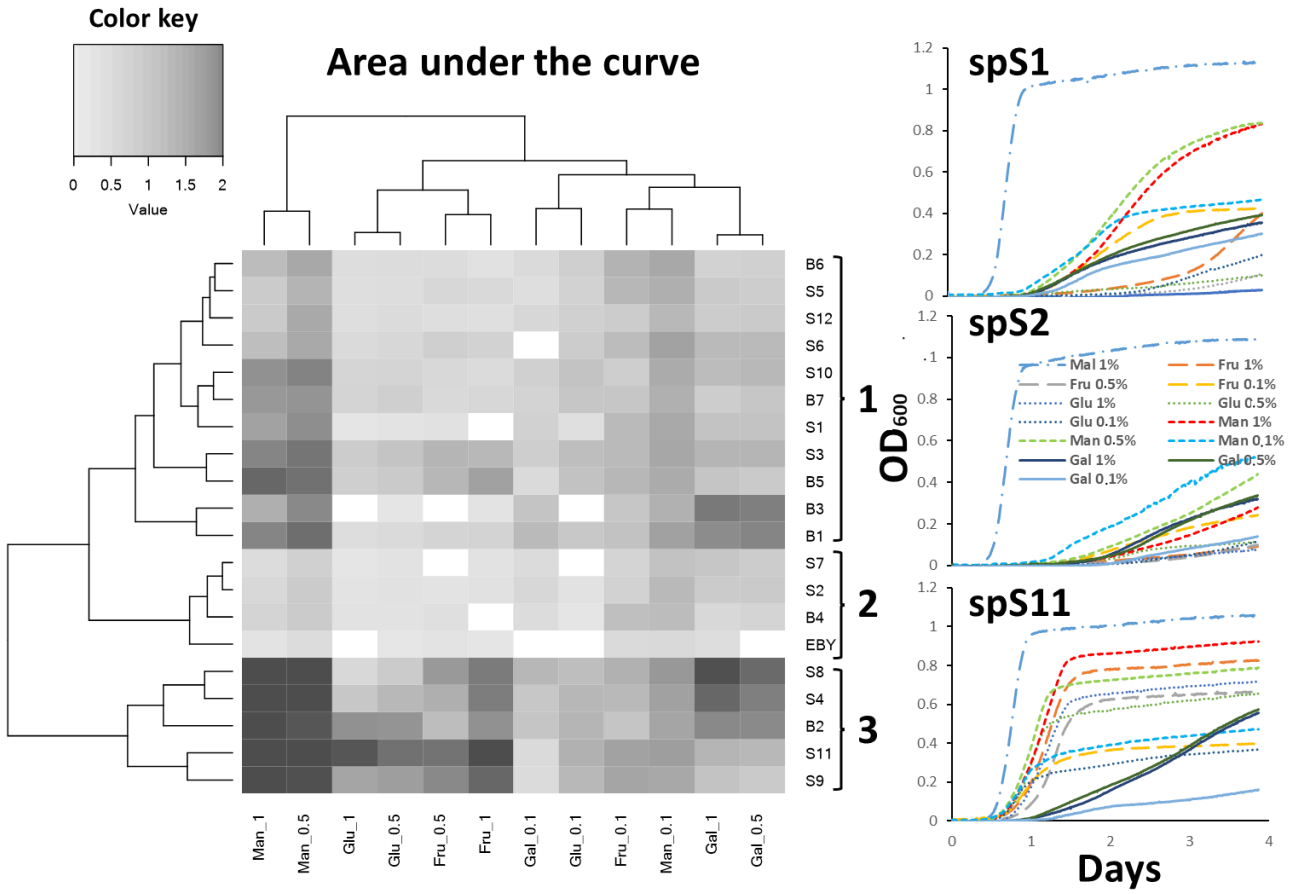
12  
13  
14 833 **Fig. 4** Mannose sugar porters spS1 and spS2 induce sugar-specific pH-dependent currents  
15  
16 834 when heterologously expressed in *Xenopus laevis* oocytes. (A,B) Representative examples of  
17  
18  
19 835 mannose-induced currents in oocytes expressing spS1 (A) or spS2 (B), respectively, recorded  
20  
21  
22 836 by two-electrode voltage-clamp in presence of 10 mM mannose at pH 5.5. To a similar but  
23  
24 837 slightly minor extent, also other substrates are transported, as fructose by spS1 (A), or glucose  
25  
26  
27 838 by spS2 (B). (C) Mean current amplitudes induced by different sugar substrates in oocytes  
28  
29 839 expressing spS1 or spS2. Current amplitudes induced by different substrates have been  
30  
31 840 determined at -100 mV after subtracting initially endogenous background currents recorded in  
32  
33  
34 841 control solution without the added substrate. Mannose-induced currents measured at pH 5.5  
35  
36 842 (“mann” S1 n=24, N=7; S2 n=16, N=7) are shown to be pH-dependent as no current induction  
37  
38  
39 843 was observed at pH 7.5 (“mann 7,5” S1 and S2 n=3, N=2) and to be conformation-dependent  
40  
41 844 (“Lmann” S1 n=3, S2 n=4). Glucose was transported by spS2 (“gluc” S1 n=10, N=4; S2 n=6,  
42  
43  
44 845 N=5), fructose by spS1 (“fruct” S1 n=5, N=3; S2 n=6, N=5). Galactose (“gal” S1 and S2 n=3)  
45  
46 846 and xylose (“xyl” S1 and S2 n=3) induced currents by both transporters.  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65



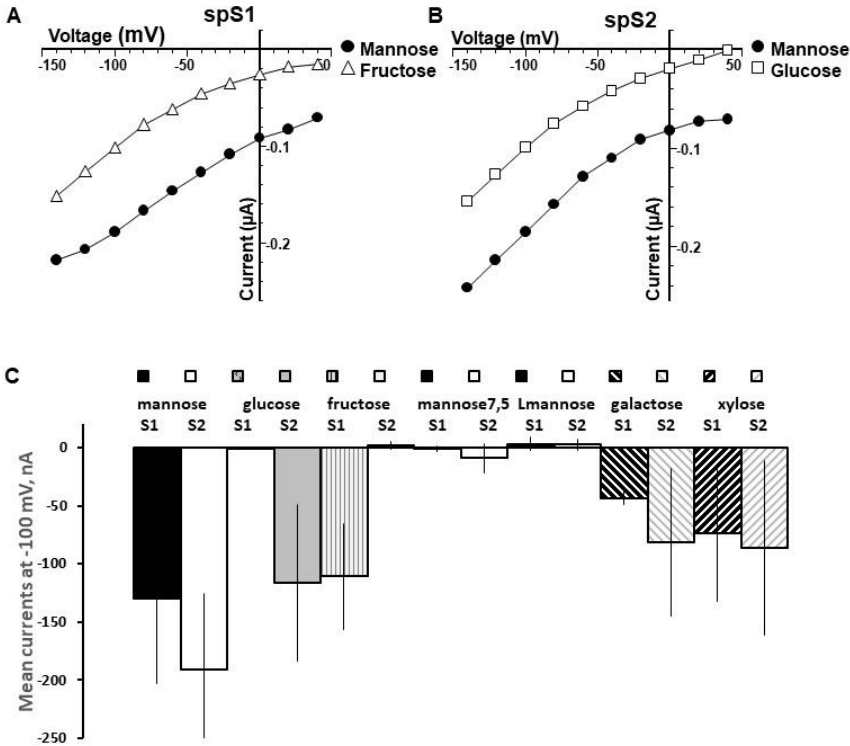




# Fig. 3



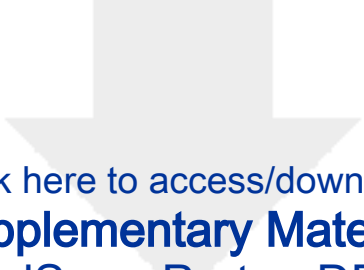
# Fig. 4





Click here to access/download  
**Supplementary Material**  
SugarTransporterOR.pdf





Click here to access/download  
**Supplementary Material**  
FungalSugarPortersDB.xlsx

