FSY1, a horizontally transferred gene in the *Saccharomyces cerevisiae* EC1118 wine yeast strain, encodes a high-affinity fructose/H⁺ symporter

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Transport of glucose and fructose in the yeast *Saccharomyces cerevisiae* plays a crucial role in controlling the rate of wine fermentation. In *S. cerevisiae*, hexoses are transported by facilitated diffusion via hexose carriers (Hxt), which prefer glucose to fructose. However, utilization of fructose by wine yeast is critically important at the end of fermentation. Here, we report the characterization of a fructose transporter recently identified by sequencing the genome of the commercial wine yeast strain EC1118 and found in many other wine yeasts. This transporter is designated Fsy1p because of its homology with the *Saccharomyces pastorianus* fructose/H⁺ symporter Fsy1p. A strain obtained by transformation of the V5 *hxt1-7Δ* mutant with *FSY1* grew well on fructose, but to a much lesser extent on glucose as the sole carbon source. Sugar uptake and symport experiments showed that *FSY1* encodes a proton-coupled symporter with high affinity for fructose (K_m 0.24 ± 0.04 mM). Using real-time RT-PCR, we also investigated the expression pattern of *FSY1* in EC1118 growing on various carbon sources. *FSY1* was repressed by high concentrations of glucose or fructose and was highly expressed on ethanol as the sole carbon source. The characteristics of this transporter indicate that its acquisition could confer a significant advantage to *S. cerevisiae* during the wine fermentation process. This transporter is a good example of acquisition of a new function in yeast by horizontal gene transfer.

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INTRODUCTION

The ability of the yeast *Saccharomyces cerevisiae* to degrade sugars into ethanol and carbon dioxide has been used by humans for several millennia for the fermentation of wine and beer, and in baking. Indeed, grape must contains large amounts of glucose and fructose, up to 300 g l⁻¹, in equimolar quantities. *S. cerevisiae* prefers glucose over fructose, and glucose is hence the first sugar to be depleted from the medium during fermentation (Berthels *et al.*, 2004; Gafner & Schütz, 1996). A high fructose/glucose ratio may therefore cause sluggish and stuck fermentations with high levels of fructose remaining in the finished wines, which is a major problem in the wine industry (Bisson, 1999; Gafner & Schütz, 1996).

The first essential step in the utilization of hexose sugars is their uptake by yeast cells. In yeast, there are two types of hexose transporters (Leandro *et al.*, 2009): (i) facilitated diffusion carriers, which use an energy-independent

mechanism that mediates transport of sugar molecules down their concentration gradient, and (ii) energy-dependent active proton-sugar symporters, which operate only when sugar concentration is low, and which couple the uptake of sugar molecules to the uptake of protons. The latter uptake system becomes important during growth at low extracellular sugar concentrations.

Hexose transport in *S. cerevisiae* occurs only via facilitated diffusion carriers and these are encoded by several genes, including the *HXT* genes (Kruckeberg, 1996; Lagunas, 1993). There are 17 *HXT* genes in *S. cerevisiae*, but only seven of these, *Hxt1p*–*Hxt7p*, are required for growth on glucose or fructose (Boles & Hollenberg, 1997; Luyten *et al.*, 2002; Reifemberger *et al.*, 1997). The proteins encoded by these seven genes have different kinetic properties (K_m from 1 to 300 mM) and are subject to different modes of regulation. All of the hexose transporters in *S. cerevisiae* can also transport fructose, but they

generally have a high K_m for this sugar, indicating glucose to be the preferential sugar for Hxt carriers (Bisson & Fraenkel, 1983; Reifemberger *et al.*, 1997).

In contrast, *Zygosaccharomyces bailii*, a fructophilic yeast, has a low-affinity facilitator protein specific for fructose, the Ffz1p transporter (Piña *et al.*, 2004). Fructose may also be transported by other transport systems, such as Fsy1p in *Saccharomyces pastorianus*, the gene of which was the first encoding a specific fructose permease to be identified in yeast. This transporter mediates active fructose uptake by a proton symport mechanism and does not accept glucose as a substrate (Gonçalves *et al.*, 2000). Two other specific fructose/H⁺ symporters were subsequently characterized in *Kluyveromyces lactis* (Frt1p) and in the filamentous fungus *Botrytis cinerea* (Diezemann & Boles, 2003; Doehlemann *et al.*, 2005). Studies have aimed to establish the repertoire of sugar transporters in yeast, by using functional characterization of proteins or phylogenetic analyses. They have shown that such active fructose/H⁺ symporters, although absent in the model yeast *S. cerevisiae* (Rodrigues de Sousa *et al.*, 1995), are actually present in other hemiascomycetous yeasts and in filamentous fungi (Leandro *et al.*, 2009; Palma *et al.*, 2007).

We have recently reported the complete genome sequence of the commercial wine yeast strain EC1118 (Novo *et al.*, 2009). Comparative analysis of this genome with that of the laboratory strain S288C revealed the presence of 34 new genes, grouped into three large clusters located in subtelomeric or internal chromosomal positions. Phylogeny and synteny analyses showed that these gene clusters were acquired by several events of horizontal transfer, two of them (regions A and B) from non-*Saccharomyces* species and the third (region C) from a species closely related to *S. cerevisiae*. These genes potentially have key functions in fermentation, such as metabolism of sugars and nitrogen. Among them, *IO4_6634g* from region C, encoded a protein highly similar to that encoded by the *S. pastorianus*-specific fructose symporter *FSY1* gene (Novo *et al.*, 2009). The aim of the present study was to characterize the *EC-IO4_6634g* gene, which we designated *FSY1*. We report the functional analysis of this gene and demonstrate that it encodes a high-affinity fructose/H⁺ symporter. In addition, we studied the regulation of this gene during growth on various carbon sources by quantitative RT-PCR. We show that *FSY1* expression is repressed by high concentrations of glucose or fructose and is induced by ethanol as the sole carbon source.

METHODS

Yeast strains and culture conditions. The yeast strains used in this study are listed in Table 1. Yeast cells were grown at 28 °C on YP medium (1 % Bacto yeast extract, 2 % Bacto peptone) or on synthetic (SD) minimal medium (0.67 % yeast nitrogen base without amino acids, 50 mg uracil l⁻¹) containing either 2 % maltose or different amounts of various carbon sources.

For drop-test assays, cells in the exponential phase were pelleted and resuspended in sterile water to an OD₆₀₀ of 1. Cells were serially

diluted in 10-fold steps, and 10 µl of each dilution was spotted onto a control SD agar plate containing 2 % (w/v) maltose and onto SD agar plates containing 0.2, 2 or 20 % (w/v) of either fructose or glucose.

For ¹⁴C-labelled sugar uptake and symport experiments, cells were cultured in SD minimal medium supplemented with the appropriate carbon source and were subsequently harvested during the mid-exponential phase of growth (OD₆₀₀ 0.7–0.9) by centrifugation and then washed twice with demineralized ice-cold water. Cells were resuspended in citrate buffer and equilibrated at 28 °C prior to uptake experiments (Spencer-Martins & Van Uden, 1985).

V5 *hxt1-7Δ* cells were pre-grown in 2 % maltose, and resuspended to an OD₆₀₀ of ~0.5 in fresh medium containing either 0.5 % (w/v) fructose or 5 % (w/v) glucose. Cells were then incubated at 28 °C for 2 h with shaking and subsequently harvested for sugar transport assays and symport experiments.

Strain construction. The *FSY1* gene from strain EC1118 was introduced into strain V5 *hxt1-7Δ* by genomic integration at the *HXT3* locus. A 2.18 kb DNA fragment was amplified from EC1118 genomic DNA by PCR (cycling conditions: 40 s at 95 °C, 30 s at 50 °C, 5 min at 68 °C; 25 cycles) by using the Expand High Fidelity PCR system (Boehringer Mannheim) and the primers 5'-*atagaatca-caaacaatttacatctgagttaaacaatcatggactgtaataactctgaggatt*-3', corresponding to the *HXT3* promoter (italics) and to nucleotides +1 to +25 of the *FSY1* gene (underlined), and *aagtacggcgatgagtaagaa-gaaataactgactcattagatagcctagtttgggtggtcatct*, corresponding to the *HXT3* terminator (italics) and to nucleotides +2078 to +2100 of the *FSY1* gene (underlined). The PCR product was used to transform V5 *hxt1-7Δ* by the lithium acetate method (Gietz & Woods, 2002). Transformants were selected on SD agar plates containing 50 mg uracil l⁻¹ and either a low concentration of fructose (0.5 or 1 %, w/v) or 2 % (w/v) sucrose.

This construct allowed integration of *FSY1* under the control of the *HXT3* promoter. Integration of the *FSY1* ORF was verified by PCR analysis of total DNA.

Phylogenetic analysis. Protein sequence alignment was built by CLUSTAL X with homologues retrieved by BLASTP searches against the Fungal Genome and Genolevures databases. Grouping was achieved by using a threshold *E* value of <10⁻⁶⁰. For comparison, hexose transporters of *S. cerevisiae* were included. Phylogenetic analysis was done via PhyML v3.0 by using the WAG+G+I model (shape parameter, 2.282; proportion of invariant sites, 0.084) (Guindon & Gascuel, 2003).

Sugar-uptake and symport experiment. Transport of D-[U-¹⁴C]glucose and D-[U-¹⁴C]fructose was measured according to the procedures described by Spencer-Martins & Van Uden (1985). Kinetic parameters were obtained from activity measurements with fructose and glucose concentrations that ranged from 0.05 to 1.4 mM and 0.2 to 80 mM, respectively. Eady-Hofstee plots were obtained with Excel software and kinetic parameters were determined by non-linear regression (Michaelis-Menten equation) by using GraphPad Prism (version 5.02 for Windows, GraphPad Software).

All values reported are expressed as the mean ± SD of three independent experiments.

Proton symport activity was studied by using a pH electrode to record the transient alkalification of an aqueous cell suspension following the addition of sugar, as described by Loureiro-Dias & Peinado (1984).

Quantitative real-time RT-PCR. Yeast cells were grown at 28 °C on YP medium with different amounts of various carbon sources to an OD₆₀₀ of 0.9 or 3. Total RNA was extracted from 10⁹ yeast cells, by using TRIzol reagent (Invitrogen), and was purified on RNeasy spin

Table 1. *S. cerevisiae* strains

Strain	Description	Reference/source
V5	<i>MATa ura3 gal</i>	V. Galeote
V5 <i>hxt1-7Δ</i>	V5 <i>hxt514::kanMX hxt367::kanMX hxt2::kanMX</i>	Luyten <i>et al.</i> (2002)
V5 <i>hxt1-7Δ/FSY1</i>	V5 <i>hxt514::kanMX hxt367::FSY1 hxt2::kanMX</i>	This study
Lalvin EC1118	Champagne wine strain	Lallemand Inc.

columns (Qiagen). Purified RNA was quantified with a spectrophotometer (Nanodrop 1000; ThermoScientific) and its integrity was assessed by electrophoresis. Purified RNA was treated with DNase I (Invitrogen) and first-strand cDNA synthesis was carried out by using SuperScript III reverse transcriptase (Invitrogen) and random primers, in accordance with the manufacturer's protocol. Quantitative PCRs were performed in triplicate on an ABI 7300 real-time PCR system (Applied Biosystems), using Power SYBR Green PCR Master Mix (Applied Biosystems) and gene-specific primers. The *SCR1* gene was amplified as an internal control. The DNA sequences of the primers were as follows: *FSY1*, gctttgctaactcgccacat (F) and aagcagctgctgcaaatctgt (R); *SCR1*, tctggtgcggcaagtagtt (F) and caccttgctgacgtggt (R).

The comparative C_t method was used for quantification of gene expression (Livak & Schmittgen, 2001). Gene expression was normalized with respect to the expression of *SCR1*, a non-coding RNA gene with a constant level of expression (data not shown). Normalized expression levels were compared with the lowest level of expression of *FSY1*, which occurred at a concentration of 2 % glucose at an OD₆₀₀ of 0.9.

RESULTS

Phylogenetic analysis of the *FSY1* gene

Analysis of the genome sequence of strain EC1118 revealed 34 new genes that were not present in the S288C laboratory strain (Novo *et al.*, 2009). A search of the UniProt-KB, GenBank and Genolevures databases identified homologous proteins. Of these, the *S. pastorianus* fructose-specific symporter Fsy1p had 78 % amino-acid similarity with the protein encoded by strain EC1118 (1O4_6634g). This gene, designated *FSY1*, consists of a 1701 bp ORF encoding a predicted protein of 567 amino acids.

Phylogenetic analysis showed a significant level of similarity with other yeast high-affinity fructose transporters (Fig. 1). We observed two distinct clusters, one including three functionally characterized high-affinity fructose symporters (Fsy1p from *S. pastorianus* and the two Frt1p proteins from *K. lactis* and *B. cinerea*) and several non-characterized sequences, and the other including the hexose facilitator proteins. The highest level of similarity was with *S. pastorianus* and *Saccharomyces uvarum* transporters (78 % amino acid similarity), while that with *K. lactis* and *B. cinerea* Frt1p was lower (66 and 44 %, respectively).

Functional analysis of the *FSY1* gene

To examine the functionality of the *FSY1* gene, we expressed *FSY1* under the control of the *HXT3* promoter,

in strain V5 *hxt1-7Δ*. This strain lacks the hexose transporter genes *HXT1* to *HXT7* and cannot utilize glucose, fructose or sucrose as sole carbon source (Luyten *et al.*, 2002). As *S. pastorianus* Fsy1p has a low K_m for fructose (Gonçalves *et al.*, 2000), transformants were selected on minimal medium containing uracil and either a low concentration of fructose (0.5 or 1 %) or 2 % sucrose (which was converted into small amounts of glucose and fructose by invertase). Strains with the integrated *FSY1* gene were obtained on all these media. The *FSY1* gene was therefore able to complement the growth deficiency of the *hxt1-7Δ* mutant, indicating that it encodes a hexose transporter.

We examined the growth of one of these strains in comparison with V5 and V5 *hxt1-7Δ* by using drop-test assays on plates containing minimal medium with different concentrations of either glucose or fructose. As expected, V5 *hxt1-7Δ* could not grow on glucose or fructose as sole carbon source, whereas all the strains grew well on maltose, which is taken up by a specific transport system (Fig. 2). *FSY1* expression was able to complement the growth deficiency of the *hxt1-7Δ* mutant on fructose, albeit only partially on 20 % fructose medium (Fig. 2b). Similar properties have been reported for the *S. pastorianus* and *K. lactis* high-affinity fructose/H⁺ symporters (Diezemann & Boles, 2003; Gonçalves *et al.*, 2000).

Expression of the *FSY1* gene also partially restored growth of V5 *hxt1-7Δ* on glucose, although to a considerably lesser extent than on fructose (Fig. 2b). Growth of V5, V5 *hxt1-7Δ* and V5 *hxt1-7Δ/FSY1* was also monitored in liquid SD medium supplemented with uracil and containing different carbon sources (Fig. 3). *FSY1* restored the growth of V5 *hxt1-7Δ* in 0.5 and 5 % fructose and in 5 % glucose, thus confirming the result of the drop test assays.

Symport activity

Fructose/H⁺ symport activity was measured by alkalization of cell suspensions after the addition of sugars. Proton influx resulting in external alkalization was observed when fructose was added at a final concentration of 5 mM to aqueous suspensions of cells expressing *FSY1* that were pre-grown in fructose medium (Fig. 4a), as indicated by the increase in alkalization rate triggered by addition of fructose. Activation of the plasma membrane ATPase resulting from fructose metabolism was also subsequently observed, as expected. Fructose failed to elicit changes in the net rate of proton movements for V5 *hxt1-7Δ*, strongly

Fig. 1. Phylogenetic relationship between the *S. cerevisiae* Fsy EC1118 protein (in bold) and other fungal transporters, showing the Hxt and Fsy1 clusters. GenBank accession numbers or ORF codes are indicated after the species designation. Protein acronyms are only noted for transporters that have been biochemically characterized. The *Ajellomyces capsulatus* putative *myo*-inositol transporter is the closest relative of the Fsy1 cluster proteins, as judged by BLAST searches. Numbers at nodes are bootstrap values based on 1000 replicates; only values >50 % are shown.

suggesting that this strain is unable to transport or metabolize fructose (Fig. 4a). These results demonstrate that *FSY1*, like the *FSY1* gene of *S. pastorianus* and the *FRT1* gene, encodes a proton-coupled fructose transporter. Addition of L-sorbose to aqueous suspensions of cells also produced proton influx but because this sugar is not metabolized we did not observe a subsequent decrease in

pH (data not shown). Similar results have been obtained with the *FSY1* gene of *S. pastorianus* (Cason *et al.*, 1986).

Weak acidification was observed when V5 *hxt1-7Δ/FSY1* cells were grown on glucose (Fig. 4b), consistent with the partial restoration of growth observed on glucose plates or liquid medium. However, alkalization was not observed,

Fig. 2. Functional analysis of the *FSY1* fructose transporter expressed in *S. cerevisiae* *hxt1-7Δ* strain. The V5 strain (1) served as a wild-type control. *FSY1* under control of the *HXT3* promoter (3) was integrated into the isogenic *hxt1-7Δ* mutant. The V5 *hxt1-7Δ* strain (2) was used as a negative control. The different yeast strains were grown for 2 days on solid minimal medium supplemented with 2 % maltose (a) or different concentrations of fructose or glucose (b).

Fig. 3. Growth of V5 *hxt1-7Δ* expressing the *FSY1* gene on synthetic medium with different carbon sources. Growth was monitored by measuring OD₆₀₀ at the times indicated. ■, 5 % fructose; ◆, 0.5 % fructose; ▲, 5 % glucose; △, 0.5 % glucose; ●, V5 *hxt1-7Δ* on 5 % fructose. A zoom of the OD₆₀₀ area from 0 to 0.4 is also shown.

possibly because the transport capacity for glucose is very low (see next section).

Kinetic parameters of hexose transport

Sugar uptake was measured in V5 *hxt1-7Δ/FSY1* cells grown on synthetic medium containing 0.5 % fructose. As a control, sugar uptake was measured in V5 *hxt1-7Δ* cells

that were grown on the same medium containing 2 % maltose and then incubated in 0.5 % fructose for 2 h.

The kinetic parameters of fructose uptake mediated by *FSY1* were determined in strain V5 *hxt1-7Δ* expressing *FSY1*. K_m values of 0.24 ± 0.04 mM with a V_{max} of 0.93 ± 0.08 mmol h⁻¹ (g dry weight)⁻¹ were obtained. No uptake of fructose could be measured in strain V5 *hxt1-7Δ*, indicating that the transport kinetic constants determined with the V5 *hxt1-7Δ/FSY1* strain represent the kinetic properties of the *S. cerevisiae* EC1118 Fsy1p carrier. Although the K_m value was somewhat higher for *FSY1*, it is consistent with the values previously reported (0.16 ± 0.02 mM) for Fsy1p of *S. pastorianus* and for Frt1p of *K. lactis* (Diezemann & Boles, 2003; Gonçalves *et al.*, 2000). We also tried to measure glucose transport (D-[U-¹⁴C]glucose concentration between 0.2 and 80 mM) in cells pre-grown under different conditions, but no significant glucose uptake was detected (results not shown). Furthermore, in competition experiments, realized by addition of 125 mM unlabelled glucose to D-[U-¹⁴C]fructose (concentration range 0.05–1.4 mM), glucose did not inhibit [¹⁴C]fructose uptake by yeast cells expressing *FSY1*; indeed, the same K_m and V_{max} values were obtained. This may be due to the extremely low affinity of this transporter for glucose.

Fig. 4. Energy-coupled transport by Fsy1p. Effects of the addition of sugars on the pH of an aqueous suspension of (a) V5 *hxt1-7Δ* cells pre-grown on maltose and incubated for 2 h in fructose medium (grey line) and V5 *hxt1-7Δ/FSY1* grown on fructose (dark line), and (b) V5 *hxt1-7Δ/FSY1* cells grown on 5 % glucose.

Expression of *FSY1* in EC1118

Expression of the *FSY1* gene in strain EC1118 was analysed in various growth conditions by quantitative real-time RT-PCR. Total RNA was purified from strain EC1118 grown with either 0.5 or 2 % fructose or glucose, 2 % ethanol or 2 % maltose, at two different growth stages (OD₆₀₀ 0.9 and 3). Transcript concentrations of *FSY1* are shown in Fig. 5(a). A lower transcript abundance was observed in glucose- or fructose-grown cells collected at an OD₆₀₀ of

Fig. 5. Quantitative RT-PCR of *FSY1* gene expression in strain EC1118 grown in YP medium containing different amounts of various carbon sources at 28 °C. All media were inoculated (initial OD₆₀₀ 0.1) with cells pre-grown on YP plus 2 % maltose. (a) Cells were collected at OD₆₀₀ 0.9 ± 0.04 (black bars) or OD₆₀₀ 3 ± 0.08 (grey bars). RNA was extracted and analysed by quantitative RT-PCR. The relative expression of *FSY1* was normalized to its expression in 2 % glucose at OD₆₀₀ 0.9. The results are expressed as the mean ± SD of relative expression from three repeat experiments. (b) Growth on hexoses was monitored by measuring the OD₆₀₀ at the times indicated. ▲, 2 % glucose; △, 0.5 % glucose; ■, 2 % fructose; ◆, 0.5 % fructose.

0.9 whereas the gene was highly expressed on ethanol at this growth stage. Transcript levels of *FSY1* increased with depletion of hexoses from the growth medium. This increase was particularly noticeable for cells grown on 0.5 % glucose or fructose at an OD₆₀₀ of 3 (at 8 h), when most of the sugar was exhausted, as suggested by the observation of a diauxic shift shortly after the sampling time point (at around 9 h) (Fig. 5b). The difference in transcript abundance in these two conditions was probably related to a difference in the residual sugar concentration.

These data indicate that expression of *FSY1* is repressed by high glucose or fructose concentrations and is up-regulated on non-fermentable carbon sources. A higher transcript concentration of *FSY1* was observed on maltose than on glucose, consistent with maltose being less effective in catabolite repression than glucose (Eraso & Gancedo, 1984).

DISCUSSION

The present study describes the functional characterization of an *S. cerevisiae*-specific fructose transporter, Fsy1p, discovered by genome sequencing of the wine yeast strain EC1118 and also found in many other wine yeast *S. cerevisiae* strains (Novo *et al.*, 2009). So far, only hexose uniporters have been identified in *S. cerevisiae*, which makes Fsy1p the first active fructose transporter carrier to be described in this species.

Novo *et al.* (2009) have analysed the distribution of the EC1118 genomic region containing the *FSY1* gene among *S. cerevisiae* clades. They propose that this 65 kb region was

obtained by horizontal gene transfer, probably from a species closely related to the genus *Saccharomyces*. No PCR amplification was obtained with primers based on the sequence of the *FSY1* gene with the strains dispersed among the ‘*Saccharomyces* complex’ described by Kurtzman & Robnett (2003) (data not shown). However, phylogenetic analysis of Fsy1p showed that the two closest homologues were the functionally characterized fructose/H⁺ symporter Fsy1p of *S. pastorianus*, and the structurally related fructose transporter of *S. uvarum* (78 % similarity). These findings are consistent with the hypothesis that the contributor of this region may be an as yet undescribed species very closely related to the genus *Saccharomyces* (Novo *et al.*, 2009).

Functional complementation experiments in V5 *hxt1-7Δ*, a mutant strain unable to ferment hexoses, and kinetic characterization revealed that Fsy1p of *S. cerevisiae* is a high-affinity fructose/H⁺ symporter with characteristics similar to those of *S. pastorianus* Fsy1p. The low growth observed in glucose medium and the weak acidification shown in symport experiments led to the assumption that this protein can also transport glucose. However, no uptake of glucose could be measured and no inhibition of fructose transport by glucose was observed. This might be explained by a very low affinity of this transporter for glucose, in line with the slightly improved growth of the V5 *hxt1-7Δ/FSY1* strain at high glucose concentrations. To identify small differences between the kinetic properties of the different Fsy1p homologues, it will be necessary to express all the proteins from the same promoter in the same strain background.

Expression of *S. cerevisiae* *FSY1* is regulated by the carbon source and was maximal during respiratory growth on

ethanol. Although the transcriptional regulation of *FSY1* is clearly repressed by glucose or fructose, this gene was expressed at detectable levels whatever the carbon source and the amount of sugar. A different regulation has been observed for the *FRT1* gene of *K. lactis*, which had no detectable expression in ethanol-grown cells and was not subject to sugar catabolic repression (Diezemann & Boles, 2003).

Compared with its close relative *S. pastorianus*, Fsy1p seems to be able to transport glucose, albeit to a much lesser extent. On the other hand, phylogenetically more distant transporters are not repressed by glucose (*K. lactis*) and were able to rescue growth of an *hxt*-null mutant in 2% glucose medium (Diezemann & Boles, 2003; Doehlemann *et al.*, 2005). In a recent review on the evolution of hexose transport across *Ascomycota* fungi, Leandro *et al.* (2009) proposed that the *FSY1* gene appeared early during evolution of ascomycetes and has subsequently been selectively lost in some lineages of both yeasts and moulds. In hemiascomycetes, putative orthologues of *FSY1* are well conserved through the CTG clade (a monophyletic clade within the *Saccharomycotina* containing organisms that translate CTG as serine instead of leucine) (Fitzpatrick *et al.*, 2006) and *Kluyveromyces* clade. Indeed, in addition to the putative orthologues shown in Fig. 1, five other species among these two clades have homologues to Fsy1p (*Lanchancea kluyveri*, *Pichia guilliermondii*, *Candida tropicalis*, *Candida dubliniensis*, *Lodderomyces elongisporus*) (data not shown). This phylogenetic tree combined with the differential regulation of the *Saccharomyces* and *K. lactis* fructose transporter genes suggest that the fructose transporter has undergone a clear functional divergence in the evolution of these different lineages. In light of these various studies, we can hypothesize that among the 'Saccharomyces complex', fructose/H⁺ symporters have been lost or have evolved a fine-tuned regulation that led to the emergence of a new function. Subsequently, some *S. cerevisiae* oenological strains might have gained an evolved gene by horizontal gene transfer.

The discrepancy in glucose and fructose utilization by *S. cerevisiae* is an essential step during oenological fermentation. The role and importance of sugar transport in oenological conditions were emphasized by Luyten *et al.* (2002), who showed that *HXT1*, *HXT2*, *HXT3*, *HXT6* and *HXT7* are required for optimal sugar fermentation in grape must. On the other hand, Guillaume *et al.* (2007) showed that the higher fructose utilization capacity of the Fermichamp wine yeast strain results from the expression of an allelic variant of *HXT3*. As fructose utilization is rate limiting at the end of fermentation, this allele has probably been retained in some wine yeasts because it confers a selective advantage during wine fermentation.

Similarly, *FSY1* might have been selected and retained in wine yeasts because of the selective advantage it may confer. Most of the new genes acquired by strain EC1118 are present in the Champagne group containing EC1118-related strains, and in a closely related group containing flor yeast (Novo

et al., 2009). *S. cerevisiae* flor yeasts form a film (or velum) on the surface of the wine, and develop an oxidative metabolism in the presence of a high ethanol concentration and low levels of fermentable sugar, essentially fructose (Berlanga *et al.*, 2001; Charpentier *et al.*, 2009). In light of the characteristics of the Fsy1p of strain EC1118, the acquisition of *FSY1* by horizontal transfer could confer an advantage either at the end of wine fermentation, when wine yeasts have to ferment this non-preferred sugar in the presence of large amounts of ethanol, or in the oxidative flor conditions.

Overall, these data highlight the potential role of environmental pressure on the acquisition of new functions by yeast. Fructose transport capacity and resistance to ethanol are key parameters for improving the fermentation performance of wine yeasts (Guillaume *et al.*, 2007; Santos *et al.*, 2008). Future studies on the role of Fsy1p in various wine-making situations and on its sensitivity to ethanol should provide further support to the hypothesis that the acquisition of this transporter by *S. cerevisiae* has an adaptive value in the wine environment.

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