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RESEARCH ARTICLE

Identification and analysis of sugar transporters capable of co-transporting glucose and xylose simultaneously

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Abstract

Simultaneous co-fermentation of glucose and xylose is a key desired trait of engineered *Saccharomyces cerevisiae* for efficient and rapid production of biofuels and chemicals. However, glucose strongly inhibits xylose transport by endogenous hexose transporters of *S. cerevisiae*. We identified structurally distant sugar transporters (*Lipomyces starkeyi* LST1_205437 and *Arabidopsis thaliana At*SWEET7) capable of co-transporting glucose and xylose from previously unexplored oleaginous yeasts and plants. Kinetic analysis showed that LST1_205437 had lenient glucose inhibition on xylose transport and *At*SWEET7 transported glucose and xylose simultaneously with no inhibition. Modelling studies of LST1_205437 revealed that Ala335 residue at sugar binding site can accommodates both glucose and xylose. Docking studies with *At*SWEET7 revealed that Trp59, Trp183, Asn145, and Asn179 residues stabilized the interactions with sugars, allowing both xylose and glucose to be co-transported. In addition, we altered sugar preference of LST1_205437 by single amino acid mutation at Asn365. Our findings provide a new mechanistic insight on glucose and xylose transport mechanism of sugar transporters and the identified sugar transporters can be employed to develop engineered yeast strains for producing cellulosic biofuels and chemicals.

KEYWORDS
co-fermentation, rational evolution, substrate specificity, sugar membrane transporter

1 | INTRODUCTION

Glucose and xylose are the two most abundant sugars in lignocellulosic biomass.[1] The development of efficient and economical processes for the conversion of lignocellulosic biomass into various biofuels, chemicals and bioproducts requires microorganisms capable of utilizing both sugars if possible simultaneously.[2] Xylose metabolism, however, is not native to *Saccharomyces cerevisiae*, which has been used for the production of corn and sugarcane ethanol. A number of studies have demonstrated that *S. cerevisiae* can be engineered to efficiently utilize xylose.[3–10] However, xylose transport in these engineered strains is subject to glucose repression, which leads to sequential utilization of glucose and xylose rather than simultaneous co-utilization. Glucose repression in a xylose-fermenting engineered

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S. cerevisiae is initiated from glucose inhibition on xylose uptake by endogenous sugar transporters.[11–14]

S. cerevisiae has at least 18 hexose transporters. However, dedicated xylose transporters in *S. cerevisiae* have not been reported. Xylose transport in *S. cerevisiae* is facilitated by actively expressed hexose transporters *(HXT1-7 and GAL2) as HXT8-HXT17* are either inactive (not transcribed) or cryptic.[12,15,14] Although these hexose transporters can facilitate efficient xylose utilization when it is the sole sugar, the presence of glucose completely inhibits xylose uptake due to the higher affinity of the sugar transporters toward glucose.[16] As such, glucose inhibition of xylose transport has been considered as a bottleneck preventing simultaneous co-fermentation of glucose and xylose. Several attempts have been made to bypass glucose inhibition in mixed-sugar fermentations. Ha et al. developed an engineered yeast strain capable of co-fermenting cellobiose, a dimer of glucose, and xylose; thus, avoiding inhibition of xylose transport by glucose.[17] However, this strategy does not allow co-fermentation of monomeric sugars present in cellulosic hydrolysates generated by matured pretreatment and enzymatic hydrolysis processes.[18,19] Therefore, many studies have focused on identifying xylose specific transporters from xylose-fermenting yeast species, such as *Pichia stipitis* and *Candida intermedia*.[20,21] Although heterologous expression of the identified xylose transporters in a *S. cerevisiae* lacking hexose sugar transporters conferred growth on xylose, glucose inhibition on xylose transport was still observed.[20,21] In addition to bioprospecting, rational and directed-evolution approaches have led to the development of xylose transporters not inhibited by glucose.[22–26] Using rational mutagenesis, Young et al. reported a conserved amino-acid motif responsible for monosaccharide selectivity in sugar transporters conferring growth on xylose. Further, mutation of the conserved monosaccharide recognition motifs led to a designed transporter for xylose transport. However, the transporter could not transport glucose and xylose simultaneously, leaving the co-fermentation problem open.[26] Farwick et al. employed adaptive laboratory evolution of an individual sugar transporter, using a xylose-utilizing strain of *S. cerevisiae* lacking all hexose transporters and with disrupted glycolysis, to identify evolved hexose transporters insensitive to glucose repression. The authors discovered two amino-acid residues (Asn376/370 and Thr219/213) of Gal2 and Hxt7 that are essential for co-transport of glucose and xylose. However, modifying these two residues resulted in reduced rates of glucose and xylose transport.[22] Using similar approach Shin et al., identified Asn366 residue mutation (same as in *Sc*Gal2/Hxt7 Asn376/370) in Hxt11 that enabled simultaneous glucose and xylose co-fermentation.[25]

While the rational design approach led to promising results, we aimed to expand bioprospecting in the search of native glucose and xylose co-transporters. Oleaginous yeasts, such as *Rhodosporidum toruloides* and *Lipomyces starkeyi* are receiving more attention as an alternative cell factory for lipid and acetyl-CoA based products given their ability to naturally consume most of the sugars including hemicellulose derived glucose and xylose.[27,28] Recently, genome sequence of *R. toruloides* and *L. starkeyi* have been reconstructed and annotated, allowing search for putative xylose transporters.[29,30] According to our xylose transporter search criteria based on conserved motif G[G/F]XXXG[26] and Thr213 and Asn370 residues,[22] both species contained 8 putative xylose transporters.

In contrast to yeast transporters, the mechanism of xylose transport by SWEETs has not been studied so far. SWEETs are newly discovered family of transporters with distinct 7 transmembrane (TM) structure, which play a key role in plant development and sugar translocation within the plant phloem.[31] SWEETs are comprised by seven TM domains, where the N-terminal three helixes shares sequence similarity to C-terminal three helixes, connected by non-conserved fourth domain.[32–35] Previous studies on *Arabidopsis thaliana* SWEETs demonstrated functional expression of the transporters in yeast, conferring growth on glucose.[32,36,34] Recently, Podolsky et al., identified novel fungal SWEET from anaerobic fungi (Neocallimastigomycota) which demonstrated co-consumption of glucose and xylose in *S. cerevisiae*.[37]

In this study we aimed to investigate an ability of putative xylose transporters from *R. toruloides* IFO0880 and *L. starkeyi* NRRL Y-11557 and SWEET transporters from *A. thaliana* to co-ferment glucose and xylose, a desired trait for producing cellulosic biofuels by engineered *S. cerevisiae*. In the first part of the study, we expressed selected transporters in engineered *S. cerevisiae* optimized for efficient xylose fermentation lacking major hexose transporters to screen and characterize transporters that capable to co-ferment both sugars.[38] We identified that *L. starkeyi* LST1_205437 and *A. thaliana* SWEET7 have an ability to co-ferment glucose and xylose simultaneously. To understand kinetic background behind simultaneous glucose and xylose co-fermentation, we performed kinetic study using ^{14}C labeled sugars. Kinetics studies revealed that both transporters transports xylose in the presence of glucose. Cryo-EM or/and X ray crystallography of the selected transporters have not been resolved. Hence, to explain molecular basis of this unique trait observed in the selected transporters, we employed *in silico* molecular modelling and dynamics simulation (MD). Using crystal structure of *Os*SWEET2b and XylE transporters as a homology template, we performed molecular simulation of glucose and xylose transport in LST1_205437 and *A. thaliana* SWEET7.

The study demonstrated that bioprospecting approach still can be a versatile tool to identify novel transporters with unorthodox protein motifs and residues for glucose and xylose cotransport. By combining kinetics and molecular simulation study, we were able to get insights into a molecular basis and responsible amino acid residues enabling co-transport of glucose and xylose in LST1_205437 and *At*SWEET7 (Figure 1).

2 | MATERIALS AND METHODS

2.1 | Medium and cell growth conditions

Under non-selective conditions, all strains were grown YPD agar plates (2% w/v agar, 1% w/v yeast extract, 2% peptone and 2% glucose). A single colony from YPD agar plate was inoculated into 2 ml YPD liquid medium to obtain seed cultures. For growth study, the seed cultures

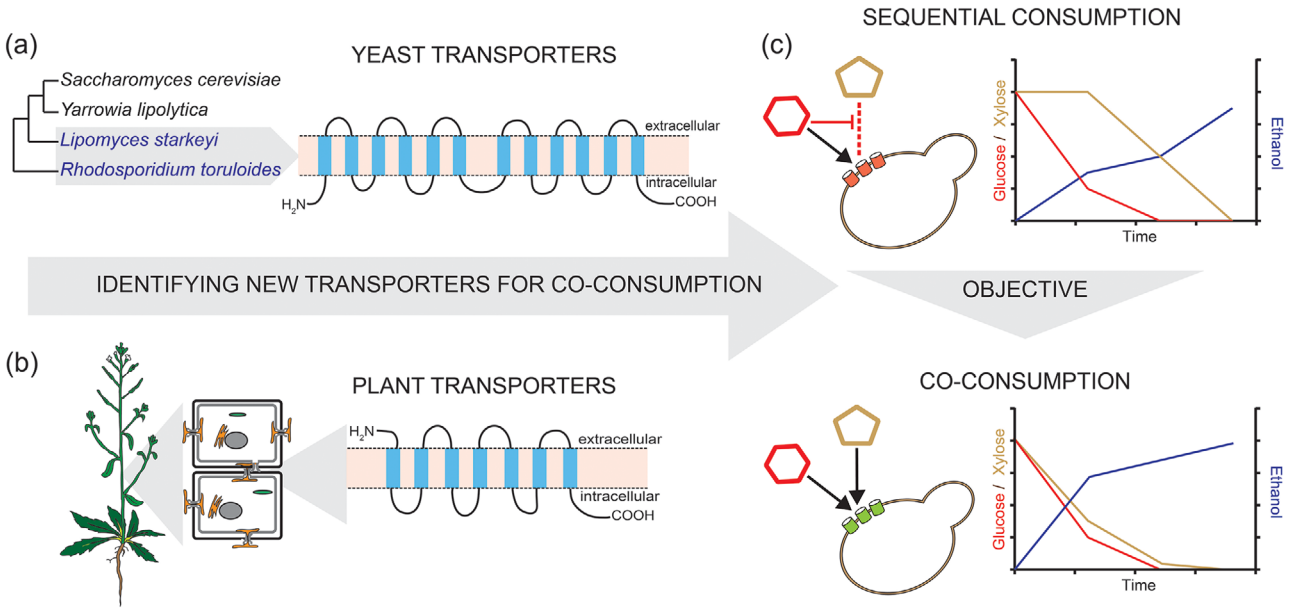

FIGURE 1 Bioprospecting strategy implemented in this study. This figure depicts the main steps applied to identify novel xylose and glucose co-transporting transporters. (a) Identification transporters from emerging oleaginous yeasts *Lipomyces starkeyi* and *Rhodosporidium toruloides*. (b) Characterization of SWEET transporters from *Arabidopsis thaliana*. (c) Schematic fermentation profile of a sugar mixture containing glucose and xylose by the engineered *S. cerevisiae*. Glucose presence inhibits xylose transport leading to sequential sugar utilization. Application of the discovered transporters relief glucose inhibition of xylose transport, leading to glucose and xylose co-consumption

were then used to inoculate 25 ml of YPD and YPX medium (10 g L^{-1} yeast extract, 20 g L^{-1} peptone, and 20 g L^{-1} xylose or glucose) in a 125 ml shake flask with a starting OD600 of 1. The cells were then grown at 30°C and 250 rpm.

For flask fermentation, a single colony was inoculated to 5 or 25 ml YPE (1% w/v yeast extract, 2% peptone, 5% ethanol) supplemented with 200 μg ml^{-1} of geneticin to obtain seed cultures. Subsequently, seed cultures were inoculated to 25 ml of YPD,YPX and YPDX medium (10 g L^{-1} yeast extract, 20 g L^{-1} peptone, and 20 g L^{-1} xylose or/and glucose) in a 125 ml shake flask with a starting OD600 of 1, 5 or 10 for flask fermentation. Flask fermentations were maintained at 30°C and 250 rpm. $CaCO_3$ at 50 g L^{-1} were added for high sugar fermentations in YPDX medium (10 g L^{-1} yeast extract, 20 g L^{-1} peptone, 70 g L^{-1} glucose and 40 g L^{-1} xylose).

A previously constructed xylose fermenting *S .cerevisiae* yeast (SR8) with *HXT1-7*Δ, *GAL2*Δ deletions was used for transporter screening and characterization (SR8D8).[238] SR8D8 was grown in YPE medium (10 g L^{-1} yeast extract, 20 g L^{-1} peptone, and 5 g L^{-1} ethanol). The codon optimized sugar transporter genes from *L. starkeyi*, *R. toruloides* and *A. thaliana* were expressed in SR8D8 using G418 resistance dominant marker harboring plasmid for glucose and/or xylose transport characterization. SR8D8 strains transformed with plasmid containing *KanMX* marker conferring resistance to G418 (geneticin) were propagated on YPE supplemented with 200 μg ml^{-1} of geneticin. For growth and flask fermentation experiments all media was supplemented with 200 μg ml^{-1} of geneticin for plasmid maintenance. Biomass was calculated from the OD600 measured using a Biomate 5 UV-visible spectrophotometer (Fisher, NY, USA). All growth rates were measured using a Bioscreen C plate reader system (Growth Curves USA, Piscataway, NJ, USA). A 2 μL inoculum of fully-grown culture was added into 200 μL YP containing 200 μg ml^{-1} Geneticin with varying concentrations of different sugars. A wide band filter (420–580 nm) was used to measure optical density. Bioscreen C values represent mean value from three biological replicates. In all cases, the Bioscreen C was set to maintain a temperature of 30°C and high aeration through high continuous shaking.

2.2 | Plasmid construction and transformation

All transporters were cloned into p42K-GPD1p-CYC1t plasmid harboring 2μ replication origin and *KanMX* marker conferring resistance to G418 (geneticin) antibiotic. For *At*SWEET transporters p42K-GPD1p-CYC1t plasmid were linearized with BamHI and XhoI enzymes. *AtSWEET*s were PCR amplified and digested with BamHI and XhoI. Linear p42K-GPD1p-CYC1t and AtSWEETs were ligated with T4 ligase according to manufacturer's protocol. For *R. toruloides* and *L. starkeyi* transporters p42K-GPD1p-CYC1t plasmid were linearized with BamHI and EcoRI enzymes. The trasnporters were PCR amplified and digested with BamHI and EcoRI. Both p42K-GPD1p-CYC1t and the transporters were ligated with T4 ligase according to manufacturer's protocol. All plasmid was transformed into *E. coli* DH5α for propagation and maintenance. SR8D8 yeast strain was grown on YPE medium for transformation. SR8D8 transformations were performed using LiAc method according to Gietz et al.[39] Transformants were selected on YPE plate supplemented with 200 μg ml^{-1} of geneticin. *At*SWEET1 and *At*SWEET mutants were synthesized as gBlocks and cloned into p42K-GPD1p-CYC1t as described before (Integrated DNA

technologies, IA, USA). Variants of LST1_205437 mutant were synthesized from Twist Biosciences (Twist Biosciences, CA, USA) and cloned as previously described.

2.3 | ^{14}C labeled sugar uptake assay

SR8D8 containing the respective plasmid was grown on selective YPE medium to an OD600 of 1-1.5, harvested by centrifugation, and washed twice in ice-cold uptake buffer (100 mM potassium phosphate, pH 6.5). ^{14}C labeled sugar uptake assay was done according to Boles and Oreb.[40] Radioactivity was analyzed in a Beckman-Coulter LS6500 multi-purpose liquid scintillation counter (Beckman-Coulter, CA, USA).

Uptake was measured at sugar concentrations 0.2, 1, 5, 25, and 100 mM for glucose and 1, 5, 25, 66, 100, 200, and 500 mM for xylose. Inhibition of xylose uptake by glucose was measured at 25, 66, and 100 mM xylose with additional 25 and 100 mM unlabeled glucose. Sugar solutions contained 0.135–0.608 μCi of D-[U-^{14}C]-glucose (290-300 mCi mmol^{-1}) or D-[1-^{14}C]-xylose (55 mCi mmol^{-1}) (PerkinElmer, MA, USA). Calculation of K_m (Michaelis constant), V_{max} (maximal initial uptake velocity), and K_i (inhibitor constant for competitive inhibition) was done by nonlinear regression analysis and global curve fitting in Prism 7 (GraphPad Software) with values of three independent measurements.

2.4 | Transporter identification

Orthologs of known sugar transporters were identified in *R. toruloides* and *L. starkeyi* using BlastP.[41] Glucose transporters from *S. cerevisiae* (Hxt7, Hxt2, Hxt1, Hxt3)[42,15] and xylose transporters from *P. stipitis* (Xut5, Xut2, Rgt2, Xut3)[43] were used as query sequences for blast search. Search results were filtered by e-value and gene regulation. MEGA X 10.0.1 tool[44] was used to perform ClustalW alignment for the filtered putative sugar transporters and identify conserved structural domains and amino acid residues. The alignment results were edited using the Jalview 2.8 tool[45] for enhanced visual presentation.

2.5 | Transporter modeling

The homology models of ScGal2, LST_205437, *At*SWEET1 and *At*SWEET7 were constructed using Modeller.[46] The OF and IF models of Scal2 and LST_205437 were built using the structural template XylE (PDB ID: 4GBZ and 4JA4).[47,48] The 3D coordinates of XylE structures are obtained from protein databank. The structural models of OC and OF states of *At*SWEET1 and *At*SWEET7 are obtained using MD predicted structures of *Os*SWEET2b as template.[36] The IF *Os*SWEET2b[34] was used to build both *At*SWEET1 and *At*SWEET7 IF models. Molecular docking was performed using Autodock software package.[49] The PDBQT format files for protein and substrate molecules were obtained using AutoDock Tools. The grid files were generated using Autogrid4 and docking was performed using Autodock4.[49] The docking files were visualized using pymol (The PyMOL Molecular Graphics System, Version 1.7, Schrodinger, 2015).

3 | RESULTS

3.1 | Identification of putative xylose transporters in *Rhodosporidium toruloides* and *Lipomyces starkeyi*

We used knowledge of existing yeast sugar transporters to identify sugar transporters in *R. toruloides* and *L. starkeyi*, which have not been searched for sugar transporters. We found multiple orthologs to HXT transporters from *S. cerevisiae* and XUT transporters from *P. stipitis*. We filtered the transporters with 12 TM domains and conserved sequence motifs (Figure 2A).[20] Recent studies have shown the involvement of the conserved motif G[G/F]XXXG,[26] and Thr213 and Asn370 residues[22] in Hxt7 towards xylose specificity. As such, we used these conserved motifs and residues to refine glucose and xylose specific transporters in *R. toruloides* and *L. starkeyi*. For *L. starkeyi*, LST1_106361 and LST1_205437 were identified as putative glucose transporters and LST1_76 was identified as a putative xylose transporter. For *R. toruloides*, RTO4_11075 and RTO4_13042 were identified as putative glucose transporters, and RTO4_13731 and RTO4_10452 were identified as putative xylose transporters (Figure 2C). The protein IDs' were picked from respective gene models at JGI mycocosm.[22,26]

3.2 | Screening of *Arabidopsis thaliana* SWEET and oleaginous yeast transporters for glucose or xylose transport

It has been well reported that SWEETs transport different sugars, which encouraged us to examine xylose and glucose transport capabilities of 17 *At*SWEET1-17. We used an engineered *S. cerevisiae* strain (SR8D8) capable of xylose fermentation which lacks the Hxt1-7 and Gal2 transporters—rendering it unable to grow on glucose or xylose—for the examination.[6,38] We measured growth kinetics of SR8D8 transformants expressing the *A. thaliana* SWEETs and putative oleaginous yeast transporters using glucose and xylose as a sole sugar (Figures 2B,C, and 3). *Sc*Gal2 expressing SR8D8 was used as a positive control. Most of the SR8D8 transformants expressing *At*SWEETs and putative oleaginous transporter were not able to grow on glucose or xylose. Only *At*SWEET4, *At*SWEET7, and LST1_205437 expressing strains exhibited robust growth on xylose and glucose (Figure 3A).

3.3 | *A. thaliana* SWEET and *L. starkeyi* LST1_205437 transporters conferred glucose and xylose cofermentation ability in engineered yeast

To test if the selected transporters can enable consumption of both sugars simultaneously upon introduction to the SR8D8 strain, we

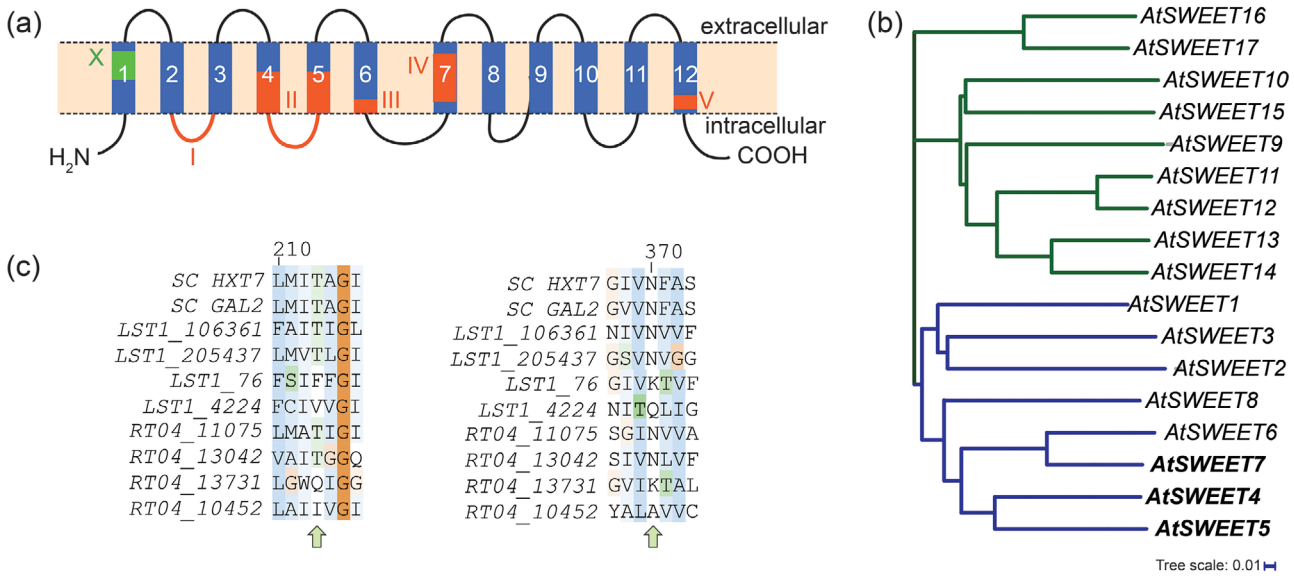

FIGURE 2 Bioinformatics analysis for transporter identification. (a) Most monosaccharide transporters in yeasts have 12 TM domains (represented in blue). The conserved motifs identified in yeasts transporters are marked in orange (I-V). Motif X (marked in green) has recently been identified as a key motif involved in xylose specificity. (b) A phylogenetic tree of the 17 *A. thaliana* SWEET transporters clusters the monosaccharide and disaccharide transporters independently. (c) Multiple sequence alignment of putative transporters: Thr213 and Ans370 are conserved in reported glucose transporters in yeasts

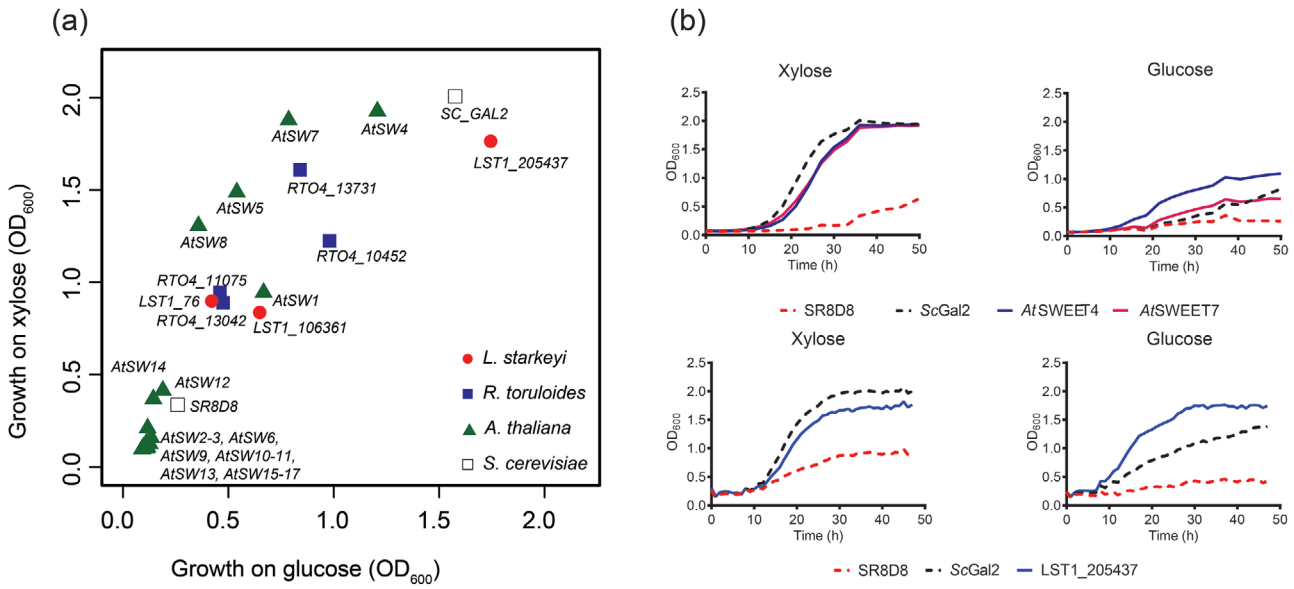

FIGURE 3 *L. starkeyi*, *R. toruloides* and *A. thaliana* SWEET transporter screening for growth on glucose or xylose. (a) Growth characteristics of the SR8D8 strain expressing transporters were summarized using a plot with. X axis represents the cell densities on glucose and Y axis represents the cell densities on xylose. Cell densities of the transporter-expressing strains at 40 h were presented. (b) Growth curves of the four strains with an overexpression cassette of *GAL2*, *At*SWEET4, *At*SWEET7, or a control plasmids (SRD8) on xylose and glucose. The dots and line lines are means from duplicated cultures

performed flask fermentations with a mixture of glucose and xylose and monitored sugar consumption over time. We used the SR8D8 expressing *GAL2* as a baseline control for determining co-consumption phenotypes, because it is known to transport both glucose and xylose in a sequential manner (Figure 4A). In addition, we included *At*SWEET1 as an additional control for *At*SWEETs, because it is most studied SWEET transporter and confers growth of SR8D8 on glucose (Figure S1A).[32,50,51] Both *At*SWEET4 and *At*SWEET7 showed simultaneous co-utilization of glucose and xylose with different rates within 24 h. While *At*SWEET1 showed a complete preference for glucose with

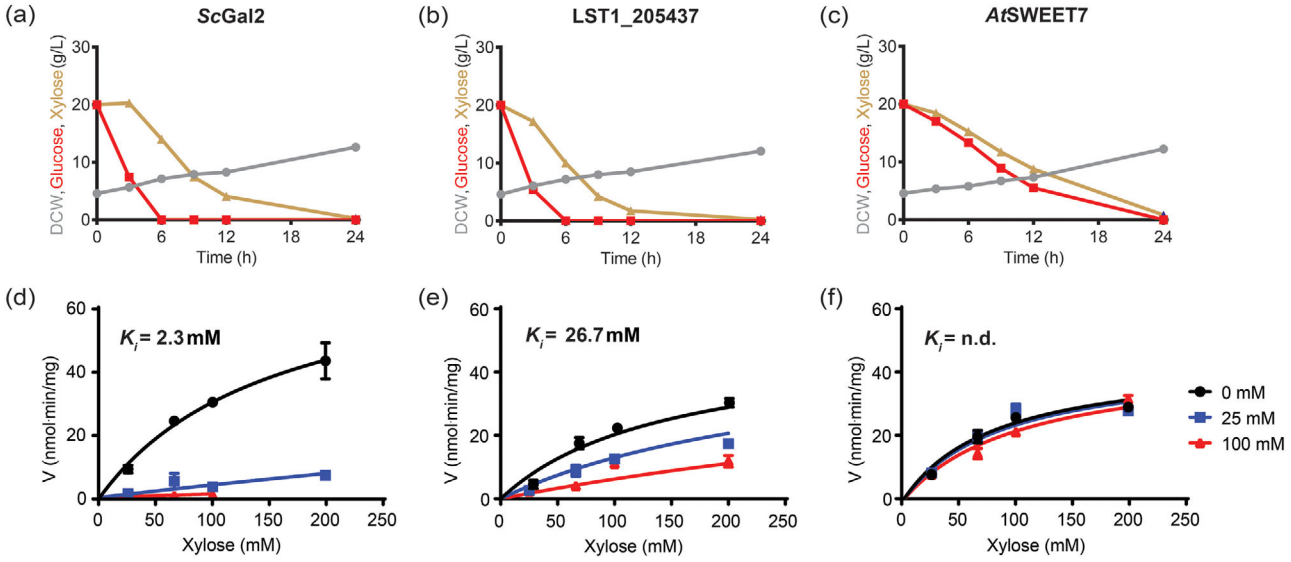

FIGURE 4 Glucose and xylose mixed sugar fermentation profile and inhibitory effect of glucose on xylose transport. 20 g L^{-1} of glucose and xylose mixed sugar fermentation by SR8D8 expressing *Sc*Gal2 (sequential fermentation) (a), LST1_205437 (partial cofermentation) (b), *At*SWEET7 (true co-fermentation) (c). Symbols: glucose (square), xylose (triangle up), DCW (circle). Inhibitory effect of 0, 25, and 100 mM glucose on xylose transport in SR8D8 expressing *Sc*Gal2 (d), LST1_205437 (e) and *At*SWEET7 (f). Global curve fitting for Michaelis–Menten kinetics with competitive inhibition was applied to data of three independent measurements at each concentration

negligible xylose consumption (Figure S1a), *At*SWEET4 showed co-consumption of glucose and xylose with a faster glucose consumption rate than that of xylose (Figure S1b). Interestingly, *At*SWEET7 enabled simultaneous co-consumption of glucose and xylose with almost same rates of sugar consumption (Figure 4c). LST1_205437 transporter from *L. starkeyi* showed co-consumption of glucose and xylose (Figure 4b) but glucose consumption was faster than xylose consumption. In further experiments, we chose *At*SWEET1 as a sole glucose transporter, *At*SWEET7 as a glucose and xylose co-transporter, and LST1_205437 as a semi glucose and xylose co-transporter. *At*SWEET7 transports both sugars simultaneously, but suffer from slow transport capacity. While LST1_205437 performs partial co-consumption, it has an efficient transport capacity for both glucose and xylose. The difference could be attributed to the structure and function of the transporters within the isolated organism.

Next, we evaluated fermentation performances of the SR8D8 transformants expressing *At*SWEET1, *At*SWEET7 and LST1_205437 under glucose or xylose conditions (Figure S2). As expected, *At*SWEET7 and LST1_205437 transporters enabled glucose and xylose fermentation, depleting all provided sugars. In contrast, *At*SWEET1 enabled robust glucose fermentation but inefficient xylose fermentation with only 5 g L^{-1} of xylose consumption within 50 h.

3.4 | Kinetic and molecular properties of *A. thaliana* SWEET7 and *L. starkeyi* LST1_205437

To understand kinetic and molecular basis of *At*SWEET7 and LST1_205437 glucose and xylose co-transport phenotypes, we performed radiolabeled sugar transport kinetics experiments, and *in silico* molecular modeling simulations with *Sc*Gal2 and *At*SWEET1 served as representative controls. *Sc*Gal2 was confirmed to be a high affinity glucose transporter (K_M = 1.613 mM, V_{max} = 38.33 nmol min-mg^{-1}), with low affinity toward xylose (K_M = 320.5 mM) (Figure S3c, and Table 1). Glucose transport kinetics of LST1_205437 was inferior to the *Sc*Gal2 transporter (K_M = 4.975 mM, V_{max} = 46.89 nmol min-mg^{-1}), whereas xylose kinetics was superior (K_M = 145.3 mM, V_{max} = 76.8 nmol min-mg^{-1}) (Figure S3e, and Table 1). These transport kinetic differences were not noticeable during sole sugar fermentation, unlike mixed sugar fermentation (Figure S2a-2b).

We then compared transport kinetic properties of *At*SWEET1 and *At*SWEET7. The results showed that *At*SWEET1 transports glucose more efficiently as compared to *At*SWEET7, with very poor xylose transport kinetics (Figure S3b and S3d). These kinetics results of *At*SWEET1 and *At*SWEET7 are consistent with the fermentation results (Figure S2a-2b) by the SR8D8 strains expressing *At*SWEET1 and *At*SWEET7.

Individual sugar uptake kinetics results of LST1_205437 supported the partial glucose and xylose co-consumption phenotype. However, the engineered yeast expressing AtSWEET7 showed apparent co-consumption of glucose and xylose, while kinetics results indicated discrepancies in K_M (K_M = 75 mM for glucose and K_M = 308 mM) (Table 1). These results prompted us to directly investigate the xylose transport rates by *Sc*Gal2, LST1_205437 and *At*SWEET7 in the presence of glucose. We performed xylose uptake assay with 25 or 100 mM glucose, similar conditions that were used in previous study.[22] As shown in (Figure 4d) xylose transport by *Sc*Gal2 was completely inhibited in the presence of glucose (K_i = 2.3 mM). This kinetic behavior of *Sc*Gal2 is consistent with the mixed sugar fermentation result (Figure 4a). Interestingly, xylose transport by LST1_205437 was less inhibited by

TABLE 1 Kinetic properties of *Sc*Gal2, *At*SWEET7, and LST1_205437

Transporter	Glucose		Xylose		
	K_m (mM)	V_{max} (nmol·min^{-1}·mg^{-1})	K_m (mM)	V_{max} (nmol·min^{-1}·mg^{-1})	K_i (mM)
*Sc*Gal2	1.6 ± 0.2	38.3 ± 1.4	320.5 ± 70	88.7 ± 10.0	2.4 ± 0.5
*[At*SWEET7	74.1 ± 13.0	110.3 ± 7.2	308.7 ± 86	100.9 ± 14.8	370.6 ± 109
LST1_205437	5.0 ± 1.0	47.0 ± 2.6	145.3 ± 43	76.8 ± 9.0	26.7 ± 6

Determined by zero-trans influx measurements with transporter-overexpressing SR8D8 and calculated with cell wet weight. SEM is indicated.

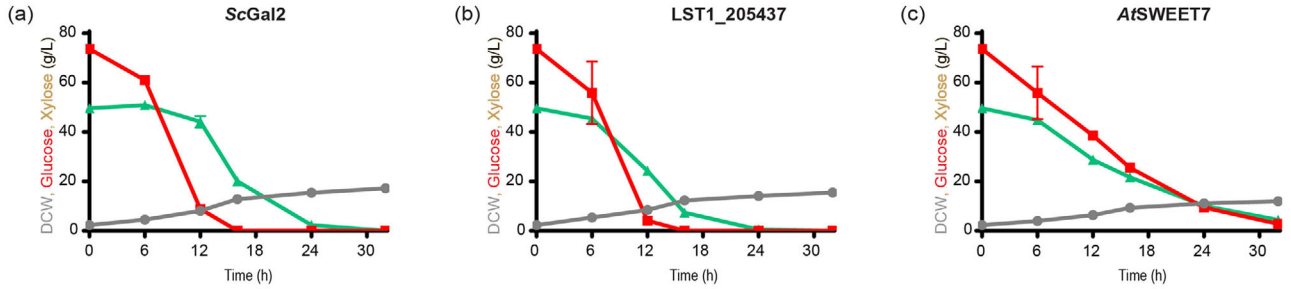

FIGURE 5 Glucose and xylose mixed sugar fermentation profile using industrially relevant sugar concentrations. 70 g L^{-1} of glucose and 40 g L^{-1} xylose mixed sugar fermentation by SR8D8 expressing *Sc*Gal2 (a), LST1_205437 (b), *At*SWEET7 (c). Symbols: glucose (square), xylose (triangle up), DCW (circle). The values are the means of two independent experiments, and the error bars indicate the standard errors

glucose than those by *Sc*Gal2 (K_i = 26.7 vs. 2.3 mM) (Figure 4e). As a result, the LST1_205437 expressing strain showed a partial co-consumption of glucose and xylose (Figure 4b). Remarkably, *At*SWEET7 showed no inhibition of xylose transport by glucose (Figure 4f, Table 1) (Figure 4c). Next, we performed a mixed sugar fermentation experiment under industrially-relevant sugar concentrations of 7% glucose and 4% xylose to validate co-fermentation of *At*SWEET7 and LST1_205437. As expected the *Sc*Gal2 expressing strain exhibited a sequential utilization of glucose and xylose (Figure 5a). The sugar utilization profile of the LST1_20437 expressing strain was consistent with the kinetics data, showing partial xylose and glucose co-consumption (Figure 5b). The *AtSWEET7* expressing strain showed co-consumption of glucose and xylose even at higher glucose concentrations, further supporting that *At*SWEET7 is indeed glucose and xylose co-transporter which is insensitive to glucose inhibition even under high glucose concentrations (Figure 5c).

To probe critical amino-acid residues responsible for the observed phenotypes—severe and partial glucose inhibition on xylose—of *Sc*Gal2 and LST1_205437, we performed in-silico docking studies to predict the preferred binding sites of glucose and xylose in *Sc*Gal2 and LST1_205437. We constructed the homology models of outward-facing (OF) and inward facing (IF) states of *Sc*Gal2 and LST1_205437 using the closest homologous structure, XylE[47,48] and docked glucose and xylose into the primary binding site (see Section 2 for details) (Figures 6 and S4-5). Glucose and xylose exhibited conserved binding mode in *Sc*Gal2 and LST1_205437 in OF states and our docked pose shows close match with previous studies based on XylE[48] (Figure 6a-d). The non-conserved residue Tyr446 in *Sc*Gal2 is involved in hydrogen bond interaction with both substrates while the equivalent residue Phe433 in LST1_205437 does not form any polar interaction. The presence of additional hydroxylmethyl moiety in glucose forms favorable contact with Thr219 (ScGal2)/Thr209 (LST1_205437) and stabilizes glucose in the binding site. However, striking differences were observed in the binding mode of substrate molecules in the IF state (Figure 6e-h). The structural transition to IF state exposes Asn346 (*Sc*Gal2) to the binding site and plays crucial role in substrate translocation. Both glucose and xylose were involved in hydrogen bond interaction with Tyr446 and Asn346 in *Sc*Gal2. In contrast, the equivalent residues Phe433 and Ala335 in LST1_205437 cannot form hydrogen bond interaction with glucose and xylose. Furthermore, dynamics involving both N- and C-terminal domains in LST1_205437 leads to co-transport of both glucose and xylose. In contrast xylose fails to form favorable contact with N-domain residues in ScGal2 which may be required for efficient transport. To validate the docking results, we constructed the SR8D8 expressing LST1_205437 with Ala335Asn mutation (LST1_205437_A335N) and examined the profile of glucose and xylose utilization. As expected, the Ala335Asn mutation of LST1_205437 increased glucose uptake and decreased the xylose uptake as compared to the wild type (Figure S6).

In contrast to *Sc*Gal2 and LST1_205437 with 12 TM domains, *At*SWEET7 with 7 TM domains showed no inhibition of xylose transport in the presence of either 25 or 100 mM glucose (Figure 4f and Table 1). This unique kinetic property of *At*SWEET7 is consistent with the fermentation result (Figures 4c and 5c). Both *At*SWEET1 and *At*SWEET7 are structurally related to each other, but when expressed

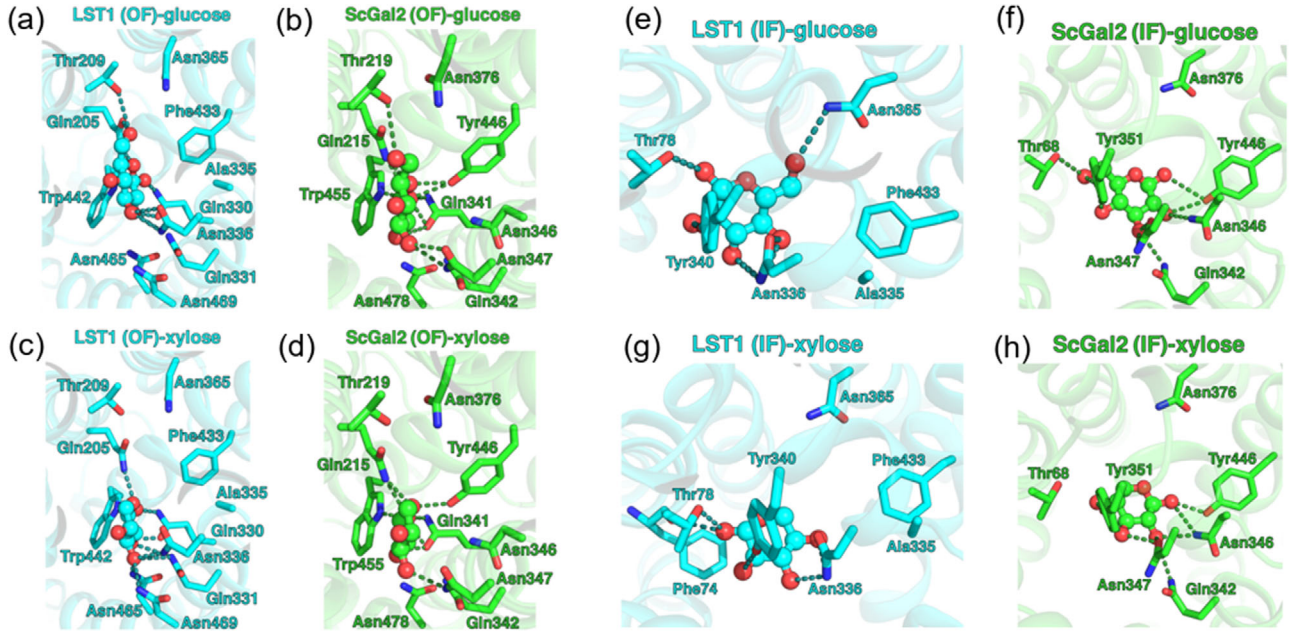

FIGURE 6 Predicted binding orientation of glucose and xylose in *Sc*Gal2 and LST1_205437. The dock poses of glucose and xylose in OF conformations for LST1_205437 (a,c) and *Sc*Gal2 (b,d), respectively. The dock poses of glucose and xylose in IF conformations for LST1_205437 (e,g) and *Sc*Gal2 (f,h), respectively

in SR8D8 they showed different mixed-sugar fermentation phenotypes (Figure S7). The *At*SWEET1 expressing strain consumed glucose rapidly but did not utilize xylose (Figure S1a). The *At*SWEET7 expressing strain consumed glucose and xylose simultaneously (Figure 4c). In a previous study, we characterized the complete glucose transport cycle in *Os*SWEET2b using molecular dynamics (MD) simulations.[36] Using the MD predicted structures of the occluded (OC) and OF states as structural templates, we constructed the homology models of intermediate conformations of *At*SWEET1 and *At*SWEET7 (Figure S8).[36] The IF models were built using an *Os*SWEET2b crystal structure.[34] The substrate molecules were docked in three different states and bound poses were predicted to be similar in both *At*SWEET1 and *At*SWEET7 (Figures 7 and S9). However, the major differences between *At*SWEET1 and *At*SWEET7 were observed in the non-conserved residues that stabilize the glucose and xylose in the binding site. The docking results reveals that substrate molecules are sandwiched between Trp59 and Trp183 in *At*SWEET7 while the equivalent residues in *At*SWEET1 are Ser54 and Trp176 cannot form a strong stacking interaction with substrates (Figure 7). Molecular simulations have also shown that the presence of two bulky aromatic residues in the binding site of bacterial SemiSWEET with one THB decreases the substrate dynamics and thereby increases the energetic barrier for substrate transport (28). Similarly, the non-conserved residues Asn145 (Ser138) and Asn179 (Cys172) in *At*SWEET7 have an extended amide group that forms favorable contact with both substrates in all three major conformational states (Figure 7) whereas the counterpart residues Ser138 and Cys172 in *At*SWEET1 cannot form favorable interactions in all the conformational states. To validate our findings, we mutated Trp59Ser in *At*SWEET7 and observed decreased xylose transport without affecting the glucose uptake (Figure S10d). We also identified secondary hydrophobic gating residues in our previous study and mutating of one of the hydrophobic residues beneath these gating residues Phe168Ala in *At*SWEET1 improves the glucose transport and allows the co-transport of xylose (Figures S9 and S10c).

3.5 | Alteration of Asn365 amino acid residue in *L. starkeyi* LST1_205437 changes sugar preference

Asn370/376 residues in *S. cerevisiae* hexose transporters Gal2 and Hxt7 play a critical role in glucose and xylose co-transport.[22] Replacing the Asn370/376 residue in Gal2 and Hxt7 with either hydrophobic or hydrophilic amino acids led to alleviation of glucose inhibition on xylose transport.[22] Interestingly, LST1_205437 transporter retains Asn365 (equivalent to Asn370 in Gal2) residue and show partial inhibition of xylose uptake by glucose (Figure 4b,e). We sought to test if alteration of Asn365 residue in LST1_205437 to phenylalanine, serine or valine would further alleviate glucose inhibition on xylose transport, allowing complete co-fermentation of glucose and xylose. We found that Asn365Phe, Asn365Ser, and Asn365Val mutations in LST1_205437 resulted in similar phenotypic changes as it was reported by Farwick et al. Particularly, Asn365Phe mutation abolished glucose transport while retaining xylose, Asn365Ser and Asn365Val showed co-fermentation phenotypes (Figure 8b-d). Our computational investigation also showed that Asn365 mutation to phenylalanine sterically hinders the binding mode of the glucose molecule and hence results in loss of transport function (Figure 7e). Altogether Asn365 residue mutation functions not only in *S. cerevisiae* transporters but also in *L. starkeyi* LST1_205437, supporting the universal importance of Asn370/376 residue in closely related yeast hexose transporters.

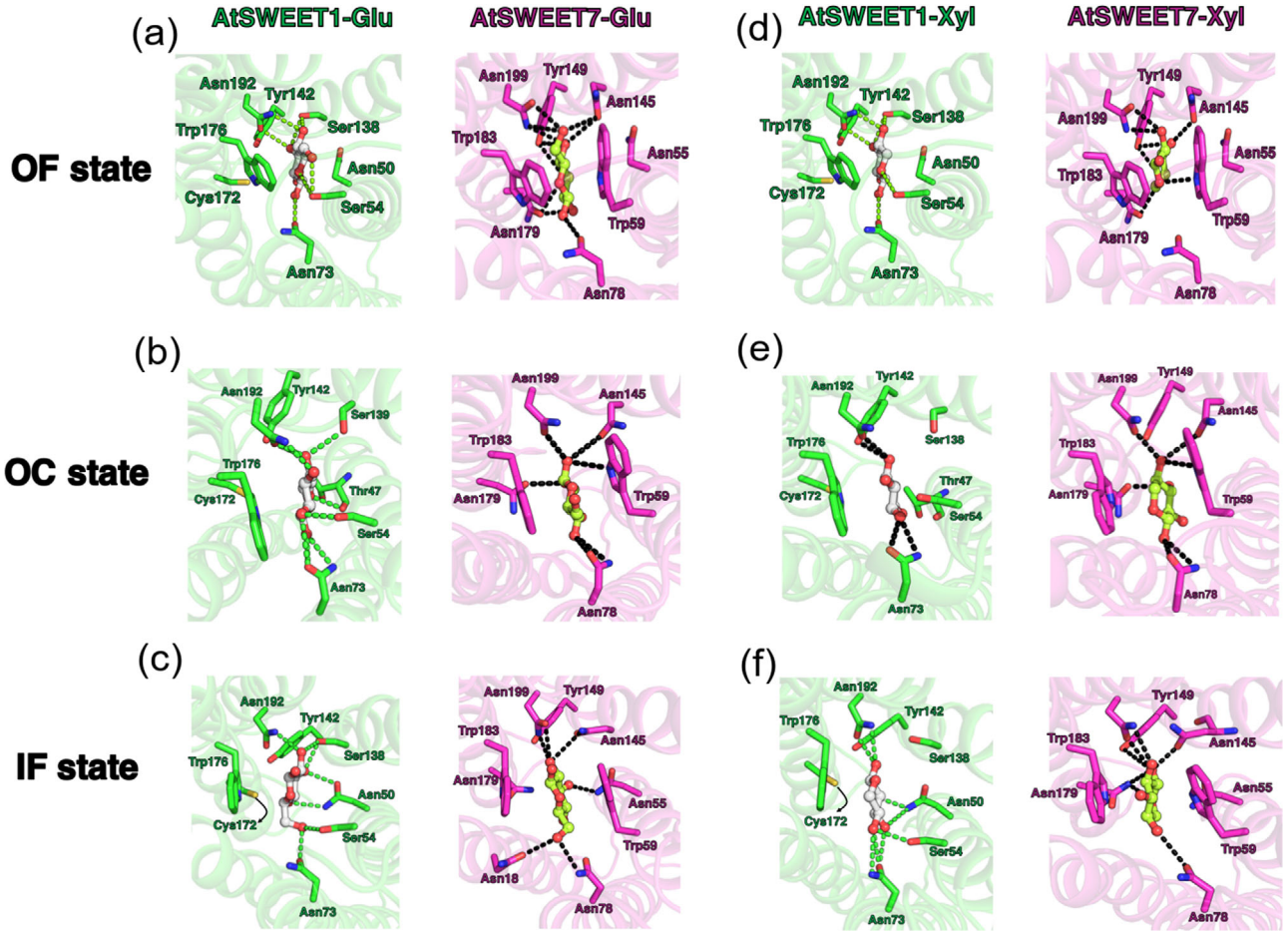

FIGURE 7 Dockposes of glucose and xylose in *At*SWEET1 and *At*SWEET7. The predicted binding mode of glucose and xylose in *At*SWEET1 and *At*SWEET7 in OF (a,d), OC (b,e) and IF (c,f) conformations

4 | DISCUSSION

The wealth of sequencing information and recently discovered SWEET family sugar transporters are still unexplored by bioprospecting for tackling glucose and xylose co-transport problem. In this study, we undertook a bioprospecting approach to identify glucose and xylose co-transporting transporters from unexplored oleaginous yeasts and plant (Figure 1). We identified 8 putative xylose transporters in *R. toruloides* and *L. starkeyi* (Figure 2c). However, experimental validation of the putative transporters using a xylose-fermenting *S. cerevisiae* lacking major hexose transporters (SR8D8) showed that only *L. starkeyi* LST1_205437 can enable robust growth on either glucose or xylose (Figure 3a). Interestingly, LST1_205437 retained conserved Thr213 and Asn370 residues, and demonstrated a partial cofermentation of glucose and xylose (Figure 4b). Furthermore, the glucose inhibition kinetics by LST_205437 showed less glucose inhibition on xylose transport whereas *Sc*Gal2 exhibited severe glucose inhibition on xylose transport even under a low glucose concentration (25 mM) (Figure 4d,e). This observation provides evidence that other than Thr213 and Asn370 residues might be involved in the partial cofermentation phenotype. *In silico* analysis reveals that the non-conserved residues Tyr446/Phe433 and Asn346/Ala335 might play crucial role in substrate binding and transport in *Sc*Gal2 and LST1_205437 (Figure 6). The increase in polarity restricts the binding of xylose only to C-terminus in *Sc*Gal2; however, dynamics involving both C and N domains is essential for efficient transport of both glucose and xylose in LST1_205437. The fermentation experiments also support our prediction and mutation of Ala335Asn decreases the xylose uptake in LST1_205437.

Most studies related to xylose transporters focused on MFS (Major Facilitator Superfamily) type transporters with 12 TM domains, and other families of sugar transporters have been overlooked. Here, we expanded bioprospecting approach toward SWEET family transporters. *A. thaliana* has 17 SWEET transporters that can transport either monosaccharides or disaccharides across a membrane via concentration gradients (Figure 2b).[52] According to Han *et. al. A. thaliana* SWEETs can be divided into two distinct groups based on conserved residues dictating sugar preference to monosaccharide or disaccharide. However, the authors discovered that this division could not reflect sugar specificity for all *At*SWEETs. In particular, Han et al. showed that *At*SWEET13 have both glucose and sucrose transport activities.[33] Therefore, in this study, we screened all 17 *At*SWEETs to identify xylose and glucose transporter. Interestingly, 17 *At*SWEETs share sequence similarity and yet showed very different sugar uptake

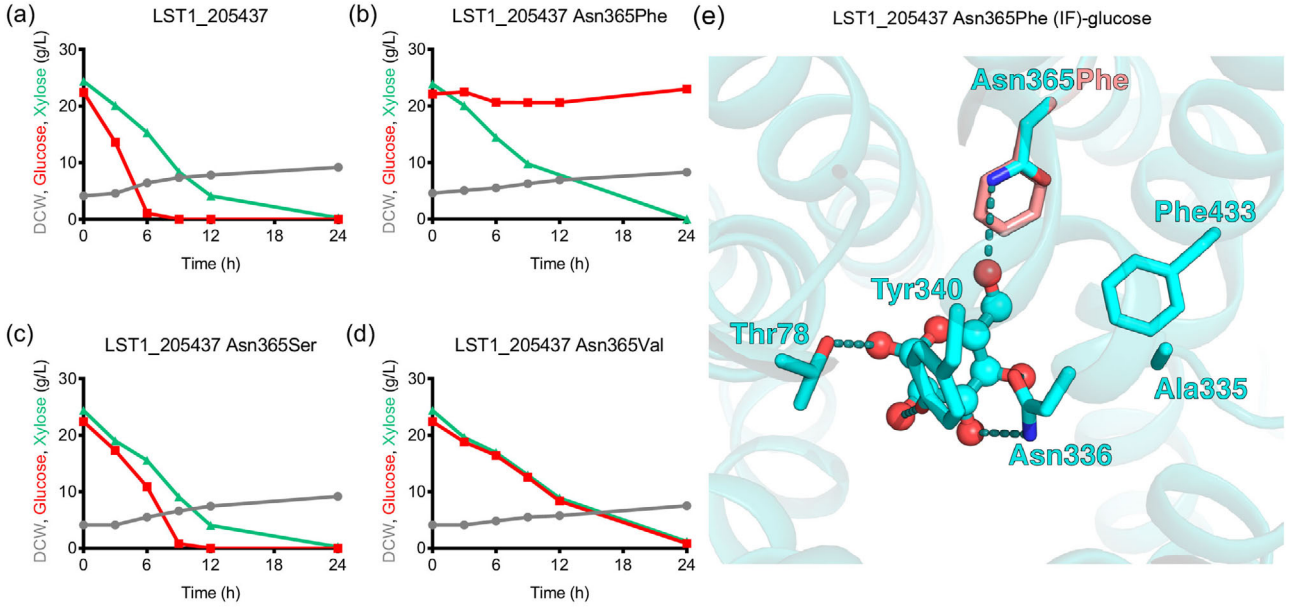

FIGURE 8 Glucose and xylose mixed sugar fermentation profile of SR8D8 expressing LST1_205437 Asn365 mutant variants and glucose dockpose of LST1_205437 Asn365Phe. LST1_205437 Asn365Phe mutant, LST1_205437 Asn365Ser and LST1_205437 Asn365Val. 20 g L^{-1} of glucose and xylose mixed sugar fermentation in YP medium of LST1_205437 wild type (a), LST1_205437 Asn365Phe (b), LST1_205437 Asn365Ser (c) and LST1_205437 Asn365Val (d). Mutation of Asn365 to phenylalanine in LST1_205437 (e). Asn365 form crucial contact with glucose molecule in stabilize the IF state. The mutation to phenylalanine results in steric clash with substrate and affects the conformational transition to intermediate states and transport. Symbols: glucose (square), xylose (triangle up), DCW (circle). The values are the means of two independent experiments, and the error bars indicate the standard errors

phenotypes on glucose or xylose. We confirmed *At*SWEET1 to be a glucose transporter with almost no xylose transport capacity, whereas *At*SWEET4 and *At*SWEET7 showed both glucose and xylose transport capacities (Figure S1 and Figure 4c). Moreover, among screened transporters, *At*SWEET7 exhibited complete co-fermentation phenotype. The kinetic analysis of *At*SWEET7 revealed no glucose inhibition of xylose transport, though the glucose and xylose transport kinetic properties were poorer than *Sc*Gal2 and LST_205437 (Figure 4f and Figure S3). Moreover, *At*SWEET7 exhibited complete co-fermentation of glucose and xylose even at high residual glucose concentrations, suggesting the transporter is completely insensitive to glucose inhibition (Figure 5c). Recently, Podolsky et al., demonstrated utility of fungal SWEET transporters to tackle glucose and xylose cotransport problem. The authors demonstrated that the wild-type NcSWEET1 and the best performing chimera derived from it allowed co-transport of glucose and xylose. However, in their experimental setup *S. cerevisiae* expressing wild type and the chimera transporter co-consumed only 20 g L^{-1} of sugars within 120 h.[37] Similar results were achieved in engineered Asn366Thr Hxt11 transporter, which belongs to MFS family, engineering of native glucose and xylose co-transporter with more simpler molecular structure than MFS might be advantageous for transporter engineering.[25]

More recently, we investigated the glucose transport cycle in *Os*SWEET2b and Bacterial SemiSWEET with 3 TMs and reported that substrate transport mechanism varies between closely related families of transporters.[36] We constructed the homology models of *At*SWEET1 and *At*SWEET7 intermediate states and docked the substrate in the binding site (Figures 7 and S9). The results revealed that the substrate molecules were sandwiched between Trp59 and Trp183 in *At*SWEET7, thereby enables the structural transition to other states for efficient transport. The lack of one of the aromatic counterpart may lead to the increase in conformation degrees of rotational freedom that could possibly affects the substrate stability in the binding site and the transport.[50] As expected, the mutation of Trp59 decreased the xylose transport in *At*SWEET7 (Figure S10d). In a previous study, we identified a hydrophobic gate at the center of transporter and opening of these gates drives the conformational transition of IF state.[36] In *At*SWEET1, Phe169 is located just beneath the hydrophobic gates and the mutation of this residue to alanine increases the glucose uptake and shows partial cotransport of xylose (Figure S10c). Although SWEETs transport both glucose and xylose via the same translocation pore, the free energy barriers and the critical residues that facilitate the transport along the pore cavity could be different. Extensive long timescale simulations are required to characterize the mechanistic difference between glucose and xylose transport that provides more insights into atomic-level details of the transport mechanism.

5 | CONCLUSION

In summary, this work demonstrates how bioprospecting can identify unique transporters for industrial applications. Availability of vast amounts of sequencing information, allowed us to identify and characterize yeast transporter LST_205437 that has partial glucose

and xylose co-consumption capacity. We found that LST_205437 has non conserved amino acid residue responsible for the phenotype. We characterized newly discovered SWEET transporters, which are structurally different from its yeast counterparts. Using *in silico* modeling, we were able to identify key amino acid residues responsible for glucose and xylose co-transport. The discovered data could be further used for rational transporter engineering of *At*SWEETs and yeast transporters to improve xylose and glucose transport characteristics. Altogether, information gathered in this study will increase the understanding of yeast hexose transporters and SWEET transporters, providing valuable information for industrial biotechnology and fundamental biology.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

N.K., L.C., D.S., C.R., and Y.J. conceived and designed the study. N.K., S.J, and J.L., performed experiments. A.D. performed bioinformatics analysis. B.S. performed *in silico* studies and docking analysis. N.K., and Y.J. analyzed and interpreted the data and wrote the manuscript in discussion with all authors.

DATA AVAILABILITY STATEMENT

Data available on request from the authors.

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REFERENCES

1. Carroll, A., & Somerville, C. (2009). Cellulosic biofuels. *Annual Review of Plant Biology*, *60*, 165–182.
2. Kim, S. R., Ha, S. J., Wei, N., Oh, E. J., & Jin, Y. S. (2012). Simultaneous co-fermentation of mixed sugars: A promising strategy for producing cellulosic ethanol. *Trends in Biotechnology*, *30*, 274–282.
3. Brat, D., Boles, E., & Wiedemann, B. (2009). Functional expression of a bacterial xylose isomerase in *Saccharomyces cerevisiae*. *Applied and Environmental Microbiology*, *75*, 2304–2311.
4. Jeffries, T. W., & Jin, Y. S. (2004). Metabolic engineering for improved fermentation of pentoses by yeasts. *Applied Microbiology and Biotechnology*, *63*, 495–509.
5. Jin, Y. S., Ni, H., Laplaza, J. M., & Jeffries, T. W. (2003). Optimal growth and ethanol production from xylose by recombinant *Saccharomyces cerevisiae* require moderate D-xylulokinase activity. *Applied and Environmental Microbiology*, *69*, 495–503.
6. Kim, S. R., Skerker, J. M., Kang, W., Lesmana, A., Wei, N., Arkin, A. P., & Jin, Y. S. (2013). Rational and evolutionary engineering approaches uncover a small set of genetic changes efficient for rapid xylose fermentation in *Saccharomyces cerevisiae*. *Plos One*, *8*, e57048.
7. Kotter, P., Amore, R., Hollenberg, C. P., & Ciriacy, M. (1990). Isolation and characterization of the Pichia stipitis xylitol dehydrogenase gene, XYL2, and construction of a xylose-utilizing *Saccharomyces cerevisiae* transformant. *Current Genetics*, *18*, 493–500.
8. Kuyper, M., Harhangi, H. R., Stave, A. K., Winkler, A. A., Jetten, M. S., de Laat, W. T., den Ridder, J. J., Op den Camp, H. J., van Dijken, J. P., & Pronk, J. T. (2003). High-level functional expression of a fungal xylose isomerase: The key to efficient ethanolic fermentation of xylose by *Saccharomyces cerevisiae*? *Fems Yeast Research*, *4*, 69–78.
9. Kwak, S., & Jin, Y. S. (2017). Production of fuels and chemicals from xylose by engineered *Saccharomyces cerevisiae*: A review and perspective. *Microbial cell factories*, *16*, 82.
10. Zhou, H., Cheng, J. S., Wang, B. L., Fink, G. R., & Stephanopoulos, G. (2012). Xylose isomerase overexpression along with engineering of the pentose phosphate pathway and evolutionary engineering enable rapid xylose utilization and ethanol production by *Saccharomyces cerevisiae*. *Metabolic Engineering*, *14*, 611–622.
11. Gardonyi, M., Jeppsson, M., Liden, G., Gorwa-Grauslund, M. F., & Hahn-Hagerdal, B. (2003). Control of xylose consumption by xylose transport in recombinant *Saccharomyces cerevisiae*. *Biotechnology and Bioengineering*, *82*, 818–824.
12. Hamacher, T., Becker, J., Gardonyi, M., Hahn-Hagerdal, B., & Boles, E. (2002). Characterization of the xylose-transporting properties of yeast hexose transporters and their influence on xylose utilization. *Microbiology*, *148*, 2783–2788.
13. Parachin, N. S., Bergdahl, B., van Niel, E. W., & Gorwa-Grauslund, M. F. (2011). Kinetic modelling reveals current limitations in the production of ethanol from xylose by recombinant *Saccharomyces cerevisiae*. *Metabolic Engineering*, *13*, 508–517.
14. Sedlak, M., & Ho, N. W. (2004). Characterization of the effectiveness of hexose transporters for transporting xylose during glucose and xylose co-fermentation by a recombinant Saccharomyces yeast. *Yeast*, *21*, 671–684.
15. Ozcan, S., & Johnston, M. (1999). Function and regulation of yeast hexose transporters. *Microbiology and Molecular Biology Reviews*, *63*, 554–569.
16. Subtil, T., & Boles, E. (2012). Competition between pentoses and glucose during uptake and catabolism in recombinant *Saccharomyces cerevisiae*. *Biotechnology for Biofuels*, *5*, 14.
17. Ha, S. J., Galazka, J. M., Kim, S. R., Choi, J. H., Yang, X., Seo, J. H., Glass, N. L., Cate, J. H., & Jin, Y. S. (2011). Engineered *Saccharomyces cerevisiae* capable of simultaneous cellobiose and xylose fermentation. *Proceedings of the National Academy of Sciences of the United States of America*, *108*, 504–509.
18. Cheng, M. H., Dien, B. S., Lee, D. K., & Singh, V. (2019b). Sugar production from bioenergy sorghum by using pilot scale continuous hydrothermal pretreatment combined with disk refining. *Bioresource Technology*, *289*, 121663.
19. Shirkavand, E., Baroutian, S., Gapes, D. J., & Young, B. R. (2016). Combination of fungal and physicochemical processes for lignocellulosic biomass pretreatment – A review. *Renewable & Sustainable Energy Reviews*, *54*, 217–234.
20. Leandro, M. J., Fonseca, C., & Goncalves, P. (2009). Hexose and pentose transport in ascomycetous yeasts: An overview. *Fems Yeast Research*, *9*, 511–525.
21. Young, E., Poucher, A., Comer, A., Bailey, A., & Alper, H. (2011). Functional survey for heterologous sugar transport proteins, using *Saccharomyces cerevisiae* as a host. *Applied and Environmental Microbiology*, *77*, 3311–3319.

22. Farwick, A., Bruder, S., Schadeweg, V., Oreb, M., & Boles, E. (2014). Engineering of yeast hexose transporters to transport D-xylose without inhibition by D-glucose. *Proceedings of the National Academy of Sciences of the United States of America*, *111*, 5159–5164.
23. Li, H., Schmitz, O., & Alper, H. S. (2016). Enabling glucose/xylose co-transport in yeast through the directed evolution of a sugar transporter. *Applied Microbiology and Biotechnology*, *100*, 10215–10223.
24. Reider Apel, A., Ouellet, M., Szmidt-Middleton, H., Keasling, J. D., & Mukhopadhyay, A. (2016). Evolved hexose transporter enhances xylose uptake and glucose/xylose co-utilization in *Saccharomyces cerevisiae*. *Scientific Reports*, *6*, 19512.
25. Shin, H. Y., Nijland, J. G., de Waal, P. P., de Jong, R. M., Klaassen, P., & Driessen, A. J. (2015). An engineered cryptic Hxt11 sugar transporter facilitates glucose-xylose co-consumption in *Saccharomyces cerevisiae*. *Biotechnology for Biofuels*, *8*, 176.
26. Young, E. M., Tong, A., Bui, H., Spofford, C., & Alper, H. S. (2014). Rewiring yeast sugar transporter preference through modifying a conserved protein motif. *Proceedings of the National Academy of Sciences of the United States of America*, *111*, 131–136.
27. Adrio, J. L. (2017). Oleaginous yeasts: Promising platforms for the production of oleochemicals and biofuels. *Biotechnology and Bioengineering*, *114*, 1915–1920.
28. Zhang, S., Skerker, J. M., Rutter, C. D., Maurer, M. J., Arkin, A. P., & Rao, C. V. (2016). Engineering Rhodosporidium toruloides for increased lipid production. *Biotechnology and Bioengineering*, *113*, 1056–1066.
29. Coradetti, S. T., Pinel, D., Geiselman, G. M., Ito, M., Mondo, S. J., Reilly, M. C., Cheng, Y. F., Bauer, S., Grigoriev, I. V., Gladden, J. M., Simmons, B. A., Brem, R. B., Arkin, A. P., & Skerker, J. M. (2018). Functional genomics of lipid metabolism in the oleaginous yeast *Rhodosporidium toruloides*. *Elife*, *7*, e32110.
30. Riley, R., Haridas, S., Wolfe, K. H., Lopes, M. R., Hittinger, C. T., Goker, M., Salamov, A. A., Wisecaver, J. H., Long, T. M., Calvey, C. H., Aerts, A. L., Barry, K. W., Choi, C., Clum, A., Coughlan, A. Y., Deshpande, S., Douglass, A. P., Hanson, S. J., Klenk, H. P.,… Jeffries, T. W. (2016). Comparative genomics of biotechnologically important yeasts. *Proceedings of the National Academy of Sciences of the United States of America*, *113*, 9882–9887.
31. Jeena, G. S., Kumar, S., & Shukla, R. K. (2019). Structure, evolution and diverse physiological roles of SWEET sugar transporters in plants. *Plant Molecular Biology*, *100*, 351–365.
32. Chen, L. Q., Hou, B. H., Lalonde, S., Takanaga, H., Hartung, M. L., Qu, X. Q., Guo, W. J., Kim, J. G., Underwood, W., Chaudhuri, B., Chermak, D., Antony, G., White, F. F., Somerville, S. C., Mudgett, M. B., & Frommer, W. B. (2010). Sugar transporters for intercellular exchange and nutrition of pathogens. *Nature*, *468*, 527–532.
33. Han, L., Zhu, Y., Liu, M., Zhou, Y., Lu, G., Lan, L., Wang, X., Zhao, Y., & Zhang, X. C. (2017). Molecular mechanism of substrate recognition and transport by the AtSWEET13 sugar transporter. *Proceedings of the National Academy of Sciences of the United States of America*, *114*, 10089–10094.
34. Tao, Y., Cheung, L. S., Li, S., Eom, J. S., Chen, L. Q., Xu, Y., Perry, K., Frommer, W. B., & Feng, L. (2015). Structure of a eukaryotic SWEET transporter in a homotrimeric complex. *Nature*, *527*, 259–263.
35. Xuan, Y. H., Hu, Y. B., Chen, L. Q., Sosso, D., Ducat, D. C., Hou, B. H., & Frommer, W. B. (2013). Functional role of oligomerization for bacterial and plant SWEET sugar transporter family. *Proceedings of the National Academy of Sciences of the United States of America*, *110*, E3685–3694.
36. Selvam, B., Yu, Y. C., Chen, L. Q., & Shukla, D. (2019). Molecular basis of the glucose transport mechanism in plants. *ACS Central Science*, *5*, 1085–1096.
37. Podolsky, I. A., Seppala, S., Xu, H., Jin, Y. S., & O'Malley, M. A. (2021). A SWEET surprise: Anaerobic fungal sugar transporters and chimeras enhance sugar uptake in yeast. *Metabolic Engineering*, *66*, 137–147.
38. Xu, H. (Urbana, Illinois 2015). *Engineering Saccharomyces cerevisiae for cellulosic ethanol production*. University of Illinois at Urbana-Champaign.
39. Gietz, R. D., & Schiestl, R. H. (2007). High-efficiency yeast transformation using the LiAc/SS carrier DNA/PEG method. *Nature Protocols*, *2*, 31–34.
40. Boles, E., & Oreb, M. (2018). A growth-based screening system for hexose transporters in yeast. *Methods in Molecular Biology*, *1713*, 123–135.
41. Altschul, S. F., Gish, W., Miller, W., Myers, E. W., & Lipman, D. J. (1990). Basic local alignment search tool. *Journal of Molecular Biology*, *215*, 403–410.
42. Lewis, D. A., & Bisson, L. F. (1991). The HXT1 gene product of *Saccharomyces cerevisiae* is a new member of the family of hexose transporters. *Molecular and Cellular Biology*, *11*, 3804–3813.
43. Jeffries, T. W., Grigoriev, I. V., Grimwood, J., Laplaza, J. M., Aerts, A., Salamov, A., Schmutz, J., Lindquist, E., Dehal, P., Shapiro, H., Jin, Y. S., Passoth, V., & Richardson, P. M. (2007). Genome sequence of the lignocellulose-bioconverting and xylose-fermenting yeast *Pichia stipitis*. *Nature Biotechnology*, *25*, 319–326.
44. Kumar, S., Stecher, G., & Tamura, K. (2016). MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution*, *33*, 1870–1874.
45. Waterhouse, A. M., Procter, J. B., Martin, D. M., Clamp, M., & Barton, G. J. (2009). Jalview Version 2–a multiple sequence alignment editor and analysis workbench. *Bioinformatics*, *25*, 1189–1191.
46. Fiser, A., & Sali, A. (2003). Modeller: Generation and refinement of homology-based protein structure models. *Methods in Enzymology*, *374*, 461–491.
47. Quistgaard, E. M., Low, C., Moberg, P., Tresaugues, L., & Nordlund, P. (2013). Structural basis for substrate transport in the GLUT-homology family of monosaccharide transporters. *Nature Structural & Molecular Biology*, *20*, 766–768.
48. Sun, L., Zeng, X., Yan, C., Sun, X., Gong, X., Rao, Y., & Yan, N. (2012). Crystal structure of a bacterial homologue of glucose transporters GLUT1-4. *Nature*, *490*, 361–366.
49. Morris, G. M., Huey, R., Lindstrom, W., Sanner, M. F., Belew, R. K., Goodsell, D. S., & Olson, A. J. (2009). AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. *Journal of Computational Chemistry*, *30*, 2785–2791.
50. Cheng, K. J., Selvam, B., Chen, L.-Q., & Shukla, D. (2019). Distinct Substrate Transport Mechanism Identified in Homologous Sugar Transporters. *The Journal of Physical Chemistry B*, *123*(40), 8411–8418. http://doi.org/10.1021/acs.jpcb.9b08257.
51. Eom, J. S., Chen, L. Q., Sosso, D., Julius, B. T., Lin, I. W., Qu, X. Q., Braun, D. M., & Frommer, W. B. (2015). SWEETs, transporters for intracellular and intercellular sugar translocation. *Current Opinion in Plant Biology*, *25*, 53–62.
52. Chen, L. Q., Cheung, L. S., Feng, L., Tanner, W., & Frommer, W. B. (2015). Transport of sugars. *Annual Review of Biochemistry*, *84*, 865–894.

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