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REVIEW

Xylose transport in yeast for lignocellulosic ethanol production: Current status

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Lignocellulosic ethanol has been considered as an alternative transportation fuel. Utilization of hemicellulosic fraction in lignocelluloses is crucial in economical production of lignocellulosic ethanol. However, this fraction has not efficiently been utilized by traditional yeast *Saccharomyces cerevisiae*. Genetically modified *S. cerevisiae*, which can utilize xylose, has several limitations including low ethanol yield, redox imbalance, and undesired metabolite formation similar to native xylose utilizing yeasts. Besides, xylose uptake is a major issue, where sugar transport system plays an important role. These genetically modified and wild-type yeast strains have further been engineered for improved xylose uptake. Various techniques have been employed to facilitate the xylose transportation in these strains. The present review is focused on the sugar transport machineries, mechanisms of xylose transport, limitations and how to deal with xylose transport for xylose assimilation in yeast cells. The recent advances in different techniques to facilitate the xylose transportation have also been discussed.

[**Key words:** Bioethanol; Engineered ethanologenic yeast; *Saccharomyces cerevisiae*; Pentose sugar; Xylose transporter]

Environmental and socio-economic issues need the replacement of fossil fuels to environment friendly and sustainable alternative fuels like biofuels. Sustainable ethanol production as a biofuel from lignocellulosic residues is current focus in renewable energy society. Lignocellulosic biomass (LCB) produced from agricultural and forestry residues including cotton stalks, bagasse, straw (wheat and rice), maize cobs, coconut shells, jute sticks, rice husks, wood chips, sawdust and bark have been considered as a generous source, which does not compete with food requirements (1–3). For example, in the US, LCB (one billion dry tons per year, predicted to increase to 1.6 billion tons by 2030) has the potential to replace national petroleum consumption by about 30% (4). However, there are so many hurdles in each step to convert lignocellulosic sugars into ethanol. Out of those, complexity in structure, inhibitor produced during pretreatment and utilization of pentose sugars are the major hurdles in the process. LCB composed of lignin, cellulose and hemicelluloses, out of which cellulose and hemicelluloses cover 50–70% of cell wall of biomass (5,6). Followed by different pretreatment methods including acid and alkali and enzymatic hydrolysis, complex polysaccharides (cellulose and hemicelluloses) get converted into simple sugars (glucose and xylose) (5,7). Besides, hydrolysis of LCB also releases various inhibitors such as furan derivatives (furfural and 5-hydroxymethylfurfural), phenolic compounds and organic acids (levulinic, formic, and acetic acid) (5,8). These inhibitors affect viability of microbes and resultantly product yield and productivity get reduced. Numerous detoxification approaches including use of resins (ion-exchange), charcoal and/or alkali pretreatment and enzymatic detoxification can neutralize inhibition of such inhibitors (5,7). On the other hand, after successful utilization of complete sugars is also another major issue for sustainable process development.

In the complex LCB, xylose is the second most abundant sugar after glucose. Lignocellulosic glucose can be efficiently fermented to ethanol (6). However, the economic feasibility of lignocellulosic ethanol production depends on the utilization of xylose as well (7). Various ascomycetes have been used for efficient ethanol production, out of which many do not utilize xylose as carbon source. The traditional yeast, *Saccharomyces cerevisiae*, has been used widely for ethanol production from glucose as carbon source. However, the efforts have been made to modify *S. cerevisiae* genetically for efficient utilization of xylose (7,9–14). Ethanol production from xylose fermentation has been carried out successfully using recombinant *S. cerevisiae*, which was constructed through the administration of heterologous metabolic enzymes xylose reductase (XR) and xylitol dehydrogenase (XDH) from *Scheffersomyces stipitis, Candia intermedia* and other fungal strains (15,16). In addition *xylA* gene (encoding for xylose isomerase) has also been expressed in *S. cerevisiae* for direct conversion of xylose into xylulose without xylitol formation, which could be easily fermented (10). Moreover, enhancement of lignocellulosic ethanol titer was also achieved through co-fermentation of glucose and xylose (17). For example, SVSCF (Same Vessel Saccharification and Co-fermentation) process was performed using mixed culture of *S. cerevisiae* and *S. stipitis* and reported 5.73 g ethanol with an yield of 14.07 g ethanol/100 g raw solid (rapeseed straw) through co-fermentation (17). A novel strategy has also been applied for augmentation of xylose

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assimilation to ethanol using *S. stipitis* on hemicellulosic hydrolysate (18). As reported, growth of *S. stipitis* was enhanced along with increment of 20–51% of ethanol yield by adding hydrolyzed rice straw in hemicellulosic hydrolysate from different LCBs including plywood, bagasse and bamboo (19). Table 1 (19–31) summarizes the ethanol titers from LCB using xylose assimilating recombinant and wild-type yeasts.

Apart from bioconversion of xylose to ethanol, other issues like co-utilization of glucose and xylose as well as enhanced rate of xylose uptake, which are comparatively low and less-efficient due to presence of low affinity xylose specific transporters, need to be resolved (32,33). Past three decades of research have been focused on improving xylose catabolic pathways in recombinant industrial strains (34). However, less work has been focused on the xylose transport in the cells, which is a major limitation in the efficient conversion of lignocellulosic sugars to ethanol. Literature suggests that inhibition of sugar transporters has been highlighted as a major bottleneck to produce ethanol from LCB. In line to overcome this issue, many studies have been carried out through various approaches including systematic engineering, evolutionary engineering and inverse metabolic engineering for enhanced xylose transport (34–38).

Xylose can be transported either through naturally occurring specific transporter or through hijacking the hexose transporters (39). High-affinity xylose transport systems have been reported in few xylose-fermenting types of yeast such as *S. stipitis* and *C. intermedia* (40,41). Although, identified xylose-specific transporters were functionally expressed in these strains but they are neither selective nor have affinity towards xylose, which are the major causes for competitive inhibition by glucose (41–45). However, selective non-specific hexose transporters, i.e., Hxt1p, Hxt2p and Hxt7 transport xylose in the cell but with a very poor affinity. In genetically modified *S. cerevisiae*, xylose transport occurred by hexose transporter family including Hxt1p, Hxt2p, Hxt5p and Hxt7p, which are capable of transporting xylose at a very slow rate (46). The xylose uptake depends on the flux in the downstream pathway and xylose concentration in the surrounding (47).

The present review is focused toward the recent progress on xylose transport in yeasts with the available hexose/pentose transporters, mechanism and function of xylose transport in co-fermentation, the glucose repression through sugar transporter, and protein engineering of xylose specific transporters. Further, the obstacles and their solutions for efficient xylose utilization have also been discussed. Most of the research for genetic manipulation is focused on *S. cerevisiae* as a model organism for xylose uptake for ethanol fermentation. However, other ethanologenic yeasts are also used for genetic alteration. The present review is focused on the sugar transport machineries, mechanisms of xylose transport, limitations and how to deal with xylose transport for xylose assimilation in yeast cells. The recent advances in different techniques to facilitate the xylose transportation have also been discussed.

XYLOSE TRANSPORT SYSTEM: MACHINERIES AND MECHANISM

The efforts have been made to find out xylose specific transporter proteins in different species. However, the huge numbers of transporters are either less efficient or non-functional for xylose transport (48). Moreover, most of the identified transporters in yeasts for xylose uptake confer better affinity to glucose (49). Various xylose transporters have been identified in different yeasts, plants and bacterial cells, which play distinct role in different systems as described in Table 2 (34,41,43,44,50–57). In the yeasts including *S. cerevisiae*, *S. stipitis*, *C. intermedia* and *Debaryomyces hansenii*, the sugar transport is either carried out by energy-independent facilitated diffusion or energy dependent proton symport system (Fig. 1). Various hexose transporters participate in facilitated diffusion system, which have higher affinity for glucose than xylose (58). On the other hand, the xylose specific transporters participate in proton symport system, which helps to assimilate xylose (59). The machineries and their regulation depend on a number of factors such as yeast species, availability of particular sugar molecule as carbon source, and the physical conditions under which the yeasts are grown (42,43,60). In some species, the sugar transport also depends upon aerobic and anaerobic conditions or other stress systems (61). Various strategies have been applied for establishment of xylose metabolic pathway in *S. cerevisiae* including cloning of heterologous xylose metabolic genes such as *XYL1*, *XYL2*, *XYL3* and *xylA* encoding for xylose reductase, xylitol dehydrogenase, xylulokinase and xylose isomerase, respectively, from model xylose utilizing yeast, fungi, plants and bacteria (10,11,41,62). The ethanol conversion rate depends upon the sugar uptake rate, i.e., high or low and hence, the sugar transporter system plays an important role, which varies from species to species and substrate to substrate (53). Sugar transport in *S. cerevisiae* is exclusively mediated by facilitated diffusion, whereas, in some yeasts, plant and bacterial cells, the sugar is transported by proton-

TABLE 1. Ethanol titer of xylose assimilating yeast strain by using lignocellulosic biomass and simple xylose.

Xylose assimilating strain	Biomass	Sugar	Sugar consumed	Ethanol yield ($g\ g^{-1}$)	Reference
C. tropicalis S4	–	Xylose	56 $g\ l^{-1}$	0.1	20
C. tropicalis FP	–	Xylose	84 $g\ l^{-1}$	0.05	20
K. marxianus NIRE-K1.1	–	Xylose	$0.063 \pm 0.01\ g\ g^{-1}\ h^{-1}$	0.11	21
S. cerevisiae GS1.11-26	Arundo donax	Glucose and Xylose	Glucose = 59.34 $g\ l^{-1}$ Xylose = 22.18 $g\ l^{-1}$	0.47 ± 0.01	22
S. cerevisiae GS1.11-26	Spruce	Glucose and Xylose	Glucose = 48.91 $g\ l^{-1}$ Xylose = 10.09 $g\ l^{-1}$	0.43 ± 0.00	22
S. cerevisiae GS1.11-26	Wheat straw/hay	Glucose and Xylose	Glucose = 68.22 $g\ l^{-1}$ Xylose = 28.22 $g\ l^{-1}$	0.48 ± 0.02	22
S. stipitis	Over limed hydrolysate of rise husk	Glucose and Xylose	Xylose = $0.48 \pm 0.05\ g\ h^{-1}$	0.43 ± 0.03	19
C. shehatae NCIM3501	Sugarcane bagasse	Pentose	30.29 $g\ l^{-1}$	0.48	23
S. stipitis NCIM 3499	De-oiled rice bran	Pentose	$38.50 \pm 0.45\ g\ l^{-1}$	0.42 ± 0.021	24
S. stipitis NCIM 3498	Saccharum spontaneum	Pentose	$32.15 \pm 0.22\ g\ l^{-1}$	0.36 ± 0.011	25
S. cerevisiae IBB10B05	Wheat straw	Xylose	0.71 $g\ g\ h^{-1}$	0.30	26
K. marxianus ATCC 36907	Hemicellulosic sugarcane bagasse hydrolysates	Glucose and Xylose	Glucose = 4.00 $g\ l^{-1}$ Xylose = 21.18 $g\ l^{-1}$	0.33	27
C. tropicalis NBRC NBRC 0618	Olive tree pruning	NM	Total sugar = 0.23 $g\ g^{-1}$	0.27–0.38	28
C. tropicalis NCIM 3119 and *S. cerevisiae* NCIM 3090	Rice straw	Glucose and Xylose	Glucose = $7 \pm 0.4\ g\ l^{-1}$ Xylose = $46.2 \pm 1.5\ g\ l^{-1}$	0.44 ± 0.05	29
S. cerevisiae SXA-R2P-E	–	Xylose	$0.077\ g\ OD^{-1}\ h^{-1}$	0.45	30
S. cerevisiae TMB 3504	–	Xylose	0.760 ($g\ g^{-1}\ h^{-1}$)	0.40	31

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