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MINI-REVIEW

Sugar transporters in efficient utilization of mixed sugar substrates: current knowledge and outlook

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Abstract There is increasing interest in production of transportation fuels and commodity chemicals from lignocellulosic biomass, most desirably through biological fermentation. Considerable effort has been expended to develop efficient biocatalysts that convert sugars derived from lignocellulose directly to value-added products. Glucose, the building block of cellulose, is the most suitable fermentation substrate for industrial microorganisms such as *Escherichia coli*, *Corynebacterium glutamicum*, and *Saccharomyces cerevisiae*. Other sugars including xylose, arabinose, mannose, and galactose that comprise hemicellulose are generally less efficient substrates in terms of productivity and yield. Although metabolic engineering including introduction of functional pentose-metabolizing pathways into pentose-incompetent microorganisms has provided steady progress in pentose utilization, further improvements in sugar mixture utilization by microorganisms is necessary. Among a variety of issues on utilization of sugar mixtures by the microorganisms, recent studies have started to reveal the importance of sugar transporters in microbial fermentation performance. In this article, we review current knowledge on diversity and functions of sugar transporters, especially those associated with pentose uptake in microorganisms. Subsequently, we review and discuss recent studies on engineering of sugar transport as a driving force for efficient bioconversion of sugar mixtures derived from lignocellulose.

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Introduction

Recent concerns regarding global climate change and energy security necessitate intensified research in renewable energy resources in order to alleviate the prevailing over-dependence on fossil fuels. The heightened interest in production of fuels and chemicals from lignocellulosic biomass that these concerns have elicited is focused on biological fermentation. Considerable efforts expended so far to develop efficient biocatalysts that convert sugars derived from lignocellulose directly to value-added products aim to develop technologies which will eventually enable the unlocking of the unlimited potential of biomass as a resource for commodity chemicals and energy.

Lignocellulosic biomass derived from agricultural and forestry residues is the most promising candidate feedstock since it is widely abundant, renewable, and can also contribute to the development of rural economies (Ragauskas et al. 2006). Its principal lignocellulose components cellulose, hemicellulose, and lignin are tightly bound by hydrogen and covalent bonds (Malherbe and Cloete 2002), contributing to its recalcitrance to enzymatic hydrolysis (Himmel et al. 2007; Porter et al. 2007; Zhang et al. 2007). The proportion of each of the three fractions varies among hardwoods, softwoods, and grasses (Betts et al. 1991). The complex phenolic polymer, lignin, glues the other fractions together and is exceedingly resistant to hydrolysis (Crawford 1981; Sarkanen and Ludwig 1971). Since most microorganisms cannot effectively utilize raw cellulose or hemicellulose because of their recalcitrance to biodegradation, biological production of fuels and chemicals from lignocel-

lulosic biomass necessitates depolymerization of lignocellulose by enzymatic or chemical hydrolysis in a saccharification process. The mostly monomeric resultant sugars can then be converted to desired products by microbial action. Even though saccharification can add significantly to the final production costs of biomass-based processes, the influence of the efficiency of microbial conversion of sugars to product on these costs cannot be gainsaid. The ability of a host organism to efficiently uptake the saccharified sugars greatly impacts microbial efficiency. Whereas transporters for glucose derived mainly from the cellulosic fraction are common across most microbial species, those for other hexose (e.g. mannose and galactose) and pentose (e.g. xylose and arabinose) that are derived mainly from the hemicellulosic fraction are much less common. Since lignocellulosic biomass hydrolysates are complex mixtures of mostly pentose and hexose monomers, ideal microorganisms for bio-based process should possess the ability to metabolize mixed sugar substrates efficiently and completely. To this end, engineering of microorganisms that can completely and efficiently utilize a variety of mixed sugar substrates is only now starting to enable such interesting concepts as the biorefinery (Lynd et al. 1999).

Glucose is not only the building block of cellulose, but it also serves as the optimal fermentation substrate for most microorganisms, including the major industrial microorganisms *Escherichia coli*, *Corynebacterium glutamicum*, *Bacillus subtilis*, *Zymomonas mobilis*, and *Saccharomyces cerevisiae*. In contrast, the pentoses xylose and arabinose that are major building blocks of hemicellulose are generally much less susceptible to bacterial action. Only a few microorganisms like *E. coli* and *B. subtilis* possess the natural ability to utilize pentoses. Irrespective of this natural ability of microorganisms to metabolize pentoses, productivity and yield from these sugars are invariably low even when genes for their catabolizing enzymes are overexpressed and fully functional. This has been attributed to a variety of phenomena including carbon catabolite repression (CCR; Deutscher 2008; Gancedo 1998; Gorke and Stulke 2008), cellular redox imbalance in pentose metabolism, and inefficient uptake of some sugars (Hahn-Hägerdal et al. 2007; Jeffries and Jin 2004; Matsushika et al. 2009; van Maris et al. 2007). The importance of each of these varies with the microorganism in question.

As microbial preference for glucose would imply incomplete and/or slow utilization of lignocellulosic hydrolysates, genetic transformation of industrial strains with heterologous genes for pentose metabolic pathways is a popular way to enable, say, ethanol production from lignocellulose hydrolysates (Hahn-Hägerdal et al. 2007; Jeffries 2006; Rogers et al. 2007; van Maris et al. 2007). Invariably, the rates of pentose consumption by such engineered strains are generally lower than their corresponding glucose consumption rates. CCR is a prevailing obstacle that must be overcome in all industrial microorganisms (Bruckner and Titgemeyer 2002; Gancedo 1998). In effect, efficiency of sugar utilization remains a significant bottleneck to economical utilization of the mixed sugars of lignocellulosic hydrolysates.

For economically feasible production of fuels and chemicals from lignocellulosic biomass, continued improvements in more facets of microbial utilization of sugar mixtures must be made. For this to occur, technical challenges that vary depending on the inherent natural versatility of each host organism to utilize disparate sugars must be overcome (Fig. 1). For example, whereas deregulation of CCR in microorganisms that are capable of utilizing pentoses is the primary challenge, functional expression of genes encoding pentose-metabolizing enzymes and pentose transporters is of paramount importance in pentose-incompetent microorganisms. Moreover, one consequence of the differences in pentose metabolism pathways between bacteria and yeasts (Fig. 2) is that construction of pentose-metabolizing pathways is markedly more problematic in yeasts. It is therefore difficult to introduce functional bacterial pathways into yeasts, implying that advances made with bacteria are mostly inapplicable in yeasts (Sarthy et al. 1987). Nevertheless, numerous efforts to construct functional pentose-metabolizing pathways in *S. cerevisiae* have been made are well reviewed elsewhere (Hahn-Hägerdal et al. 2007; Jeffries 2006; Matsushika et al. 2009; van Maris et al. 2007). Recent studies on microbial sugar transport in general, and pentose transport in particular, have started to reveal the importance of sugar transporters in microbial fermentation performance (Kuyper et al. 2005; Pitkänen et al. 2005; Wahlbom et al. 2003). This review examines the recent progress made in engineering of sugar transport and the implication of such progress on development of microorganisms that can efficiently utilize sugar mixtures.

Bacterial sugar transporters in lignocellulosic hydrolysate utilization

Bacterial transporter proteins are crucial for selective permeability of microbial cells to nutrients and metabolites in general. They specifically mediate both passive and active transport of small solutes, including monosaccharide sugars, across membranes. In industrial bacteria, the role of transporters in sugar uptake is central to the microorganisms' ability to utilize lignocellulosic substrates. Elucidation of the roles of transporters was continued to improve markedly with the complete sequencing of an increasing number of genomes. Information derived from these sequences confirmed earlier suspicions that structurally and functionally different sugar transporters are largely

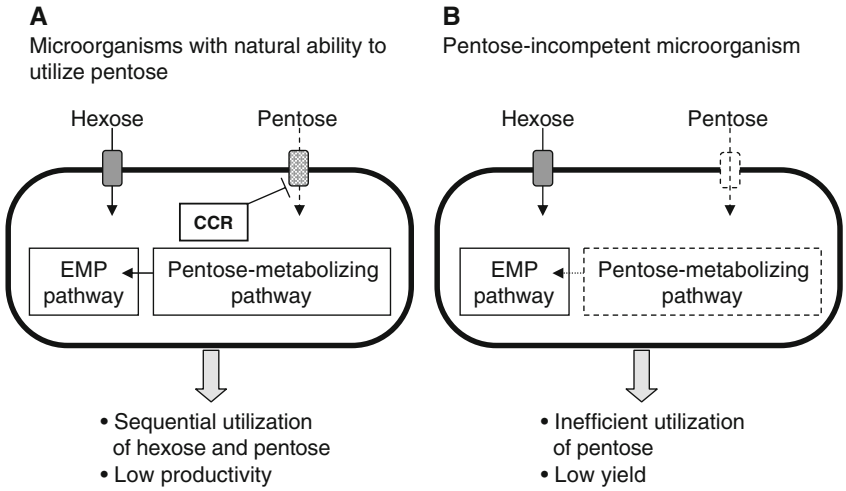

Fig. 1 Technical challenges to development of biocatalysts for producing value-added chemicals and fuels from lignocellulosic hydrolysates. Group A microorganisms include *E. coli* and some *Bacillus*. Due to CCR, they uptake pentose sugar only after hexoses have been exhausted. Group B microorganisms on the other hand lack efficient pentose uptake and/or metabolism enzymes

homologous to corresponding transporters across other life forms (Maiden et al. 1987). Bacterial sugar transporters as membrane proteins have evolved in nature into a few main groups. The largest and most diverse of these groups encompasses ATP binding cassette (ABC) transporters (Tarr et al. 2009). Universally distributed among living organisms, they function in many different aspects of bacterial physiology by coupling ATP hydrolysis to the uptake and efflux of solutes across the cell membrane (Davidson et al. 2008). The online repertoire called the ABCdb that aggregates ABC transporter information on fully sequenced

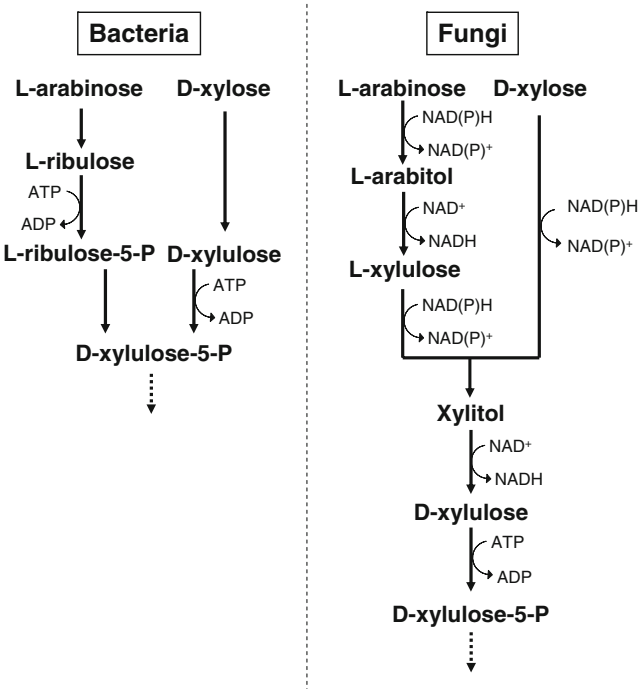

Fig. 2 Metabolic pathways for xylose and arabinose found in bacteria and fungi

archeal and bacterial genomes attests to the importance attached to this group of transporters (Fichant et al. 2006). The major facilitator superfamily (MFS) of transporters is another group that is ubiquitous in bacteria, archaeae, and eukaryotes (Pao et al. 1998). It encompasses H^+-linked symporters (Henderson 1990; Hofer and Misra 1978), Na^+-linked symporters/antiporters and uniporters (Goswitz and Brooker 1995), with recent evidence indicating a shared three-dimensional structure among all MFS antiporters (Law et al. 2008). Whereas ABC and MFS transporters are ubiquitous in all life forms, the phosphoenolpyruvate (PEP):carbohydrate phosphotransferase system (PTS) has been identified only in bacteria. Indeed it is through the PTS that most monosaccharides, disaccharides, and other sugar derivatives are phosphorylated and transported (Cases et al. 2007; Lengeler and Jahreis 2009; Lengeler et al. 1990; Saier et al. 2005; Saier and Reizer 1994). The localization, organization, and presumed mechanism of action of these transporters are depicted in Fig. 3. A more thorough transporter classification database that collates information on all transporter proteins is available online (Saier et al. 2006).

Uptake of hexoses in bacteria occurs predominantly through the PTS (Postma et al. 1993; Simoni et al. 1976), although hexoses not transported through the PTS (non-PTS sugars) are not rare. In the PTS, carbohydrates are phosphorylated at the expense of PEP as the product is transported across the cell membrane. The prominence of the PTS in bacterial sugar metabolism has generated a lot of interest leading to a deep understanding of its composition, structure, and organization (Cases et al. 2007; Deutscher et al. 2006). Phosphorylation requires at least three distinct proteins. Two general proteins, enzyme I (EI) and histidine-containing phosphocarrier protein (HPr), are common to phosphorylation of all sugars. The other protein forms the sugar-specific permease (EII) complex (Simoni et al. 1976).

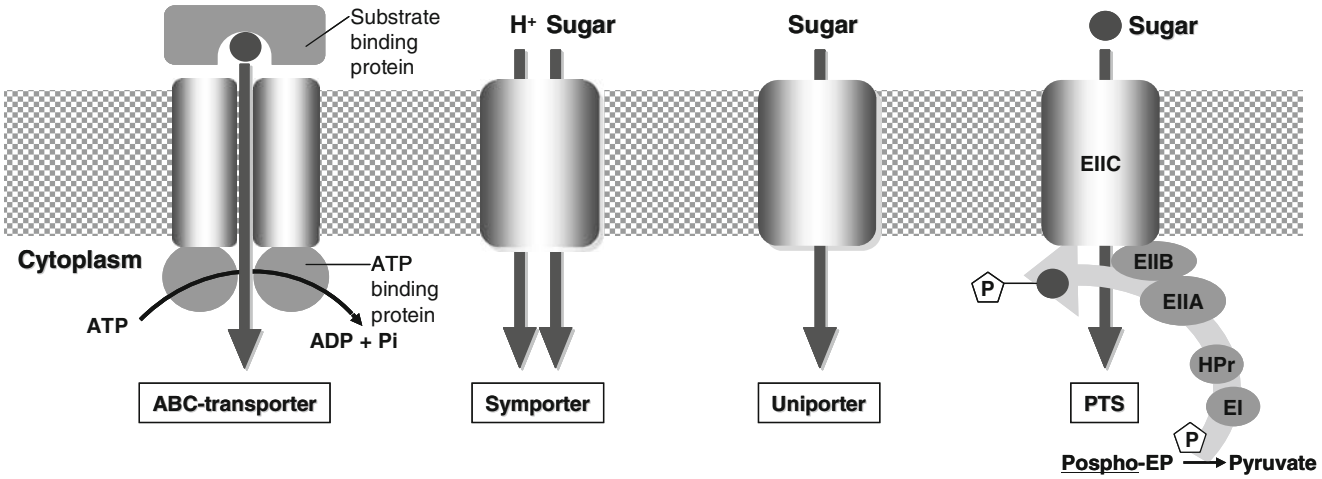

Fig. 3 General view of the organization and the mode of action of sugar transporters. In the PTS, phosphoenolpyruvate donate a phosphoryl group to EI of the PTS. HPr delivers the phosphoryl group to EIIA. Sugar eventually accepts the phosphoryl group from EIIB. Phospho-EP indicates phosphoenolpyruvate

The number of EII subunits encoded on the genome of a microorganism exerts considerable influence on the metabolic versatility of a given species. Nevertheless, even with exceedingly EII-rich microorganisms like *E. coli* that possesses at least 15 EIIs (Deutscher et al. 2006), mutations that alter some of the EII substrate specificities have the potential to appreciably improve microbial productivity of disparate compounds (Aboulwafa et al. 2003; Notley-McRobb and Ferenci 2000). Glucose and mannose are the main lignocellulose hydrolysate sugars transported into cells through the PTS.

The few bacteria capable of utilizing pentoses such as the arabinose and xylose that derive from hemicellulose hydrolysis usually possess metabolic pathways for degradation of these sugars. Moreover, they use distinct transporters other than the PTS for pentose uptake. Though specialized strains such as *Clostridium thermocellum* express specialized ABC transporters for pentose oligosaccharides (Nataf et al. 2009) the more common genes encode the uptake of given pentose monosaccharides. In *E. coli* separate ABC transporters for xylose (XylFGH) and arabinose (AraFGH; Horazdovsky and Hogg 1987; Sumiya et al. 1995) exist alongside a proton-linked xylose (XylE) and a distinct proton-linked arabinose (AraE) symporter (Davis and Henderson 1987; Maiden et al. 1988). A proton-linked GalP that catalyzes the uptake of galactose also transports xylose (Henderson 1990). ABC transporters for C5 sugars in general exhibit higher affinity (lower K_M) for their substrates than proton-linked transporters and uniporters. Table 1 summarizes biochemical characterization of sugar transporters associated with pentose uptake in selected microorganisms. This higher ABC transporter substrate affinity coupled with their elaborate genetic structure has meant that ABC transporters are attractive targets for genetic manipulation of bacterial pentose transport (Bohm et al. 2002).

Hexose and pentose transport systems in yeasts

Like bacteria, yeasts consume mono- and disaccharides preferentially to other carbohydrates. In yeasts however, irrespective of recent description of a glucose/xylose symporter (Leandro et al. 2006), the major sugar transporters of yeasts work by an energy-independent, facilitated diffusion mechanism. These so-called hexose (Hxt) and galactose (Gal2) transporters naturally exhibit different affinities and specificities for their substrates. They are abundant in *S. cerevisiae* but much less so in other industrial important yeast strains including *Kluveromyces lactis* and *Pichia stipitis* (Boles and Hollenberg 1997; Wieczorke et al. 1999). Besides D-glucose, many Hxt proteins transport D-fructose as well as D-mannose (Leandro et al. 2009), indicating that substrate specificities of Hxt proteins in yeast are generally broad.

Traditionally used in industrial production of ethanol, *S. cerevisiae* is unable to utilize either xylose or arabinose as fermentative substrates (Hahn-Hägerdal et al. 2007; Jin et al. 2004). Interestingly, it is able to uptake xylose even though the sugar is not a natural substrate (Hamacher et al. 2002). *S. cerevisiae* Gal2, Hxt1, Hxt2, Hxt4, Hxt5, and Hxt7 catalyze the uptake of xylose (Hamacher et al. 2002; Saloheimo et al. 2007; Sedlak and Ho 2004; Table 1). However, their affinity for xylose is much lower than that for glucose and the xylose uptake by the transporters is strongly inhibited by glucose (Saloheimo et al. 2007).

Table 1 Biochemical characteristics of pentose transporting proteins

Organism	Transporter	Type[a]	Substrate(s)	K_M (mM)[b]	Reference[c]
Prokaryote					
Escherichia coli	AraE	Symp	ara	$150–320\times10^{-3}$	Daruwalla et al. 1981
	AraFGH	ABC	ara	$4.1–6.1\times10^{-3}$	
	XylE	Symp	xyl	$63–170\times10^{-3}$	Henderson 1990
	XylFGH	ABC	xyl	$0.2–4.0\times10^{-3}$	Sumiya et al. 1995
Bacillus subtilis	AraE		ara, gal, xyl	ND	Krispin and Allmansberger 1998
Eukaryote					
Saccharomyces cerevisiae	Gal2	Unip	ara, gal, glu, xyl	ND (xyl)	Hamacher et al. 2002; Kou et al. 1970
	Hxt1	Unip	fru, glu, man, xyl	880 (xyl)	Saloheimo et al. 2007
	Hxt2	Unip	fru, glu, man, xyl	260 (xyl)	
	Hxt4	Unip	fru, glu, man, xyl	170 (xyl)	
	Hxt5	Unip	fru, glu, man, xyl	ND (xyl)	
	Hxt7	Unip	fru, glu, man, xyl	130 (xyl)	
Pichia stipitis	SUT1	Unip	fru , glu, xyl	1.5 (glu), 145 (xyl), 36 (fru)	Weierstall et al. 1999
	SUT2	Unip	glu, xyl	1.1/55 (glu)[d], 49 (xyl)	
	SUT3	Unip	fru, gal, glu, xyl	0.8/31(glu)[d], 103 (xyl), 49(fru), 176 (gal)	
Candida intermedia	GXS1	Symp	glu, xyl	0.2 (glu), 0.4 (xyl)	Leandro et al. 2006
	GXF1	Unip	glu, xyl	2 (glu), 49 (xyl)	

ara arabinose, *fru* fructose, *gal* galactose, *glu* glucose, *man* mannose, *ND* not determined, *xyl* xylose

[a] Type of transporters, symporter (Symp), ABC transporter (ABC), and uniporter (Unip) are indicated

[b] K_M for substrate indicated in parentheses is shown

[c] References for kinetic studies are indicated

[d] Two K_M values were determined due to their biphasic kinetics

P. stipitis is the best studied xylose-fermenting yeast (Jeffries 2006). Although its genome reveals a number of putative genes for sugar transporters (Jeffries et al. 2007), they are yet to be fully biochemically characterized. Three glucose transporters, encoded by the genes *SUT1*, *SUT2*, and *SUT3*, have been experimentally characterized in *P. stipitis* (Weierstall et al. 1999). Wild-type *P. stipitis* shows biphasic kinetics in the uptake of xylose whereas a *SUT1* mutant loses the low-affinity component, suggesting that Sut1 protein achieves xylose uptake using the low-affinity component.

Few high-affinity xylose transporters have been identified in yeast despite early evidence of the existence of proton-linked symporters in some strains (Hofer and Misra 1978; Lee et al. 2002). The rapid growth on xylose of *Candida intermedia* PYCC4715 was eventually attributed to one such glucose/xylose proton-linked symporter (Gardonyi et al. 2003; Leandro et al. 2006). The symporter protein produced in *S. cerevisiae* (Leandro et al. 2008) showed much higher affinity to xylose than xylose-transporting uniporters from *S. cerevisiae* and *P. stipitis* (Table 1). However, affinity of the protein for glucose is still higher than that for xylose.

Improved mixed-sugar utilization through modification of transporter genes in bacteria with impaired pentose uptake

Gram-positive *C. glutamicum* is widely used for the industrial production of amino and organic acids (Kinoshita 1985; Terasawa and Yukawa 1993). Owing to the ability of *C. glutamicum* cells packed to a high cell density under oxygen-deprived conditions to remain metabolically active despite cessation of cellular growth (Inui et al. 2004b), minimal genetic engineering has resulted in strains that can efficiently produce ethanol (Inui et al. 2004a), succinic acid (Okino et al. 2008a), and D-lactic acid (Okino et al. 2008b) from glucose. Though the versatility of *C. glutamicum* to produce an array of compounds from glucose may not end with these three compounds, the glaring inability of either wild-type *C. glutamicum* or any of the improved strains to utilize pentoses is an obvious bottleneck in the application of this microorganism in conversion of mixed sugars. In one study to address this, Yukawa and co-workers demonstrated that introduction of *E. coli* genes responsible for arabinose (*araBAD)* or xylose (*xylAB)* assimilation enabled *C. glutamicum* R strain to utilize the respective

sugars under both aerobic and oxygen-deprived conditions (Kawaguchi et al. 2006, 2008). Moreover, on screening numerous *C. glutamicum* strains, they discovered that *C. glutamicum* ATCC31831 has exceptional ability to metabolize arabinose. The gene cluster responsible for this arabinose metabolism in strain ATCC31831 consisted of the arabinose transporter gene (*araE*) and the metabolism operon, *araBAD* (Kawaguchi et al. 2009). By expressing only *araE* in recombinant *C. glutamicum* R harboring arabinose-catabolizing genes from *E. coli* (Sasaki et al. 2009), they were able to demonstrate a threefold increase in the arabinose consumption rate over a strain without *araE*. Moreover, *araE* introduction also enhanced xylose consumption threefold at low xylose concentrations in a recombinant *C. glutamicum* with a heterologous xylose-catabolizing pathway (Sasaki et al. 2009). This dual role of AraE has also been observed in *B. subtilis* (Krispin and Allmansberger 1998), even though amino acid similarity between the two AraE proteins from *C. glutamicum* and *B. subtilis* is only 34%. Particularly noteworthy is the observation in the studies of *C. glutamicum* that pentose consumption by either growing or oxygen-deprived cells of recombinant strains expressing *araE* was not repressed in the presence of glucose.

Lignocellulose hydrolysates contain not only monosaccharides but also oligosaccharides from partial degradation of polysaccharides. Besides the ability to utilize mixed sugar monosaccharides therefore, the ability to utilize oligosaccharides can help to reduce production costs associated with saccharification of lignocellulosic feedstocks. In a demonstration of microbial production of ethanol from cellobiose, Yanase et al. (2005) developed a cellobiose-utilizing *Z. mobilis* strain by introducing the gene for β-glucosidase from *Ruminococcus albus* that is localized in the periplasm or on the cell surface. Although the recombinant *Z. mobilis* consumed cellobiose, the sugar consumption rate decreased with decreasing cellobiose concentration, inviting the conclusion that the affinity of *R. albus* β-glucosidase for cellobiose was too low (K_M= 26 mM; Ohmiya et al. 1985).

In a different approach to confer cellobiose utilization ability to a microorganism, Sasaki et al. (2008) constructed a recombinant *C. glutamicum* strain harboring mutated PTS EIIBAC genes isolated from an adaptive mutant of *C. glutamicum*. The adaptive mutant was capable of consuming cellobiose due to a positional switch of Val-317 to Ala or Met in its β-glucoside-specific EII (Kotrba et al. 2001, 2003). In the resultant recombinant *C. glutamicum* strain, cellobiose was transported and phosphorylated through the mutated PTS, and subsequently degraded by phospho-β-glucosidase in the cell. The recombinant *C. glutamicum* consumed cellobiose without obvious dependence on cellobiose concentration under oxygen deprivation (Sasaki et al. 2008). It is likely that the affinity of the mutated EII for cellobiose is relatively high.

Development of recombinant yeast strains to improve utilization of sugar mixtures by modification of pentose transport

Xylose is a less efficient fermentative substrate than glucose even for recombinant xylose-utilizing strains of *S. cerevisiae*. Analyses of mutant strains showing better growth on xylose suggest that the uptake of xylose contributes to flux control of fermentation under certain conditions (Kuyper et al. 2005; Wahlbom et al. 2003). Moreover, since xylose transport in *S. cerevisiae* is inhibited by glucose (Saloheimo et al. 2007), introduction of xylose-specific transporters is considered an effective way to improve xylose utilization, especially in a sugar mixture containing glucose.

In an early study, *S. cerevisiae* Hxt genes involved in xylose uptake were sequentially overexpressed in strains lacking any *HXT* in an attempt to restore their ability to grow on xylose (Hamacher et al. 2002). This study revealed that expression of a single *HXT* (*HXT4*, *HXT5*, *HXT7*, or *GAL2*) restored aerobic growth on xylose. Sedlak and Ho (2004) consequently examined xylose consumption on a glucose–xylose mixture by a recombinant *S. cerevisiae* strain overproducing xylose-transporting Hxts and found that overexpression of *HXT5* or *HXT7* was more effective than *HXT4* or *GAL2* to stimulate xylose consumption. Recently, heterologous uniporter genes, *P. stipitis SUT1* (Katahira et al. 2008) and *C. intermedia GXF1* (Runquist et al. 2009) were overexpressed in a xylose-utilizing recombinant *S. cerevisiae* strain with all *HXTs*. A *SUT1*-expressing strain consumed 1.15-fold more xylose than the parental strain. Meanwhile a *GXF1*-expressing strain showed a twofold lower K_M in xylose transport kinetics compared to the parental strain. Under anaerobic conditions, xylose consumption at low concentrations was improved by expression of *GXF1*.

The above studies demonstrate that overproduction of a uniporter improves xylose utilization, albeit to a limited extent only. Since the affinity for xylose of the uniporters examined in these studies are relatively low (K_M>49 mM; Table 1), xylose consumption rates should decrease at low xylose concentrations. To circumvent the problem of reduced xylose uptake at low xylose concentrations, several studies have investigated overproduction of a sugar/proton symporter with higher affinity to xylose. The genes for symporters *xylE* (*E. coli*), *AtSTP2*, *AtSTP3* (*Arabidopsis thaliana*), and *Hup1* (*Chlorella kessleri*) were overexpressed in mutant strain TMB3201 of *S. cerevisiae* lacking all *HXT* genes but with a functional xylose utilization pathway (Hamacher et al. 2002). Neither the resultant

strains nor the parental strain were able to grow on xylose. Gsx1 symporter isolated from *C. intermedia* exhibited substrate specificity for xylose and glucose (Leandro et al. 2006). It was expressed from a high- or low-copy plasmid in the strain TMB3201. However, none of the strains showed growth on xylose even though the expressed Gxs1 protein was functional (Leandro et al. 2006). Further investigation confirmed that the Gxs1 protein was abundantly expressed and correctly targeted to the plasma membrane in *S. cerevisiae* (Leandro et al. 2008). In addition, presence of glucose/xylose uniporters strongly reduced *GXS1* mRNA level. Further discussion of the effects of *GXS1*-expression on growth on xylose is found in the reference (Leandro et al. 2008).

Hector et al. (2008) examined overexpression of genes for putative xylose/proton symporters, At5g59250 and At5g17010 from *A. thaliana* in a xylose-utilizing recombinant *S. cerevisiae*. Western analyses showed that the transporters with correct molecular mass were detected and these proteins were localized in the peripheral region of the cells. The *At5g59250*-expressing strain showed 2.5-fold increase of xylose utilization in the presence of glucose, but rate of xylose consumption approximately 80% decreased after glucose depletion.

An interesting observation was reported by Saloheimo et al. (2007) who identified a novel transporter Trxlt1 from *Trichoderma reesei*. When the *xlt1* gene was expressed in a recombinant xylose-utilizing *S. cerevisiae* lacking major *HXT* genes, no significant growth on xylose was observed. However, after long lag period (2–3 weeks), the recombinant *S. cerevisiae* expressing *xlt1* was able to grow on a medium containing 20 g/l of xylose and 0.3 g/l of maltose whereas no growth was observed on a medium containing only xylose (Saloheimo et al. 2007). The isolated strain from the xylose and maltose culture described above grew on xylose as the sole carbon source after a much shorter lag period. The authors attributed this observation to an adaptive mutation in the host strain.

Improved mixed-sugar utilization through release of carbon catabolite repression in *E. coli*

We have so far reviewed studies on improvement of xylose utilization of microorganisms incapable of xylose assimilation or fermentation. *E. coli* on the other hand can utilize almost all the sugar components of lignocellulose. The strain has a preferred order of sugar utilization when more than one sugar is present due to the regulatory mechanism of CCR (Deutscher et al. 2006; Gorke and Stulke 2008). One well-known example of CCR is the lactose–glucose diauxie where lactose consumption starts only after depletion of glucose in a growth medium. Therefore, CCR causes delayed fermentation of lignocellulose hydrolysates. Inducer exclusion is considered to be the major mechanism of CCR in *E. coli* (Deutscher et al. 2006). It is mediated via a component of the PTS which transports and phosphorylates carbohydrates. The PTS components form a protein phosphorylation cascade. When *E. coli* grows rapidly on preferred carbon sources, such as glucose, glucose-specific EII (EII^{glu}) is preferentially dephosphorylated. The dephosphorylated EII^{glu} interacts with transporters for non-PTS sugars, such as lactose, maltose, and melibiose and inhibits their translocation activity (Deutscher et al. 2006). Consequently, non-PTS sugars are less present in the cells to induce their catabolic genes. Thus, in an *E. coli*-based fermentation process sugars derived from lignocellulose are sequentially consumed, i.e. when *E. coli* is used for fermentation of lignocellulose hydrolysates, glucose is consumed in the beginning of fermentation and consumption of xylose and arabinose are repressed during glucose is present. In fact, it has been observed that sugars in a glucose–xylose mixture are sequentially utilized by ethanologenic *E. coli* (Lawford and Rousseau 1991).

As described above, PTS plays a central role in CCR of *E. coli*. Therefore, the effects of mutation in *ptsG* encoding EII^{glu} on sugar mixture utilization have been investigated. Although a PTS mutant of *E. coli* showed poor growth on glucose, a $glucose^+$ revertant of a PTS mutant with a similar growth rate to wild-type was isolated by a continuous culture method (Flores et al. 1996) and overexpression of the galactose permease (GalP) and glucokinase (Glk) genes (Hernández-Montalvo et al. 2003). A recombinant ethanologenic *E. coli* with *ptsG* mutation was constructed and its utilization of sugar mixtures was investigated (Nichols et al. 2001). A wild-type strain showed sequential glucose–pentose utilization, while the corresponding *ptsG* mutant consumed these sugars simultaneously and produced ethanol at comparable yields with the parental strain. A similar study was performed with a PTS^- $glucose^+$ mutant strain (Hernández-Montalvo et al. 2001). When the mutant was grown in a medium containing 1 g/l each of glucose, arabinose, and xylose, glucose and arabinose was simultaneously consumed to produce mixed organic acids. Meanwhile, xylose consumption by the mutant was repressed until depletion of glucose and arabinose. The authors showed that the uptake of ^{14}C-labeled xylose by the PTS^- $glucose^+$ mutant was inhibited by glucose.

The *ptsG* mutation in *E. coli* is also examined in lactic acid production from sugar mixture (Dien et al. 2002). L-lactate dehydrogenase gene from *Streptococcus bovis* was expressed in a *ptsG* mutant of *E. coli* with an additional mutation (Δ*frdABCD*). Although the resulting strain FBR19 consumed xylose in the presence of glucose and produced L-lactic acid, rate of xylose consumption gradually decreased and part of xylose remained not to ferment in a medium. When FBR19 was used to ferment a variety of

glucose (0–40 g/l) and xylose (40 g/l), the yields of lactic acid were decreased with increasing glucose concentration. These results are inconsistent with the result obtained from the ethanologenic *ptsG* mutant that completely consumes xylose in a mixture of three sugars as described above (Nichols et al. 2001). Osmotic stress brought from the addition of base to maintain pH is speculated by the authors because much less base is required for maintenance of pH in ethanol fermentation than in lactic acid fermentation. Understanding of detailed mechanisms for xylose utilization in *ptsG* mutant will provide insight into development of a better strain for conversion of lignocellulose hydrolysate.

Xylitol can be produced in a biological process where xylose is reduced by the action of NAD(P)H-dependent xylose reductase. Coenzyme regeneration is therefore required in this production system. To study of xylitol production by *E. coli*, glycolysis is used for the coenzyme regeneration. This process demands simultaneous xylose and glucose transport into cells. Khankal et al. (2008) constructed a recombinant *E. coli* strain in which either xylose/proton symporter or xylose ABC transporter genes and xylose reductase gene are expressed under control of the *tac* promoter. The resulting recombinant strain showed significantly improved xylose consumption in the presence of glucose compared to a strain with only xylose reductase.

Outlook

Although steady progress has been made in improvement of mixed-sugar utilization by microorganism, more work is required across the board if utilization of lignocellulosic biomass resources using microorganisms is to be economically feasible. For example, the consumption rate of xylose by the most efficient recombinant *S. cerevisiae* is still lower than that of glucose and gradually decreases with decreasing xylose concentration. As a result, complete consumption of xylose in mixed sugar substrates is often difficult. Positive results on engineering of sugar transport for improvement of sugar utilization have been increasingly reported, though inadequate knowledge of sugar transporters hampers further progress. Surprisingly, even in *E. coli* few studies on biochemical and molecular biological characterization of xylose transporters have been carried out. This is bound to change now that renewable energy resources are a primary focus of researchers worldwide.

Another hurdle to be cleared in the quest for efficient microbial utilization of mixed sugar substrates is the absence of a versatile method for overproduction of membrane proteins. To date, membrane proteins from phylogenetically very close organisms are likely to function well in select host microorganisms as was observed in *C. glutamicum* by Sasaki et al. (2009) and in *E. coli* by Khankal et al. (2008). In this respect, more efforts to explore and characterize sugar transporters should be expended. Hopefully, attempts to alter substrate specificities of sugar transporters by protein engineering will become more successful (Kasahara et al. 2007; Soberon and Saier 2006) in order to improve utilization of pentoses. Finally, new methods that enable fast and simple assay of sugar uptake will help to facilitate finding and engineering of sugar transporters (Chen et al. 2009).

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