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Optimization of Culture Conditions for D-Ribose Production by Transketolase-Deficient *Bacillus subtilis* JY1

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Abstract D-Ribose is a five-carbon sugar used for the commercial synthesis of riboflavin, antiviral agents, and flavor enhancers. Batch fermentations with transketolase-deficient *B. subtilis* JY1 were carried out to optimize the production of D-ribose from xylose. The best results for the fermentation were obtained with a temperature of 37°C and an initial pH of 7.0. Among various sugars and sugar alcohols tested, glucose and sucrose were found to be the most effective for both cell growth and D-ribose production. The addition of 15 g/l xylose and 15 g/l glucose improved the fermentation performance, presumably due to the adequate supply of ATP in the xylose metabolism from D-xylulose to D-xylulose-5-phosphate. A batch culture in a 3.7-l jar fermentor with 14.9 g/l xylose and 13.1 g/l glucose resulted in 10.1 g/l D-ribose concentration with a yield of 0.62 g D-ribose/g sugar consumed, and 0.25 g/l-h of productivity. Furthermore, the sugar utilization profile, indicating the simultaneous consumption of xylose and glucose, and respiratory parameters for the glucose and sucrose media suggested that the transketolase-deficient *B. subtilis* JY1 lost the glucose-specific enzyme II of the phosphoenolpyruvate transferase system.

Key words: D-Ribose, xylose, *Bacillus subtilis*, transketolase, batch fermentation

D-Ribose, a five-carbon sugar, is used as an intermediate in the synthesis of vitamin B2, nucleoside antiviral agents, and nucleotide flavor enhancers. The end products resulting from chemical conversion of D-ribose and their roles in the human body have already been well summarized in a review [6]. D-Ribose supplementation has also been used to increase the skeletal muscle adenine salvage rates during recovery from intense contractions and the subsequent muscle performance [30]. D-Ribose is known to exert a cardioprotective effect on the adenine nucleotide metabolism in the heart muscle [31]. As such, donor treatment and metabolic support with D-ribose during organ preservation may help prolong the preservation time of donor hearts by maintaining the ATP at a higher level [21].

Transketolase is a metabolic enzyme involved in the non-oxidative pentose phosphate (PP) pathway and creates

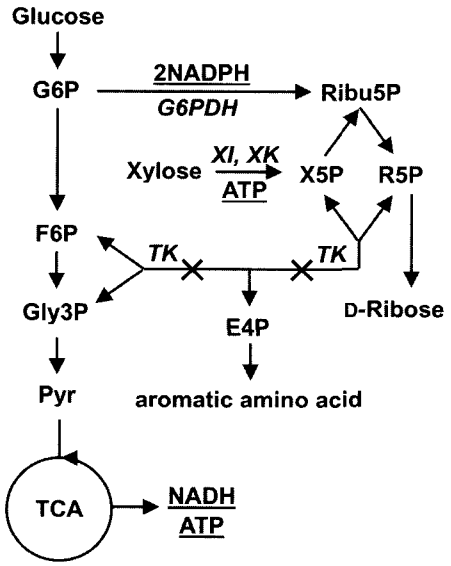

Fig. 1. Metabolic network of transketolase-deficient *B. subtilis* JY1.
The bold and underlined compounds show the cofactors produced or consumed in the catabolic steps. The bold and italic letters indicate the enzymes involved in the D-ribose production based on the following abbreviations: XI, xylose isomerase; XK, xylulokinase; G6PDH, glucose-6-phosphate dehydrogenase; TK, transketolase. The abbreviations for the other metabolites are: G6P, glucose-6-phosphate; F6P, fructose-6-phosphate; Gly3P, glyceraldehyde-3-phosphate; Pyr, pyruvate; Ribu5P, ribulose-5-phosphate; X5P, xylulose-5-phosphate; R5P, ribose-5-phosphate; E4P, erythrose-4-phosphate. The cross presents the disruption of transketolase, leading to the blocking of the non-oxidative PP pathway.

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a reversible link with transaldolase between glycolysis and the PP pathway, as shown in Fig. 1. Therefore, if transketolase is deactivated, none of the glucose metabolites catalyzed by glucose-6-phosphate dehydrogenase will be directed to glycolysis. In addition, five-carbon sugars, including xylose and arabinose, that are metabolized by their operon enzymes will not be used as the sole carbon source, but rather accumulated as an intermediate, ribulose-5-phosphate or ribose-5-phosphate, in the PP pathway due to the blocking of the non-oxidative PP pathway. The biological production of D-ribose is carried out by the dephosphorylation of D-ribose-5-phosphate. Transketolase-deficient strains have already been constructed in several microorganisms. For example, *E. coli* mutants resulting from ethyl methane sulfonate treatment and the penicillin G method were selected and characterized as transketolase mutants [15], which require aromatic amino acids and certain vitamins or shikimic acid to grow on a minimal medium. However, no accumulation of D-ribose was detected either intracellularly or extracellularly [16]. A transketolase-negative mutant of *Salmonella typhimurium* and double-deletion mutant of two transketolases (*TKL1* and *TKL2*) in *Saccharomyces cerevisiae* exhibited the same auxotroph type as other organisms [9, 25], and a transketolase mutant of *Corynebacterium glutamicum* produced 2 g/l D-ribulose without the accumulation of D-ribose [12]. Several *Bacillus subtilis* and *B. pumilus* strains have also been selected to produce D-ribose and characterized as transketolase mutants [24]. In the production of D-ribose, the addition of organic acids into the culture broth has been found to improve D-ribose productivity, while decreasing the by-product formation [7]. Gluconic acid has also been used as a good alternative carbon source [8]. Recently, we developed a biological process for producing D-ribose from xylose and glucose using a transketolase mutant of *B. subtilis* [27].

Therefore, the current study has been focused on characterizing the fermentation properties of transketolase-deficient *B. subtilis* JY1 to determine the optimal environmental conditions for the efficient production of D-ribose from xylose, a major component of a cellulosic biomass [19].

MATERIALS AND METHODS

Bacterial Strain

A D-ribose-producing *B. subtilis* strain isolated from a highly concentrated sugar solution was donated by Bolak Co. (Kyonggi, Korea). The strain was characterized as a transketolase-deficient mutant, like other D-ribose-producing stains, and named *B. subtilis* JY1.

Medium and Culture Conditions

A Luria broth (5 g/l yeast extract, 10 g/l tryptone, and 10 g/l NaCl) containing 15 g/l agar and 20 g/l glucose was used as the solid medium for storing *B. subtilis* JY1 at 4°C. The batch cultures were incubated at 37°C and 250 rpm using a shaking incubator (HK-S125C, Hankook Mechanics Co., Korea). A 500-ml baffled flask (Nalge Nunc Int., U.S.A.) contained 200 ml of the culture medium, consisting of 10 g/l Bacto yeast extract (BD, U.S.A.), 5 g/l KH_2PO_4, 5 g/l K_2HPO_4, 1 g/l $MgSO_4 \cdot 7H_2O$ [5], and some carbon sources. To determine the optimal environmental conditions, the culture temperatures were varied from 30°C to 40°C and the initial pHs from 5.5 to 7.5. Carbon mixtures including 15 g/l xylose were tested with 15 g/l hexoses (glucose, fructose, and mannose), 7.5 g/l disaccharides (sucrose and maltose), and polyols (15 g/l mannitol and sorbitol, and 30 g/l glycerol). The optimal ratio of xylose to glucose was determined by adding 5 g/l, 10 g/l, and 15 g/l glucose into the culture medium containing 15 g/l xylose. The batch culture was performed in a 3.7-l jar fermentor (Type ALF, Bioengineering AG, Sweden) at 37°C, 600 rpm agitation, and 1 vvm air-flow rate. One liter of the culture medium with a mixture of xylose and glucose or sucrose was used, and the pH was controlled at 7.0±0.05 with a 2 N HCl and ammonia solution. The concentrations of oxygen and carbon dioxide in the off-gas from the jar fermentor were monitored using a gas analyzer (LKM2000-03, Lokas, Korea). The gas rate was converted to SI units by multiplying the percentage of each concentration with 3.56×10^{-1} mmol/l-min for oxygen or 2.59×10^{-1} mmol/l-min for carbon dioxide [1]. An antifoaming agent (Antifoam 289, Sigma, U.S.A.) was added to reduce the formation of foam.

Dry Cell Mass and Carbohydrate Concentration

The dry cell mass in the culture broth was determined by multiplying a conversion factor of 0.33 with the optical density measured at 600 nm using a spectrophotometer (Ultrospec 2000, Amersham Biosciences, Sweden). The culture broth was harvested and the supernatant was extracted and diluted appropriately for further analysis. The concentrations of carbohydrates in the culture broth were determined using a high performance liquid chromatography system (M930, Younglin Co., Korea) [14]. To measure the amount of monosaccharides, disaccharides, and polyols, 20 µl of the diluted sample was injected into a Carbohydrate Analysis column (Waters, U.S.A.) at room temperature, including 80% acetonitrile at a flow rate of 1.5 ml/min. The acetic acid and acetone were separated using an Aminex HPX-87H ion exclusion column (300 mm×7.8 mm, Bio-Rad Co., U.S.A.) heated at 60°C, and a 5 mM H_2SO_4 solution was used as the solvent at a flow rate of 0.6 ml/min. Detection was carried out using a reflective index detector (Knauer Co., Germany).

Xylulokinase Activity and Protein Concentration

After centrifuging 1 ml of the culture broth, the cell pellet was washed once and resuspended in resuspension buffer

(60 mM $Na_2HPO_4 \cdot 2H_2O$, 40 mM $Na_2H_2PO_4 \cdot 2H_2O$, 10 mM KCl, 1 mM $MgSO_4 \cdot 7H_2O$, and 0.5 mM phenylmethylsulfonyl fluoride, Sigma, U.S.A.) at pH 7.0. Cells were disrupted using an ultrasonic processor (CP50T, ColeParmer, U.S.A.) at 40% output for 4 min at 4°C, then the suspension was centrifuged at 15,000 rpm for 15 min at 4°C. The supernatant was used as a crude enzyme solution to determine the xylulokinase activity and protein concentration. An assay of the xylulokinase activity was performed using a modified Enzymatic BioAnalysis of glycerol (Roche, Germany) to monitor the NADH oxidation. A 200 µl of solution 1 (glycylglycin buffer, pH 7.4, 0.2 mM NADH, 1.1 mM ATP, 2.3 mM phosphoenolpyruvate, and $MgCl_2$), 700 µl of water, and 3 µl of solution 2 (1.8 U pyruvate kinase and 1.7 U lactate dehydrogenase) were mixed and maintained at 25°C for 3 min. A 100 µl of the crude enzyme solution diluted appropriately was then added to the above solution and incubated at 25°C for 5 min. The enzyme reaction was initiated by the addition of 50 µl of a xylulose solution (3.0 mM) and monitored using a spectrophotometer at 340 nm. Controls were obtained without the addition of the xylulose solution. One unit of xylulokinase was defined as the amount of enzyme able to oxidize 1 µmol of NADH at 25°C and pH 7.0. The protein concentration in the crude enzyme solution was determined using a Bio-Rad Protein Assay kit (Bio-Rad, Richmond, CA, U.S.A.) based on the Bradford method. The assay was performed three times independently.

RESULTS AND DISCUSSION

Identification of D-Ribose-Producing *B. subtilis* JY1

To test the ability of the transketolase-deficient *B. subtilis* JY1 as regards D-ribose production, a batch culture was carried out with 5 ml of the culture medium containing 5 g/l xylose and 5 g/l glucose. After 48 h of cultivation at 37°C with shaking at 250 rpm, the sugar composition of the culture broth was analyzed by HPLC, as depicted in Fig. 2. The xylose was completely consumed, while a slight amount of glucose remained. A single peak with a retention time of 4.5 min was obtained and identified as D-ribose, thereby confirming that *B. subtilis* JY1 could produce D-ribose from xylose and glucose.

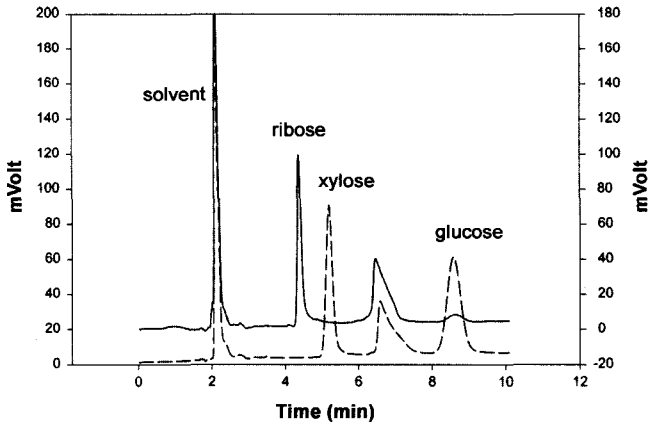

Fig. 2. HPLC chromatogram of *B. subtilis* JY1 culture broth.
The dashed line indicates the profile of the culture medium containing 5 g/l xylose and 5 g/l glucose, while the solid line presents the sample analysis after 48 h of cultivation.

Optimization of Environmental Conditions

Batch fermentations at different culture temperatures and initial pHs were carried out to determine the optimal values for the production of D-ribose from 15 g/l xylose and 15 g/l glucose. Figure 3(a) shows the final results after 48 h of fermentation with *B. subtilis* JY1 at various temperatures ranging from 30°C to 40°C. A maximum dry cell mass of 4.0 g/l and D-ribose concentration of 6.0 g/l were obtained at 37°C. From 30°C to 37°C, the fermentation performance improved gradually, yet the cell growth and production of D-ribose decreased sharply at 40°C, suggesting that *B. subtilis* JY1 is a mesophilic

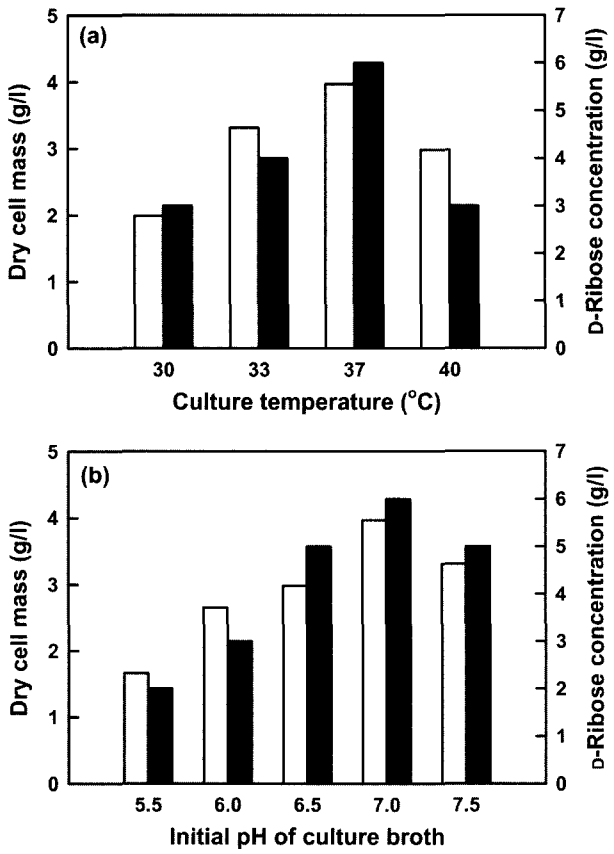

Fig. 3. Effect of culture temperature (a) and initial pH (b) of culture broth on cell growth and D-ribose production.
The white bar shows the dry cell mass, while the black bar indicates the D-ribose concentration in the culture broth after 48 h of cultivation.

bacterial strain. As shown in Fig. 3(b), the batch cultures performed with various initial pHs from 5.5 to 7.5 produced the best result at pH 7.0. Thus, a temperature of 37°C and a pH of 7.0 were used in all subsequent experiments.

Determination of Co-substrates Suitable for D-Ribose Biosynthesis

Xylose by itself is not utilized by transketolase mutants, since the non-oxidative PP pathway is knocked out. As such, other carbon sources must be added to the culture broth as a co-substrate to supply ATP for cell growth and xylose metabolism [2, 17]. Batch fermentations with various monosaccharides (glucose, fructose, and mannose), disaccharides (sucrose and maltose), and polyols (mannitol, sorbitol, and glycerol) were performed to identify the best carbon source for ATP supplementation, and the results are summarized in Table 1. With regards the dry cell mass, mannose produced the highest cell mass of 2.18 g/l, while glucose, fructose, sucrose, and sorbitol all produced similar values of around 1.93(±0.02) g/l. For D-ribose production, most of the sugars yielded final concentrations of 6.33–7.82 g/l, except for fructose (4.34 g/l) and glycerol (2.48 g/l). The yields of D-ribose relative to the sugar consumed were between 0.34 and 0.62 g/l, and the maximum value was obtained with glucose. For byproduct formation, glucose (0.77 g/l) and maltose (0.56 g/l) mediated lower concentrations of acetate and acetoin than the other sugars. The specific xylulokinase (XK) activity after 30 h, at the highest point of D-ribose production, was analyzed to investigate the effect of sugar on the conversion of xylose to D-ribose in *B. subtilis* JY1 (Table 1). XK is a key enzyme in the xylose metabolism and catalyzes the irreversible conversion of xylulose to xylulose-5-phosphate by consuming one mole of ATP (Fig. 1). Except in the case of fructose, mannitol, and glycerol, the specific XK activity was between $0.95(\pm0.02)\times10^{-1}$ and $1.54(\pm0.03)\times10^{-1}$ U/mg protein. PTS-sugars, such as glucose, fructose, and mannitol, are usually known to be transported into *B. subtilis* according to their specific phosphoenolpyruvate transferase systems (PTS) [23, 28]. In PTS, the HPr protein phosphorylated serine at residue 46 triggers the catabolite repression of the xylose operon and hence inhibits the transcription of the *xylB* gene encoding XK [13, 26, 28]. As such, the cultures with fructose and mannitol resulted in low specific XK activities of $0.22(\pm0.02)\times10^{-1}$ and $0.57(\pm0.01)\times10^{-1}$ U/mg protein, respectively. This decrease in specific XK activity then caused a reduction in D-ribose production. In the case of glucose, the specific XK activity of $0.95(\pm0.02)\times10^{-1}$ U/mg protein was higher than that with fructose and mannitol. As such, this unusual observation of a high XK activity with glucose suggests that the PTS of glucose is different from that of other sugars. It has been reported that the *ptsHI* (two elements of PTS) deletion mutant of *B. megaterium* exhibited reduced catabolite repression in the xylose operon by a factor of 3 to 16 [29], while catabolite derepression in the *ptsH* disrupted mutant of *B. subtilis* allowed the same gluconate kinase activity under catabolite repression conditions [4]. Glycerol, which diffuses into cells facilitatively, is not good for cell growth and D-ribose production owing to its insufficient supply of ATP and building blocks for cell component construction, including XK expression [23, 28]. However, glucose and sucrose were both found to be good co-substrates for producing D-ribose from xylose in terms of cell growth, D-ribose, and byproduct production.

Optimal Mixture Composition of Xylose and Glucose

The supplementation of ATP plays an important role in the conversion of xylose to D-ribose. Therefore, batch fermentations were carried out to determine the optimal composition of a mixture of xylose and glucose. The glucose concentrations were varied from 5 g/l to 15 g/l in a medium containing 15 g/l xylose, and the experimental results are presented in Fig. 4. Glucose was used as an energy source to produce ATP. A high glucose level improved the fermentation parameters, except for the D-ribose yield (0.41 g/l). The addition of 15 g/l glucose and 15 g/l xylose led to a 4.12 g/l D-ribose concentration and 0.098 g/l cell-h D-ribose productivity, which were 1.2- and

Table 1. Results of batch cultivation with monosaccharides, disaccharides, and sugar alcohols.

Carbon source	Dry cell mass (g/l)	D-Ribose concentration (g/l)	Yield (g ribose/g sugar)	Byproduct concentration (g/l)		Specific xylulokinase activity ($\times10^{-1}$ U/mg protein)
				Acetate	Acetoin	
Glucose	1.95	7.03	0.62	0.50	0.27	0.95(0.02)
Fructose	1.93	4.34	0.34	0.47	2.80	0.22(0.01)
Mannose	2.18	7.74	0.41	0.42	1.99	1.02(0.02)
Sucrose	1.91	7.82	0.56	0.77	0.87	1.04(0.03)
Maltose	1.75	6.88	0.54	0.36	0.20	1.13(0.03)
Mannitol	1.55	6.33	0.53	0.50	1.48	0.57(0.01)
Sorbitol	1.95	7.08	0.45	0.51	1.96	1.54(0.03)
Glycerol	1.29	2.48	0.58	1.36	0.54	0.30(0.02)

The values in parenthesis show the standard deviation.

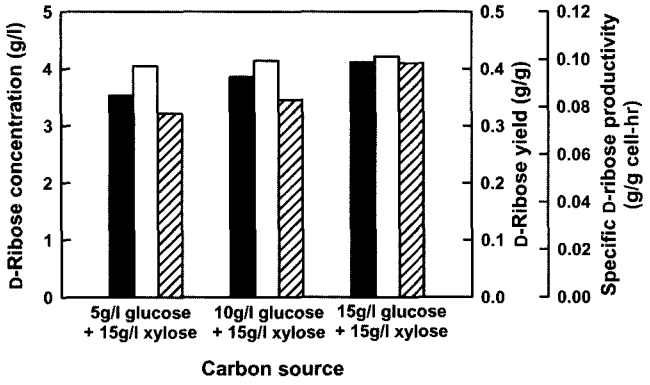

Fig. 4. Results of batch fermentation with various compositions of xylose and glucose in *B. subtilis* JY1 obtained after 30 h of cultivation at 37°C and 250 rpm.
The black bar indicates the D-ribose concentration, while the white bar indicates the D-ribose yield based on the consumed xylose and glucose. The slash bar represents the specific D-ribose productivity.

1.3-fold higher, respectively, than that in a batch culture with 5 g/l glucose and 15 g/l xylose. The enhanced D-ribose production based on increasing glucose concentration seemed to be related to the adequate supply of ATP for the conversion of D-xylulose to D-xylulose-5-phophate in the xylose metabolism. A similar result based on providing a co-substrate for xylose utilization was also reported [3, 20], where feeding glucose at a basal level increased the xylitol productivity in a cell recycle fermentation of *Candida tropicalis* and a fed-batch cultivation of a recombinant *S. cerevisiae* harboring the xylose reductase gene, since an adequate amount of NAD(P)H was regenerated with the glucose metabolism and NAD(P)H is a key cofactor in transforming D-xylose into xylitol.

Batch Fermentations of *B. subtilis* JY1

More fermentations were undertaken in a jar fermentor to determine the optimal composition of the sugar mixture, i.e. xylose and glucose or xylose and sucrose. A batch fermentation profile using 14.9 g/l xylose and 13.1 g/l glucose is depicted in Fig. 5. The dry cell mass increased exponentially at a specific growth rate of 0.65 h^{-1} for up to 8 h, then remained constant until 40 h. Xylose and glucose were consumed simultaneously and D-ribose was produced after 4 h until 40 h. As a result, a dry cell mass of 2.30 g/l and D-ribose concentration of 10.1 g/l were obtained. The oxygen uptake rate (OUR) increased rapidly during the exponential phase and reached 0.12(±0.015) mmol/l-min. While D-ribose was produced during the stationary phase, oxygen was taken up at a constant rate of 0.056(±0.007) mmol/l-min, and the carbon dioxide production exhibited the same profile as the oxygen consumption. The carbon dioxide evolution rate (CER) was maintained at 0.025(±0.005) mmol/l-mim during D-ribose production. Xylose is usually metabolized after glucose depletion,

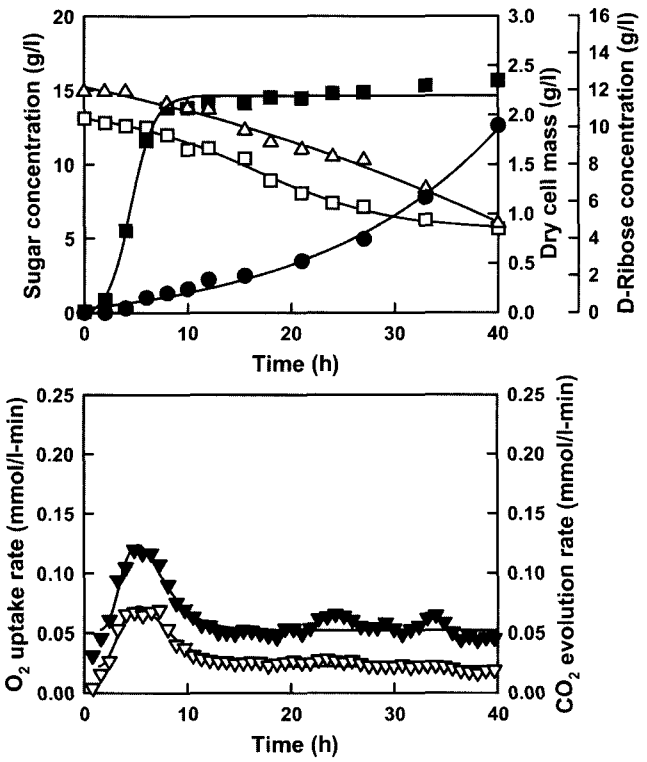

Fig. 5. Batch fermentation of *B. subtilis* JY1 with 14.9 g/l xylose and 13.1 g/l glucose in jar fermentor at 37°C, 600 rpm, pH 7.0, and 1 vvm.
■, dry cell mass; □, glucose concentration; △, xylose concentration; ●, D-ribose concentration; ▼, oxygen consumption rate; ▽, carbon dioxide evolution rate.

because glucose represses the transcription of the xylose operon genes in microorganisms [10, 28]. However, the transketolase-deficient *B. subtilis* JY1 exhibited the unusual behavior of the simultaneous utilization of xylose and glucose. Similar results have also been reported for other transketolase mutant *B. subtilis* strains, probably due to the low intracellular level of fructose-1,6-bisphosphate, a catabolite repression element [5]. The simultaneous consumption of xylose and glucose was also observed in a PTS mutant of *E. coli* [11]. Thus, it seemed that certain elements involved in the catabolite repression mechanism were disrupted in *B. subtilis* JY1.

When using sucrose as an alternative carbon source, the batch fermentation was carried out with 15.2 g/l xylose and 8.6 g/l sucrose (Fig. 6). The cells grew rapidly at a specific growth rate of 0.69 h^{-1}, then remained at a constant level of 2.48 g/l after 8 h. Xylose was metabolized during the stationary phase to produce 12.0 g/l D-ribose. An analysis of the off-gas during the D-ribose production stage revealed 0.053(±0.024) mmol/l-min of OUR and 0.027(±0.015) mmol/l-min of CER, which was similar to the values for the xylose and glucose mixture. Sucrose is transported by the sucrose-specific PTS system or hydrolyzed into glucose

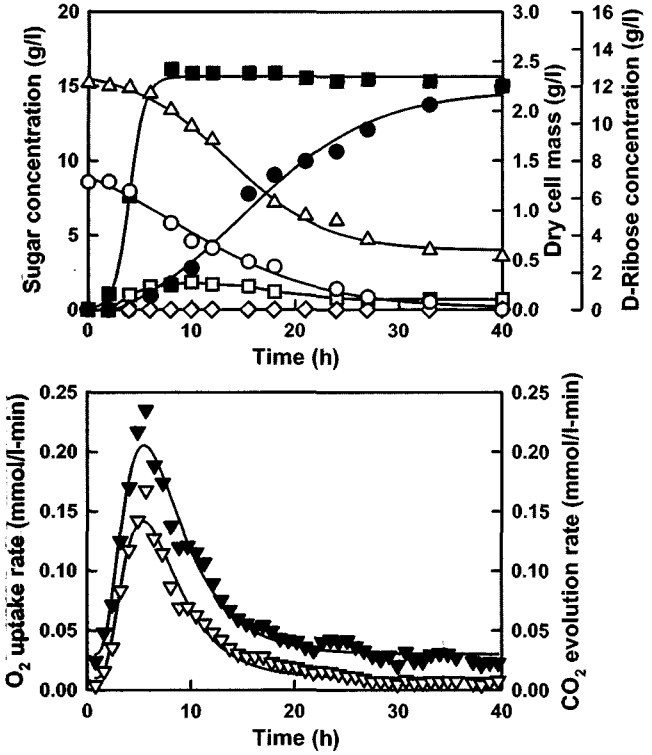

Fig. 6. Batch fermentation of *B. subtilis* JY1 with 15.2 g/l xylose and 8.6 g/l sucrose in jar fermentor at 37°C, 600 rpm, pH 7.0, and 1 vvm.

■, dry cell mass; ○, sucrose concentration; □, glucose concentration; △, xylose concentration; ●, D-ribose concentration; ◇, fructose concentration; ▼, oxygen consumption rate; ▽, carbon dioxide evolution rate.

and fructose by the extracellular levansucrase in *B. subtilis*, which typically prefers glucose to fructose as a carbon source [18, 28]. In contrast to the glucose effect, fructose was consumed quickly by *B. subtilis* JY1 with an accumulation of 1.85 g/l glucose. Two elements of PTS, enzyme I and the HPr protein encoded by the *ptsHI* gene, act together to transport most PTS sugars. Enzyme II is only induced by its specific sugar [28]. Thus, when considering the specific xylulokinase activity in the glucose or fructose medium (Table 1), simultaneous consumption of xylose and glucose (Fig. 5), and faster utilization of fructose relative to glucose (Fig. 6), it would seem that the transketolase-deficient *B. subtilis* JY1 lost the glucose-specific enzyme II (EII^{Glc}) of PTS, resulting in the glucose being transported into the cells by a PTS-independent glucose uptake system, such as GlcP or GlcU [22, 29].

The fermentation parameters for the D-ribose production and respiratory capability in jar fermentations are summarized in Table 2. The batch fermentation with 15.2 g/l xylose and 8.6 g/l sucrose resulted in a D-ribose concentration of 12.0 g/l and productivity of 0.30 g/l-h, which was a 1.2-fold increase compared with the corresponding values obtained with the xylose and glucose medium. The high performance with the xylose and sucrose medium was seemingly related to a high consumption rate of xylose. However, the yield of 0.62 g D-ribose/g sugar with the xylose and glucose medium was higher than that with the xylose and sucrose medium, due to the fact that the fructose hydrolyzed from sucrose was more efficiently converted to the cell mass (Figs. 5 and 6). The different carbon sources of glucose and sucrose facilitated changes in the respiratory capability of *B. subtilis* JY1. The OUR and CER in the xylose and sucrose medium indicated 1.96- and 2.47-fold increases, respectively, compared with the corresponding values in the xylose and glucose medium. Furthermore, the respiratory quotient during the cell growth stage was higher in the xylose and sucrose medium. As such, it seems that a large amount of fructose in the xylose and sucrose medium is rapidly transported into *B. subtilis* JY1 via the fructose-specific PTS system, resulting in a high respiratory performance, which also coincides with the previous hypothesis of EII^{Glc} mutation in PTS.

Table 2. Summary of fermentation parameters in terms of D-ribose production and respiratory capability in batch cultivations of *B. subtilis* JY1.

Parameter \ Carbon source		14.9 g/l xylose +13.1 g/l glucose	15.1 g/l xylose +8.6 g/l sucrose
D-Ribose concentration (g/l)		10.1	12.0
Yield (g D-ribose/g sugar)		0.62	0.43[a]
D-Ribose production rate (g/l-h)		0.25	0.30
Xylose consumption rate (g/l-h)		0.22	0.29
O_2 uptake rate[b] ($\times 10^{-1}$ mmol/l-min)		1.20 (0.15)	2.35 (0.11)
CO_2 evolution rate[b] ($\times 10^{-1}$ mmol/l-min)		0.68 (0.08)	1.68 (0.13)
Respiratory quotient (mol CO_2/mol O_2)	Exponential[c]	0.58 (0.06)	0.65 (0.07)
	Stationary[d]	0.53 (0.03)	0.54 (0.06)

The D-ribose yield (a) was obtained based on considering the molecular weight of sucrose, thus the sucrose concentration was multiplied by two in order to provide a comparison with the glucose concentration under equimolar conditions. The gas rate (b) represents the maximum value during the exponential phase. The respiratory quotient was obtained during the exponential phase (c), between 2 to 8 h of culture, and during the stationary phase (d), between 8 to 25 h. The values in parenthesis show the standard deviation.

However, similar respiratory quotients were obtained for the two media during the D-ribose production stage. Therefore, further research is needed to analyze the sugar transport mechanism in *B. subtilis* JY1 in order to design an optimal sugar feeding strategy for the maximum production of D-ribose in fed-batch fermentation.

Acknowledgment

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REFERENCES

1. Atkins, P. W. 1998. *Physical Chemistry*, pp. 14-44. 6th Ed. Oxford University Press, Great Clarendon Street, Oxford, U.K.
2. Chandrakant, P. and V. S. Bisaria. 2000. Application of a compatible xylose isomerase in simultaneous bioconversion of glucose and xylose to ethanol. *Biotechnol. Bioprocess Eng.* **5:** 32-39.
3. Choi, J. H., K. H. Moon, Y. W. Ryu, and J. H. Seo. 2000. Production of xylitol in cell recycle fermentations of *Candida tropicalis*. *Biotechnol. Lett.* **22:** 1625-1628.
4. Deutscher, J., J. Reizer, C. Fischer, A. Galinier, M. H. Saier, Jr., and M. Steinmetz. 1994. Loss of protein kinase-catalyzed phosphorylation of HPr, a phosphocarrier protein of the phosphotransferase system, by mutation of the *ptsH* gene confers catabolite repression resistance to several catabolic genes of *Bacillus subtilis*. *J. Bacteriol.* **176:** 3336-3344.
5. De Wulf, P., W. Soetaert, D. Schwengers, and E. J. Vandamme. 1996. D-Glucose does not catabolite repress a transketolase-deficient D-ribose-producing *Bacillus subtilis* mutant strain. *J. Ind. Microbiol.* **17:** 104-109.
6. De Wulf, P. and E. J. Vandamme. 1997. Production of D-ribose. *Appl. Microbiol. Biotechnol.* **48:** 141-148.
7. De Wulf, P., W. Soetaert, D. Schwengers, and E. J. Vandamme. 1997. Specific organic acids enhance the D-ribose productivity of a transketolase-defective *Bacillus subtilis* strain. *J. Chem. Technol. Biotechnol.* **70:** 311-315.
8. De Wulf, P., W. Soetaert, D. Schwengers, and E. J. Vandamme. 1997. Optimization of D-ribose production with a transketolase-affected *Bacillus subtilis* mutant strain in glucose and gluconic acid-base media. *J. Appl. Microbiol.* **83:** 25-30.
9. Eidels, L. and M. J. Osborn. 1971. Lipopolysaccharide and aldoheptose biosynthesis in transketolase mutants of *Salmonella typhimurium*. *Proc. Nat. Acad. Sci. USA* **68:** 1673-1677.
10. Ha, G. S., I. D. Choi, and Y. J. Choi. 2001. Carbon catabolite repression (CCR) of expression of the xylanaseA gene of *Bacillus stearothermophilus* No. 236. *J. Microbiol. Biotechnol.* **11:** 131-137.
11. Hernández-Montalvo, V., F. Valle, F. Bolivar, and G. Gosset. 2001. Characterization of sugar mixtures utilization by an *Escherichia coli* mutant devoid of the phosphotransferase system. *Appl. Microbiol. Biotechnol.* **57:** 186-191.
12. Ikeda, M., K. Okamoto, and R. Katsumata. 1998. A transketolase mutant of *Corynebacterium glutamicum*. *Appl. Microbiol. Biotechnol.* **50:** 375-378.
13. Jacob, S., R. Allmansberger, D. Gärtner, and W. Hillen. 1991. Catabolite repression of the operon for xylose utilization from *Bacillus subtilis* W23 is mediated at the level of transcription and depends on a *cis* site in the *xylA* reading frame. *Mol. Gen. Genet.* **229:** 189-196.
14. Jin, Y. S., T. H. Lee, Y. D. Choi, Y. W. Ryu, and J. H. Seo. 2000. Conversion of xylose to ethanol by recombinant *Saccharomyces cerevisiae* containing genes for xylose reductase and xylitol dehydrogenase from *Pichia stipitis*. *J. Microbiol. Biotechnol.* **10:** 564-567.
15. Josephson, B. and D. G. Frankel. 1969. Transketolase mutants of *Escherichia coli*. *J. Bacteriol.* **100:** 1289-1295.
16. Josephson, B. and D. G. Frankel. 1974. Sugar metabolism in transketolase mutants of *Escherichia coli*. *J. Bacteriol.* **118:** 1082-1089.
17. Kim, M. S., Y. S. Chung, J. H. Seo, D. H. Jo, Y. H. Park, and Y. W. Ryu. 2001. High-yield production of xylitol from xylose by a xylitol dehydrogenase defective mutant of *Pichia stipitis*. *J. Microbiol. Biotechnol.* **11:** 564-569.
18. Klier, A. F. and G. Rapoport. 1988. Genetics and regulation of carbohydrate catabolism in *Bacillus*. *Annu. Rev. Microbiol.* **42:** 65-95.
19. Lee, T. Y., M. D. Kim, K. Y. Kim, K. M. Park, Y. W. Ryu, and J. H. Seo. 2000. A parametric study on ethanol production from xylose by *Pichia stipitis*. *Biotechnol. Bioprocess Eng.* **5:** 27-31.
20. Lee, W. J., M. D. Kim, M. S. Kim, Y. W. Ryu, and J. H. Seo. 2003. Effects of xylose reductase activity on xylitol production in two-substrate fermentation of recombinant *Saccharomyces cerevisiae*. *J. Microbiol. Biotechnol.* **13:** 725-730.
21. Muller, C., H. G. Zimmer, M. Gross, U. Gresser, I. Brotsack, M. Wehling, and W. Pliml. 1998. Effect of ribose on cardiac adenine nucleotides in a donor model for heart transplantation. *Eur. J. Med. Res.* **16:** 554-558.
22. Paulsen, I. T., S. Chauvaux, P. Choi, and M. H. Saier, Jr. 1998. Characterization of glucose-specific catabolite repression-resistant mutants of *Bacillus subtilis*: Identification of a novel hexose:H^+ symporter. *J. Bacteriol.* **180:** 498-504.
23. Saier, Jr., M. H., M. J. Fagan, C. Hoischen, and J. Reizer. 1993. Transport mechanisms, pp. 133-156. *In* A. L. Sonenshein, J. A. Hoch, and R. Losick (eds.), *Bacillus subtilis and Other Gram-Positive Bacteria*. American Society for Microbiology, Washington.
24. Sasajima, K. and M. Yoneda. 1971. Carbohydrate metabolism-mutants of a *Bacillus* species. Part II. D-Ribose accumulation by pentose phosphate pathway mutant. *Agr. Biol. Chem.* **35:** 509-517.
25. Schaaff-Gerstenschläger, I., G. Mannhaupt, I. Vetter, and F. K. Zimmermann. 1993. *TKL2*, a second transketolase gene of *Saccharomyces cerevisiae*. Cloning, sequence and

deletion analysis of the gene. *Eur. J. Biochem.* **217:** 487-492.
26. Schmiedel, D. and W. Hillen. 1996. Contribution of XylR, CcpA and *cre* to diauxic growth of *Bacillus megaterium* and to xylose isomerase expression in the presence of glucose and xylose. *Mol. Gen. Genet.* **250:** 259-266.
27. Seo, J. H., Y. C. Park, S. Y. Kim, J. K. Lee, S. J. Ha, and S. K. Kim. 2002. A biological method for producing D-ribose from D-xylose using a *Bacillus subtilis* mutant. Korea Patent. Filed No. 10-2002-057580.
28. Stülke, J. and W. Hillen. 2000. Regulation of carbon catabolism in *Bacillus* species. *Annu. Rev. Microbiol.* **54:** 849-880.
29. Wagner, A., E. Kuster-Schock, and W. Hillen. 2000. Sugar uptake and carbon catabolite repression in *Bacillus megaterium* strains with inactivated *ptsHI*. *J. Mol. Microbiol. Biotechnol.* **2:** 587-592.
30. Zarzeczny, R., J. J. Brault, K. A. Abraham, C. R. Hancock, and R. L. Terjung. 2001. Influence of ribose on adenine salvage after intense muscle contractions. *J. Appl. Physiol.* **91:** 1775-1781.
31. Zimmer, H. G. 1983. Normalization of depressed heart function in rats by ribose. *Science* **220:** 81-82.