Improvement of NADPH-Dependent Bioconversion by Transcriptome-Based Molecular Breeding^{∇†}

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Transcriptome data for a xylitol-producing recombinant *Escherichia coli* were obtained and used to tune up its productivity. Structural genes of NADPH-dependent D-xylene reductase and D-xylene permease were inserted into an *Escherichia coli* chromosome to construct a recombinant strain producing xylitol from D-xylene for use as a model system for NADPH-dependent bioconversion. Transcriptome analysis of xylitol-producing and nonproducing conditions for the recombinant revealed that xylitol production down-regulated 56 genes. These genes were then selected as candidate factors for suppression of the NADPH supply and were disrupted to validate their functions. Of the gene disruptants, that resulting from the deletion of *yhbC* showed the best bioconversion rate. Also, the deletion accelerated cell growth during log phase. The features of the mutant could be maintained in jar fermenter-scale production of xylitol. Thus, our novel molecular host strain breeding method using transcriptome analysis was fully effective and could be applied to improving various industrial strains.

Bioconversion using recombinant cells is a useful method for synthesis of valuable chemical compounds and drug metabolites. Growing cells are preferable in cases where the supply of cofactors is critical for biosynthetic reactions, because cofactors are expensive to add externally (5, 12, 20). Growing cells also make it possible to maintain unstable enzymes such as membrane-associated multiprotein complexes (21).

Bioconversion using oxidoreductases is generally coupled with enzymatic cofactor regeneration systems (29). In this case, resting cells were used and NAD⁺ or NADP⁺ was externally added to stimulate reactions. Formate dehydrogenase for NADH regeneration (14) and glucose dehydrogenase for NAD(P)H regeneration (23) have often been used in resting-cell oxidoreductase reactions. Although the resting-cell reaction is certainly a powerful tool for short-term reactions, several disadvantages emerge when it is applied to longer-term reactions on an industrial scale. In particular, NAD⁺ or NADP⁺ may cause a considerable increase in production costs. In contrast, growing cells can supply cofactors effectively via their own cellular metabolism. Thus, this native cofactor supply system is particularly useful for larger industrial reaction volumes. However, the activity of this endogenous cofactor regeneration system is strictly controlled by the robustness of the cell physiology. There is little research on increasing NAD(P)H availability for bioconversion in growing cells.

In this research, we obtained specialized host cells for NADPH-dependent bioconversion by using a transcriptome data set. Transcriptome analysis is a powerful tool for functional analysis of genes in metabolic networks (18, 19, 28). We

used a recombinant *Escherichia coli* strain producing xylitol from D-xylene as a model of NADPH-dependent bioconversion. In the recombinant, chromosomal *xylA* (D-xylene isomerase gene for D-xylene utilization) was disrupted. And the *E. coli* xylene permease gene (*xylE*) and *Kluyveromyces lactis* NADPH-dependent xylene reductase gene (*XYL1*) were inserted into the chromosome of the recombinant. *E. coli* is a good host for functional analysis of genes, because a collection of single-gene-knockout (single-gene-KO) mutants is available. Transcription data sets from xylitol-producing and nonproducing conditions of the recombinant strain were compared. Genes down-regulated during xylitol production were hypothesized as suppressors of the supply of NADPH, and their defective mutants were tested for xylitol production. Among the tested mutants, a *yhbC*-deficient strain showed improvement of xylitol production and additional acceleration of log-phase growth. Thus, the combination of transcriptome analysis and phenotype tests of single-gene-knockout mutants is a good method for screening important negative regulators for strictly controlled cofactor supply.

MATERIALS AND METHODS

Construction of xylitol-productive strain AK1. The primers and plasmids used for construction of AK1 were shown in Table 1 and 2. *XYL1* (GenBank accession no. L36993), *xylE* (GenBank accession no. U00096) and a tetracycline-resistant gene (*Tc^r*; GenBank accession no. AY528506) were amplified by PCR with primers in Table 2. A *XYL1* fragment was digested with BspHI and BamHI, a *xylE* fragment containing a Shine-Dalgarno sequence at its 5' terminus was digested with BamHI, and a *Tc^r* fragment was digested with NheI. The digested fragments of *XYL1* and *xylE* were tandem inserted between the NcoI and BamHI sites of pQE60 expression vector (Qiagen); also, the *Tc^r* fragment was inserted at the NheI site. The resulting plasmid was designated pXKET6 in that T5 promoter, and the *lac* operator, *XYL1*, *xylE*, and *Tc^r* were concatenated in that order to compose a xylitol-production operon. This synthetic operon was amplified using PCR with 40-bp homologous sequences at both ends. The amplified fragment was transformed to replace a chromosomal *xylA* gene (10) to construct a xylitol-producing strain, AK1. The resulting chromosomal region for xylitol production was easily transferred into other strains by P1-phage transduction (17).

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TABLE 1. Strains and plasmids used in this study

Strain or plasmid	Description ^a	Reference or source
Strains		
2359/152	<i>K. lactis</i>	ATCC 76492
BW25113	<i>F⁻ Δ(araD-araB)567 ΔlacZ₄787(::rrnB-3) lacI_p-4000(lacI^q) λ⁻ rph-1 Δ(rhaD-rhaB)568 hsdR514; E. coli strain K-12</i>	10
BW25113 <i>ΔyhbC</i>	<i>ΔyhbC::Km^r</i> , BW25113	1
TC1	BW25113/pCA24N	This study
TY1	BW25113/pCA3139	This study
TC37	BW25113 <i>ΔyhbC</i> /pCA24N	This study
TY37	BW25113 <i>ΔyhbC</i> /pCA3139	This study
AK1	<i>ΔxylA(::XYL1 xylE)</i> , Tc ^r , BW25113	This study
AK37	<i>ΔyhbC::Km^r</i> ; AK1	This study
AKP37	<i>ΔyhbC</i> (markerless), AK1	This study
Plasmids		
pCA24N	T5 promoter, <i>lac</i> operator, <i>lacI^q</i> , Cm ^r	24
pCA3139G	<i>Φ(yhbC-gfp)</i> in pCA24N, Cm ^r	24
pCA3139	<i>yhbC</i> ; <i>gfp</i> removed by NotI treatment from pCA3139G	This study
pQE60	T5 promoter, <i>lac</i> operator, Ap ^r	Qiagen
pXKET6	<i>XYL1</i> , <i>xylE</i> , Tc ^r in pQE60	This study

^a Abbreviations: Ap, ampicillin; Cm, chloramphenicol.

Bacterial strains and culture. The strains and plasmids used in this study are shown in Table 1. Single-gene-knockout mutants of *E. coli* BW25113 (1) were obtained from Hirotada Mori (Nara Institute of Science and Technology). A kanamycin-resistant (*Km^r*) gene of a *yhbC* knockout mutant was eliminated according to a method using *Saccharomyces cerevisiae* FLP recombinase (10). In all experiments, seed cultures were incubated overnight at 30°C and 250 rpm using LB-glucose medium (5 g liter⁻¹ yeast extract, 10 g liter⁻¹ peptone, 10 g liter⁻¹ NaCl, 10 g liter⁻¹ D-glucose, and proper antibiotics if necessary) and then inoculated into M9 minimal medium (pH 6.8) (6.8 g liter⁻¹ Na₂HPO₄, 3 g liter⁻¹ KH₂PO₄, 0.5 g liter⁻¹ NaCl, 1 g liter⁻¹ NH₄Cl, 0.247 g liter⁻¹ MgSO₄·7H₂O, 0.028 g liter⁻¹ FeSO₄·7H₂O, 0.015 g liter⁻¹ CaCl₂·2H₂O) for xylitol production. The cell growth was monitored by measuring optical density at 660 nm (OD₆₆₀) with a general photometer.

DNA microarray analysis. A seed culture of xylitol production strain AK1 was inoculated at 1% volume into M9 medium that included 1% glucose and 0.1 mM isopropyl-β-D-thiogalactopyranoside (IPTG), and the cells were cultured in 5-ml medium in test tubes at 30°C and 250 rpm. At the middle of the exponential phase (when the OD₆₆₀ was about 1.0), the culture was divided into two portions and D-xylose (final concentration, 2%) was added to one aliquot. After 2 h from division of the culture, cells were harvested and total RNAs were prepared separately from two divided cultures with an RNeasy Protect bacterial Mini kit (Qiagen). Fluorescently labeled cDNA was prepared from the total RNA with an RNA fluorescence labeling core kit (Takara Bio). Competitive hybridization of the cDNAs (13) onto IntelliGene *E. coli* CHIP version 2.0 (Takara Bio) was done

according to the attached instructions. The microarray was scanned with a microarray scanner (GenePix 4000B; Axon Instruments). All fluorescence intensity data were statistically analyzed with data analysis software (GeneSpring 6.1; Agilent).

Monosaccharide and sugar alcohol analysis. All cultures for the analysis were centrifuged at 16,000 × g for 5 min at 4°C in a 5415R centrifuge (Eppendorf), and their supernatants were assayed. Concentrations of glucose, xylose, and xylitol were analyzed using HPAEC-PAD and a DXc-500 system (Dionex). Analysis was performed using a Dionex CarboPac PA-1 column (4 by 250 mm) equipped with a guard column. Conditions were isocratic (50 mM NaOH for 20 min at a 1 ml min⁻¹ flow rate and 30°C).

Large-scale bioconversion of xylitol by use of jar fermentation. Cells from the seed culture in LB-glucose medium were inoculated into the M9 medium with 10 g liter⁻¹ glucose, cultivated overnight again, and inoculated at 3% volume into 1 liter of the M9 medium containing 30 g liter⁻¹ glucose and 0.1 mM IPTG in a 2-liter bench top fermenter (BMJ-PI; Able). We found this concentration of IPTG to be appropriate for the reaction. The initial fermentation conditions were 30°C, 500-rpm agitation, an aeration rate of a 1.0 ratio of air volume to liquid volume per min, and pH 7.0. The culture pH was controlled by automatic addition of 14% NH₃, and dissolved oxygen in the culture was kept above 1.0 ppm by agitation change automatically. When the OD₆₆₀ reached about 1.0, 60 g liter⁻¹ xylose was added. Also, after 28.5 h additional glucose (30 g liter⁻¹) was added.

TABLE 2. Primers used in this study

Product	Template	Primer	Sequence
XYL1	2359/152	XYL1K5 XYL1K3	TCATGACGTACTTAGCAGAAACAGTTACTTTA GGATCCCTTAGATGAAAGTTGGGAATTCGTTGT
<i>xylE</i>	BW25113 ^b	XYLE5 XYLE3	GGATCCATTAAAGAGGAGAAATTAACCATGAATA CCCAGTATAATTCCAGTTATATA GGATCCCTACAGCGTAGCAGTTTGTGTGTTTCTT
Tc ^r	<i>Tn10</i>	TETA5 TETA3	GCTAGCAGCTCTAATGCGCTGTTAAT GCTAGCAGGCCAATTTATTGCTATTT
XPC ^a	pXKET6	XKET5 XKET3	CATCCATCACCCGCGGCATTACCTGATTATGGAGT TCAATCTCGAGAAATCATAAAAAATTTATT CGCTACCGATAACCGGGCCAACGGACTGCACAGTT AGCCGTCCATTTTAGCTTCCTTAGCTCCTG

^a DNA fragment of xylitol-productive cassette for homologous recombination.

^b Chromosomal DNA was used.

FIG. 1. Xylitol-productive strain AK1. (A) Schematic illustration of homologue recombination, placing *xylA* into a synthetic xylitol-producing operon. Expression of *XYL1* and *xylE* is induced and controlled by addition of IPTG. (B) Schematic illustration of xylitol production in AK1. Xylose in culture was imported into the cell by XylE and reduced to xylitol by XYL1 and NADPH. The NADPH was supplied from glucose metabolism. IM, inner membrane.

RESULTS

Bioconversion of D-xylose to xylitol in *E. coli* AK1. AK1 is a xylitol production strain based on *E. coli* BW25113 (Fig. 1). AK1 possesses a synthetic operon consisting of the *K. lactis* NADPH-dependent xylose reductase gene *XYL1* (3) and the extra *E. coli* D-xylose permease gene *xylE* (11) instead of, and at the chromosome site of, the *E. coli* D-xylose isomerase *xylA* (Fig. 1A). Because XylA catalyzes the initial step of *E. coli* D-xylose metabolism, isomerizing it to D-xylulose (25), AK1 does not metabolize D-xylose. *XYL1* and *xylE* were placed in tandem under the control of an IPTG-inducible promoter. When the strain was cultivated in a mixed-sugar culture of D-xylose and glucose, XylE imported D-xylose into the cell and XYL1 reduced it to xylitol by use of intracellular NADPH. It should be emphasized that the NADPH was autonomously supplied through cellular glucose metabolism. An overview of xylitol production from xylose coupled with cellular NADPH recycling in AK1 is shown in Fig. 1B. In fact, the sum of the D-xylose and xylitol concentrations in AK1 culture was almost constant (about 22.5 g liter⁻¹) during a 72-hour reaction (Fig. 2), indicating that D-xylose was stoichiometrically converted to xylitol without any loss. Thus, NADPH availability for the reaction was easily assayed as xylitol productivity even in the case of long-term bioconversion using AK1.

Transcriptome analysis of AK1. Transcriptions of AK1 with and without xylitol production were compared using DNA microarray slides. It was found that expression levels of 56 genes were strongly repressed less than 0.5-fold in xylitol-pro-

FIG. 2. Stable bioconversion of xylose to xylitol in AK1. Stoichiometrical bioconversion of xylose to xylitol was performed using an AK1 reaction culture. AK1 was cultured in 5 ml of M9 minimal medium that included 10 g liter⁻¹ glucose and 22.5 g liter⁻¹ xylose in a test tube at 30°C and 250 rpm for 72 h. Concentrations of xylose (■) and xylitol (◆) and of a combination of these (▲) in the culture are shown.

ducing AK1 (see Table S1 in the supplemental material). Eight sets of genes (*cydAB*, *glf/rbfX*, *fruKB*, *atoEB*, *yhiQ/prlC*, *dppCA*, *glySQ*, and *ibpBA*) were thought to be located in the same operons (i.e., *cydA* and *cydB* in the same operon, *glf* and *rbfX* in the same operon, etc.). According to functional classification of 56 genes, many belonged to the metabolic pathway; 8 encoded energy metabolism enzymes or related transporters (*cydAB*, *ybiW*, *focA*, *gly*, *fruKB*, and *gark*), 7 encoded amino acid metabolism enzymes or related transporters (*leuL*, *glnP*, *trpC*, *sdaA*, *aroF*, *gltB*, and *glnA*), and 5 encoded fatty acid-phospholipid metabolism enzymes or related transporters (*prpE*, *atoEB*, *fabB*, and *lgt*). There were six genes in the transcription or translation categories (*rpmF*, *argS*, *galR*, *glySQ*, and *zur*). Interestingly, there were also four genes for dipeptide- or oligopeptide-related functions (*dcp*, *prlC*, and *dppCA*) and three genes for adaptation to temperature shift (*cspA* and *ibpBA*). Since there were 17 essential genes among the 56 down-regulated genes, only 39 single-gene-knockout mutants were available in a KO collection of *E. coli* gene disruptants (1). The 39 available deletions were transferred into AK1 by use of P1-phage transduction. The cell growth and xylitol productivity of 39 constructed variants of AK1 were measured and compared with those of AK1 after 36 h of cultivation (see Table S1 in the supplemental material). Xylitol accumulation in three gene disruptants, Δ *glnA*, Δ *leuL*, and Δ *trpC*, was apparently decreased because of a strong growth defect in the M9 minimal medium. These results were understandable, because *glnA* and *trpC* encode enzymes involved in amino acid biosynthesis (glutamine synthetase [8] and indole-3-glycerol phosphate synthase-phosphoribosylanthranilate isomerase [9], respectively) and *leuL* encodes a leader peptide of a leucine biosynthesis operon; thus, its disruption may affect transcription of the *leu* operon. Of these 39 variants, the *yhbC*-defective AK37 strain was the best for xylitol production (10.3 g liter⁻¹) and also reached the highest cell density (2.6 at OD₆₆₀).

Genetic features of *yhbC* and overview of NADPH-dependent bioconversion in its mutant. The *yhbC* is the second gene of the *metY-rpsO* operon and encodes a 15-kDa protein of unknown function (22). Many of the genes belonging to the

FIG. 3. Alignment of the *metY-rpsO* operon in prokaryotes and YhbC homologues. (A) Comparison of the conserved gene arrays of the *metY-rpsO* operon among various prokaryotes. (B) Sequence alignment of *E. coli* YhbC and *S. pneumoniae* SP14.3 by CLUSTALW. The underline below the SP14.3 sequence shows the N-terminal domain (solid line) and C-terminal domain (dashed line). The bottom row indicates the positions of residues which are conserved (*), strongly similar (:), and weakly similar (.).

operon aside from *yhbC* are essential to *E. coli* survival and are well conserved among representative prokaryotes (Fig. 3A). In particular, *yhbC* and *nusA* genes are perfectly conserved in those strains, including the thermophilic bacteria *Aquifex aeolicus* and *Thermotoga maritima*. The most investigated homologue of *E. coli* YhbC is that of *Streptococcus pneumoniae* SP14.3 (30), having 60% similarity (Fig. 3B). The protein structure of SP14.3 was elucidated, and a three-dimensional structural homology search showed the existence of a nucleic acid-binding domain at its C terminal and a protein-interacting domain at its N terminal. AK1 and its $\Delta yhbC$ mutant AK37 showed different profiles of growth, glucose consumption, and xylitol production in a 72-hour cultivation (Table 3). Although all measured parameters were higher in AK37 than in AK1 during the whole cultivation period, AK37 showed prominently higher activity levels at an early stage of cultivation (2.1-fold higher in its OD₆₆₀ value, 3.4-fold higher in glucose consumption, and 2.7-fold higher in xylitol production at 24 h). It was

hypothesized that enhancement of whole-cell activity, including better glucose utilization and active cell division, resulted in higher NADPH-dependent xylitol production in AK37.

Observation of cell growth in *yhbC* mutants. The results of assays of growth acceleration after *yhbC* disruption are shown in Fig. 4. The growth rates of AK1 and AK37 were measured according to xylitol-producing or nonproducing status. Under xylitol-producing conditions, two strains showed growth slower than that seen nonproducing conditions (Fig. 4A). Both with and without xylitol production, AK37 showed obvious growth improvement compared to AK1, especially in the early log phase. Thus, elimination of *yhbC* in AK1 always accelerated growth in minimal media. Next we checked the effect of *yhbC* deletion in another genetic background without *XYL1* and *xylE*. BW25113 is the parental strain of the KO collection and the parental strain of AK1. A deletion mutant of *yhbC* from BW25113 in the KO collection was designated here BW25113 $\Delta yhbC$. Growth profiles of BW25113 and BW25113 $\Delta yhbC$

TABLE 3. AK1 and AK37 during xylitol bioconversion^a

Time (h)	AK1			AK37		
	OD ₆₆₀	Glucose consumption (g liter ⁻¹)	Xylitol production (g liter ⁻¹)	OD ₆₆₀	Glucose consumption (g liter ⁻¹)	Xylitol production (g liter ⁻¹)
24	1.7 (0.03)	1.1 (0.18)	0.9 (0.01)	3.7 (0.15)	3.7 (0.17)	2.5 (0.01)
48	4.2 (0.04)	7.7 (0.02)	6.1 (0.08)	4.5 (0.04)	9.8 (0.02)	7.9 (0.11)
72	4.5 (0.03)	10.9 (0.14)	9.9 (0.12)	4.7 (0.00)	13.2 (0.06)	12.2 (0.02)

^a All values represent the results of four independent experiments; numbers in parentheses indicate standard deviations.

FIG. 4. Acceleration of log-phase growth in *yhbC* mutants. (A) Measurement of growth curves of AK1 and AK37 in two different cultures. For the first experiment, AK1 (■) and AK37 (◆) were cultured in M9 minimal medium that included 10 g liter⁻¹ glucose. For the second experiment, AK1 (□) and AK37 (◇) were cultured in M9 minimal medium that included 10 g liter⁻¹ glucose and 22.5 g liter⁻¹ xylose. (B) Measurement of growth curves of *E. coli* BW25113-based strains. The cell growth characteristics of BW25113 (△) and BW25113 $\Delta yhbC$ (○) were observed. (C) *yhbC* complementation experiment. TC1 (△), TY1 (▲), TC37 (○), and TY37 (●) were cultured in M9 minimal medium that included 10 g liter⁻¹ glucose. All strains were shaken in L-shaped test tubes at 30°C and 70 rpm.

were compared (Fig. 4B). In this case, the *yhbC*-deletion counterpart also showed a superior growth curve in the early log phase. It was concluded that *yhbC* deletion improved growth of *E. coli* regardless of the presence or absence of xylitol-producing genes. The results of a *yhbC* complementation experiment using a plasmid are summarized in Fig. 4C. A plasmid with

FIG. 5. Practical scale of xylitol bioconversion in batch-fed cultivation. (A to C) OD₆₆₀ (A), glucose consumption (B), and xylitol production (C) of AK1 (□) and AKP37 (◇) in jar fermenters are shown. In addition, xylose consumption of AK1 (■) and AKP37 (◆) is shown in panel C.

yhbC was able to restore the phenotype of BW25113 Δyhb to that of the parental strain (compare TY37 with TC1 in Fig. 4C). This result indicates that phenotypical changes of *yhbC* mutants are derived from the depletion of YhbC protein and that rearrangement of chromosomes caused by *yhbC* deletion results in little effect.

Fermentation of AKP37 in a jar fermenter. Xylitol production by a *yhbC* mutant was tested using a 2-liter jar fermenter (Fig. 5). AKP37, a variant of AK37 with kanamycin resistance (Km^r) removed, was used for the experiment to avoid involvement of a Km^r promoter in expression of downstream genes of *yhbC*. Even in a jar fermenter, the *yhbC* mutant showed better early growth (Fig. 5A), glucose consumption (Fig. 5B), and xylitol production (Fig. 5C) than the wild type, a result similar

to that observed using flask-scale bioconversion (Table 3). Xylitol production is proportional to cell density in log phase, and the slopes of these linearizations were very similar for AK1 and AKP37 (0.98 and 0.97, respectively). These results indicate that the cellular NADPH supply depends on the cell growth rate in log phase. Therefore, the growth acceleration of AKP37 directly increases the xylitol production rate. Though the final cell densities of AK1 and AKP37 were about the same (OD_{660} s of 12.9 and 13.9), total glucose consumption and xylitol accumulation levels were higher in AKP37 than in AK1. AK1 and AKP37 showed 43.4 and 51.7 g liter⁻¹ of xylitol accumulation at 64 h and 0.68 and 0.81 g liter⁻¹ h⁻¹ of xylitol productivity, respectively. These results indicate that *yhbC* disruption was still effective in accelerating cellular NADPH supply at the stationary phase as well.

DISCUSSION

We constructed a xylitol-producing strain, AK1, as a model of reductive bioconversion using an NADPH supply from glucose metabolism in *E. coli* (Fig. 1B). At an early stage of this study, it was difficult to overcome catabolite repression of *E. coli* (4). Cells expressing *XYL1* alone did not import xylose or produce xylitol at all in media containing both glucose and xylose (data not shown). Gene expression of xylose transporters, including *xylE* and ABC transporter *xylFGH*, was repressed with glucose (17, 29). AK1 did import xylose even in the presence of glucose when IPTG was added for artificial expression of *xylE* inserted in its chromosome (Fig. 1B). And our strain does produce xylitol at a level of productivity similar to that seen with another xylitol-producing *E. coli* recombinant recently reported (7). That xylitol producer possesses NADPH-dependent xylose reductase from *Candida boidinii* and exhibits a disruption of xylulokinase (*xylB*) and a mutation in the cyclic AMP receptor protein (*crp*) for avoiding catabolite repression (7).

We tried to find regulators for the NADPH supply by use of transcriptome comparison between two AK1 states with and without xylitol production. We thought that genes in which transcription levels were depressed during xylitol production were factors repressing NADPH availability under normal conditions. Using this strategy we successfully found a *yhbC* mutant with enhanced NADPH availability. This indicates that transcriptome analysis is effective as an initial screening of genes for targets of molecular breeding. Besides *yhbC*, genes *cydAB*, encoding components of terminal oxidases of *E. coli* (27), were notably down-regulated. Down-regulation of *cydAB* may result in suppression of electron transfer in the respiratory chain to save cellular reduction power. Functional classification shows that many of the genes selected (see Table S1 in the supplemental material) belonged to the metabolic pathway. This suggests that global modification of cellular metabolism occurs when NADPH is used for xylitol production.

A gene disruption test showed that deletion of *yhbC* was most effective for xylitol production. This functionally unknown gene is well conserved in many prokaryotes (Fig. 3A). Since one of YhbC homologues, *S. pneumoniae* SP14.3, has a nucleic acid-binding domain and a protein-interacting domain (Fig. 3B), YhbC would act as a transcriptional or translational regulatory factor. The *metY-rpsO* operon, including *yhbC*, con-

sists of many well-known transcription- and translation-related genes, and most of these are essential for *E. coli*. Expression of the *metY-rpsO* operon is strictly regulated by several promoters and terminators (15). It has been demonstrated that the transcriptional regulator gene *nusA* in the operon does autoregulate itself in *E. coli* (6, 16, 26). At low temperature, the expression of the *metY-rpsO* operon was increased due to the cold shock response caused by the presence of CspA-family RNA chaperones (2). This indicates that the genes belong to the *metY-rpsO* operon and act to adapt to low-temperature stress, but the adaptive mechanism is still unknown. It is possible that YhbC may also play an important role in autoregulation of the *metY-rpsO* operon to adapt to some stresses, because *yhbC* expression was repressed in oxidative conditions by NADPH consumption in our experiment.

Our molecular breeding process to increase NADPH supply for bioconversion is a completely new approach. We have successfully overcome catabolite repression by use of inducible D-xylose permease to construct a recombinant xylitol producer, AK1. AK1 is a good component of a system to analyze cellular NADPH supply. Target genes for modification were initially selected using transcriptome data sets of AK1 under two different sets of conditions with and without xylitol production. Down-regulated genes were good candidates for disruption to accelerate the NADPH supply. Consequently it was found that deletion of *yhbC* activates xylitol production. Disruption of *yhbC* can be used to improve productivity of other NADPH-dependent bioconversion activities. Since deletion of *yhbC* results in accelerated log-phase growth, combining *yhbC* mutants and cultivation methods for a longer log phase would achieve higher productivity.

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