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Deletion of *xylR* Gene Enhances Expression of Xylose Isomerase in *Streptomyces lividans* TK24

Heo, Gun-Young[1], Won-Chan Kim[1], Gil-Jae Joo[1], Yun-Young Kwak[1], Jae-Ho Shin[1], Dong-Hyun Roh[2], Heui-Dong Park[3], and In-Koo Rhee[1]*

[1]*Department of Agricultural Chemistry, Kyungpook National University, Daegu 702-701, Korea*
[2]*Department of Microbiology, Chungbuk National University, Chungju 361-763, Korea*
[3]*Department of Food Science and Technology, Kyungpook National University, Daegu 702-701, Korea*

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Glucose (xylose) isomerases from *Streptomyces* sp. have been used for the production of high fructose corn syrup for industrial purposes. An 11-kb DNA fragment containing the *xyl* gene cluster was isolated from *Streptomyces lividans* TK24 and its nucleotide sequences were analyzed. It was found that the *xyl* gene cluster contained a putative transcriptional repressor (*xylR*), xylulokinase (*xylB*), and xylose isomerase (*xylA*) genes. The transcriptional directions of the *xylB* and *xylA* genes were divergent, which is consistent to those found in other streptomycetes. A gene encoding XylR was located downstream of the *xylB* gene in the same direction, and its mutant strain produced xylose isomerase regardless of xylose in the media. The enzyme expression level in the mutant was 4.6 times higher than that in the parent strain under xylose-induced condition. Even in the absence of xylose, the mutant strain produce over 60% of enzyme compared with the xylose-induced condition. Gel mobility shift assay showed that XylR was able to bind to the putative *xyl* promoter, and its binding was inhibited by the addition of xylose *in vitro*. This result suggested that XylR acts as a repressor in the *S. lividans* xylose operon.

Keywords: Glucose (Xylose) isomerase, *xylR*, xyl operon, gene disruption, *Streptomyces lividans*

D-Xylose can be utilized as a carbon and energy source by a variety of microorganisms. The catabolism of D-xylose in bacteria involves the transport of D-xylose into the cell, isomerization of D-xylose to D-xylulose, and phosphorylation of D-xylulose to D-xylulose-5-phosphate. D-Xylulose-5-phosphate is further metabolized by the pentose phosphate and glycolytic pathways. Genes encoding the enzymes related to the above reactions are organized as an operon in bacteria [12, 13, 33, 40, 41]. There have been reports on the regulation of the *xyl* genes in bacteria. In *Escherichia coli* and *Salmonella typhimurium*, *xyl* genes are organized as an operon and their expression is regulated positively by the activator protein (XylR) [13, 22, 33, 34]. In *Bacillus* and *Lactobacillus* species, the *xyl* operon contains two genes encoding xylose isomerase (XylA) and xylulokinase (XylB), and their expression is regulated negatively by the repressor protein (XylR) [9, 12, 21, 23, 30]. Studies of *xyl* genes in *E. coli* and *Bacillus* species have progressed actively until now, and regulations of *xyl* genes in those two bacteria have been elucidated [16, 20, 26, 29, 31, 32, 34, 35].

Of those enzymes, D-xylose isomerase (the *xylA* gene product) is essential for xylose catabolism, which has been also known as the glucose isomerase for industrial merits. The isomerization of D-glucose to D-fructose using glucose isomerase is the basis for the production of high fructose corn syrup (HFCS) from starch hydrolysates [2, 3, 37]. For industrial demands, research on xylose isomerase has been largely focused on searching for an enzyme that has broad optimum pH, high glucose affinity, thermostability as well as high activity at high temperatures. As a result, various *Streptomyces* species are widely used for HFCS production because their D-xylose isomerases satisfy such demands [1, 8, 19, 24, 39]. Although many studies on the isolation of *xylA* genes and the enzyme production in *Streptomyces* sp. have been reported, little is known about the regulation of *xyl* genes so far. Previously, Tiraby and his colleagues [37] reported an open reading frame (ORF) encoding a protein of 348 amino acids, which exists 40-bp downstream from the *xylB* gene with the same orientation in *Streptomyces violaceoniger*. The gene, designated *xylX*, was presumed to be a regulatory protein [37], which was supposed to be

*Corresponding author
Phone: 82-53-950-5718; Fax: 82-53-953-7233;
E-mail: ikrhee@knu.ac.kr

an activator according to another research group [1]. A putative repressor gene (*xylR*) for the xylose catabolism has been reported in *S. coelicolor* A3(2) by the *S. coelicolor* genome project, but its function is yet to be elucidated [2].

In this study, we have isolated the *xyl* operon from *S. lividans* TK24, knocking out a putative *xylR* gene to form a deletion mutant strain, and re-introducing that gene on a plasmid in the mutant. Enzyme assays demonstrated that *xylR* is negatively associated with xylose isomerase activity, and gel mobility shift studies indicate that XylR binding with the promoter region of the *xyl* operon depends on xylose.

Materials and Methods

Bacterial Strains and Plasmids

The strains and plasmids used in this study are summarized in Table 1. *S. lividans* TK24 was used as a source of *xyl* genes [14]. *E. coli* DH5α was used as a host strain for general cloning procedures. A genomic library of *S. lividans* TK24 was constructed as per previous methods [7]. *E. coli* BM25.8 and ER1647 were used for the automatic subcloning and plating λBlueSTAR DNA library for the isolation of *S. lividans xyl* genes, respectively. The methylation-deficient *E. coli* ET12567 (*dam-13*::Tn*9*, *dcm-6*, *hsdM*, *hsdS*) was used as a host strain for the preparation of recombinant plasmids to transform into *Streptomyces* sp. [4]. Plasmid pUC119 was used as a general cloning vector and pWHM3 was used as an *E. coli-Streptomyces* shuttle vector [38].

Media and Culture Conditions

Streptomyces sp. cells were grown and handled according to the method of Hopwood *et al.* [14] and a previous study [6]. YEME and TSB media were used for the preparation of genomic DNA and protoplast, respectively. For the enzymatic assay, YEME medium was used with or without D-xylose. Antibiotics-resistant clones were selected on R2YE agar and overlaying soft-top agar with thiostrepton, apramycin, and neomycin at final concentrations of 500, 100, and 10 μg/ml, respectively. *E. coli* strains were grown in LB medium supplemented with appropriate antibiotics when required. To maintain plasmid selection in *E. coli* strains, apramycin and ampicillin were added at a final concentration of 100 μg/ml.

Sequencing of *xyl* Gene Cluster

The 11-kb BamHI DNA fragment of the recombinant plasmid pBLX1 was digested to several pieces with a variety of restriction endonucleases, and DNA fragments smaller than 600 bp in length were subcloned into pUC119 to determine their nucleotide sequences. DNA sequencing was performed using the ALFexpress DNA sequencer (Pharmacia Biotech Co., Uppsala, Sweden).

xylR Gene Deletion by Homologous Recombination

A part of the *xylR* gene (*xylR'*), truncated at both ends of the 3'- and 5'-regions, was amplified by PCR using oligonucleotide primer RSAC, 5'-GGACGAGCTCATCCGCTC-3', and primer RHID, 5'-CCCAAGCTTCGGACACCGCGTCCG-3', in the presence of 4% dimethylsulfoxide (DMSO). The primers were synthesized to generate SacI and HindIII recognition sequences in the amplified DNA fragments, respectively. PCR was carried out as follows: 30 cycles of 30 sec at 94°C, 30 sec at 60°C, and 30 sec at 72°C. The PCR products were digested with SacI and HindIII, and inserted into pUC119 to construct pUCTR20. pUCTR20 was digested with KpnI, which was located in the center of the *xylR'* gene, and its termini of both ends were blunted by T4 DNA polymerase [28] and ligated with 1 kb of neomycin resistance gene (*neo*) isolated from pFDNEO-S [10] digested with HincII, to construct pUCTR20-*neo*. The 2.1-kb SacI-HindIII fragments containing the truncated *xylR*-neomycin resistance gene cassette (*xylR'*::*neo*) from the plasmid pUCTR-*neo* were inserted into the *E. coli/Streptomyces* shuttle vector, pKC1139, which has a temperature-sensitive replication origin [4] and apramycin-resistant gene for selection marker, to yield the designated pKCTR20 plasmid. Plasmid pKCTR20 was introduced into *S. lividans* TK24 by the method of Hopwood *et al.* [14]. Transformants were selected for the resistance to apramycin (100 μg/ml) on R2YE agar plates [14]. Selected transformants were cultured on 50 ml of R2YE liquid media containing apramycin (100 μg/ml) at 30°C for two days with shaking (200 rpm). Subculture (2.5 ml) was

Table 1. Strains and plasmids used in this study.

Strain and plasmid	Relevant characteristics	Reference or sources
Strains		
S. lividans TK24	Wild type	[14]
S. lividans XRD47	Δ*xylR*::*neo*, derivative of strain TK24	This work
E. coli DH5α	General host for cloning	Laboratory stock
E. coli BM25.8	For automatic subcloning	Novagen, Germany
E. coli ER1647	For plating λBlueSTAR DNA library	Novagen, Germany
Plasmids		
pUC119	General cloning vector in *E. coli*	Laboratory stock
pWHM3	*E. coli-Streptomyces* shuttle vector	[37]
pKC1139	Suicide vector containing *oriT* for *E. coli* to *Streptomyces* conjugation	[4]
pFDNEO-S	*neo* gene as a selection marker	[10]
pUCTR20-neo	Δ*xylR::neo*, derivative of pUC119	This work
pKCTR20	Δ*xylR::neo*, derivative of pKC1139	This work
pWLR25	Recombinant plasmid containing *S. lividans* TK24 *xylR* gene in pWHM3	This work

transferred to 50 ml of R2YE medium containing neomycin (10 μg/ml) and incubated at 39°C for two days to induce the crossover between pKCTR20 and chromosomal DNA in the microbial cells. During the incubation at the shifted temperature, cells in which the crossover occurred could grow in R2YE medium containing neomycin. The other strains without crossover could not grow because pKCTR20 could not replicate at 39°C. The culture was spread, after dilution, on R2YE agar plates containing neomycin (10 μg/ml). Colonies resistant to neomycin were picked and putative crossover recombinants were confirmed by Southern hybridization using the partial *xylR* gene (500 bp of 5'-region of *xylR* structural gene) and *neo* gene as DNA probes.

Xylose Isomerase Activity Assay

The formation of D-xylulose from D-xylose by xylose isomerase was measured according to the method of previous studies [11, 36]. The enzyme reaction mixture was composed of 1 ml of substrate solution containing 100 mM xylose, 20 mM magnesium sulfate, 100 mM potassium phosphate buffer (pH 7.0), 0.2 ml of the enzyme extract, and 0.8 ml of distilled water. After the reaction mixture was incubated for 30 min at 70°C, 2 ml of 500 mM perchloric acid was added to the reaction mixture to stop the enzyme reaction. The amount of xylulose was measured using the cysteine-carbazole method [36]. The amount of xylulose was calculated from the absorbance at 540 nm. One unit of xylose isomerase activity is defined as the amount of enzyme converting 1 μmol of D-xylose to D-xylulose per min under the conditions of this study. Crude cell extract was prepared as previously described [15].

Gel Mobility Shift Assay

S. lividans TK24, *S. lividans* XRD47, and *S. lividans* XRD47/pWLR25 were cultured in modified TSB (tryptic soy broth) media (2% tryptone, 0.5% sodium chloride, 0.25% dipotassium phosphate, 0.2% xylose) at 30°C for 36 h. Cells were harvested and disrupted by ultrasonication in a buffer containing 10 mM Tris-HCl (pH 7.4), 200 mM NaCl, 1 mM EDTA, 10 mM β-mercaptoethanol, and 0.23 mM phenylmethylsulfonyl fluoride [25]. The supernatant was obtained by centrifugation at 28,000 ×*g* at 4°C for 30 min and the crude cell-free extract was used for the gel mobility shift assay. The *xyl* promoter region was amplified by PCR from the plasmid pBLX1 containing the *xyl* gene cluster of *S. lividans* TK24 using oligonucleotide primers containing BamHI sites (primer BF10, 5'-CGCGGATCCGCTGCTGACATCGGGC-3', and primer AR20, 5'-CGGGATCCGGAAGTGGGCTGGTAG-3'). DNA fragments with the expected size were isolated from an agarose gel using a DEAE-cellulose membrane. The DNA fragments were digested with BamHI and inserted into the pUC119 plasmid. Nucleotide sequences of the fragments were confirmed with DNA sequencing. For the preparation of probes for the gel retardation assay, DNA fragments containing the *xyl* promoter were isolated from the recombinant plasmid digested with BamHI, and labeled using [α-^{32}P]-dCTP. The labeled probes were purified using the AccuPrep PCR purification Kit (Bioneer Co., Chungwon, Korea). DNA-binding reaction was performed at 0°C for 20 min in 20 μl of a mixture containing 20 mM HEPES-KOH (pH 7.9), 50 mM KCl, 2 mM $MgCl_2$, 0.5 mM dithiothreitol, 0.1 μg/ml bovine serum albumin, 10% glycerol, 1 μg of poly(dI-dC)·poly(dI-dC), 1 ng of labeled probe, and 10 μl of crude cell extracts. Samples were resolved in a pre-run 6% polyacrylamide gel using 0.5× Tris-borate buffer at 4°C [27].

RESULTS AND DISCUSSION

Cloning of *xyl* Gene Cluster of *S. lividans* TK24

Genomic DNA of *S. lividans* TK24 was isolated by the method of Hopwood *et al.* [14]. Plasmids were isolated using the QUIAGEN miniprep kit for subcloning and nucleotide sequencing according to the manufacturer's recommendation. Using the *xylA* gene fragment isolated previously from *S. chibaensis* J-59 [17, 18] as a probe, Southern hybridization of the *S. lividans* TK24 chromosomal DNA was carried out in order to locate the *xyl* gene cluster. It was found that the 11-kb BamHI, 8-kb SacI, and 1-kb SalI DNA fragments of the chromosomal DNA hybridized with the *S. chibaensis* J-59 *xylA* gene (data not shown). Therefore, 11-kb DNA fragments were isolated from the *S. lividans* TK24 chromosomal DNA digested with BamHI by the method of sucrose density gradient centrifugation [27, 28]. A gene library of the isolated DNA fragments was prepared using the λBlueSTAR vector (Novagen Co., Darmstadt, Germany), an auto-subcloning vector by *cre-loxP* site-specific recombination. Plaque hybridizaton of the library was carried out using the *xylA* gene of *S. chibaensis* J-59 as a probe by the methods of Sambrook and colleagues [27, 28]. A positive plaque showing strong signal was inoculated into an *E. coli* BM25.8 culture, which was incubated at 37°C overnight. The bacterial cells were spread on an LB agar plate containing 50 μg/ml ampicillin, which was incubated at 37°C overnight to generate a closed circle plasmid by the *cre-loxP*-mediated auto-subcloning system. A recombinant plasmid containing the 11-kb BamHI DNA fragment, which hybridizes with the *xylA* gene of *S. chibaensis* J-59, was obtained and designated as pBLX1.

Structure and Nucleotide Sequences of *S. lividans xyl* Gene Cluster

Restriction enzyme analysis of the recombinant plasmid pBLX1 containing the *S. lividans* TK24 *xyl* gene cluster was carried out to construct its physical map (Fig. 1). The nucleotide sequence analysis revealed that the insert in the recombinant plasmid pBLX1 was composed of 10,754 nucleotides (GenBank Accession No. AF184899). The fragment contained 6 ORFs including *xylA*, *xylB*, and putative *xylR* genes (Fig. 1).

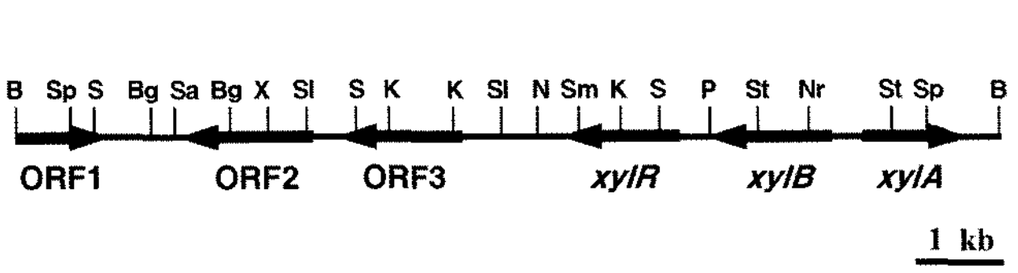

Fig. 1. Gene arrangement of the *xyl* gene cluster in *S. lividans* TK24.

Restriction endonuclease sites: B, BamHI; Bg, BglII; K, KpnI; N, NcoI; Nr, NruI; P, PstI; S, SacI; Sa, SacII; Sl, SalI; Sm, SmaI; Sp, SphI; St, StuI; X, XhoI. Frameplot 2.3 program (http://www.nih.go.jp/~jun/cgi-bin/frameplot.pl) was used to search putative open reading frames.

Fig. 2. Alignment of the deduced amino acid sequences of XylR proteins from various microbial strains.
The alignment was carried out using the Clustal W program (http://www.ebi.ac.uk/clustalw/). The asterisk, colon, and period indicate the identical amino acids, conserved substitutions, and semiconserved substitutions, respectively. The accession number for each gene is as follows: 1, *Bacillus megaterium* (CAA96093); 2, *Bacillus subtilis* (P16557); 3, *Bacillus licheniformis* (CAB02313); 4, *Anaerocellum thermophilum* (Q44406); 5, *Streptomyces coelicolor* (CAB61583); 6, *S. lividans* TK24 (AAF25624).

Alignment of the deduced amino acid sequences of XylR proteins from various microbial strains is shown in Fig. 2. The putative *xylR* gene encoded a protein composed of 402 amino acids, which is a little different from that in *S. violaceoniger*, which has been known to contain 348 amino acids [37]. In the protein motif search, XylR was presumed to contain a helix-turn-helix motif in the N-terminal region and a leucine zipper motif in the C-terminal region. Both motifs are well known for DNA binding activity. The protein has 29% to 34% identities with several *xyl* repressors of *Bacillus* spp. (GenBank Accession Nos. X57598, P16557, Z80222) and 100% identity with a putative *xyl* repressor of *S. coelicolor* A3 [2] (GenBank Accession No. AL133210). The *xylR* gene had only one different nucleotide from the putative *xyl* repressor gene of *S. coelicolor* A3 [2], whereas its amino acid sequences were exactly the same. The *xylB* gene encoded a 481 amino acid protein that showed 99% and 71% identities with the putative xylulokinase of *S. coelicolor* A3 [2] (GenBank Accession No. AL133210) and *S. rubiginosus* (GenBank Accession No. P27156), respectively. The *xylA* gene encoded a 387 amino acid protein that displayed about 79% to 97% identities with xylose isomerases of other *Streptomyces* spp. (GenBank Accession Nos. AL162460, P24300, P37031, AAF68977, P24299, P15587, AF170068, P09033, P50910, S28986, P22857). Other genes related to xylose transport were not found in this 11-kb BamHI DNA fragment. The ORF1 revealed 85% identities with the dihydroxy acid dehydratase of *S. coelicolor* A3 [2] (GenBank Accession No. AL096743) and *Schizosaccharomyces pombe* (GenBank Accession No. Q10318). The ORF2 showed 45% identity with the acetaldehyde dehydratase II of *Alcaligenes eutrophus* (GenBank Accession No. P46368) and 42% identity with the ETPC-inducible aldehyde dehydrogenase of *Rhodococcus erythropolis* (Genebank Accession No. P46369). The ORF3 exhibited 62% identity with a putative transcriptional regulator of *S. coelicolor* A3 [2] (GenBank Accession No. AL133210). The ORF3 was 975 bp apart from the putative *xylR* gene. It is ambiguous whether or not the ORF3 is related to the regulation of the *xyl* operon. The *xyl* cluster, transcriptional regulator, aldehyde dehydrogenase, and acetaldehyde dehydratase genes were arranged in the same pattern as that of *S. coelicolor* A3 [2]. However, a putative amidase gene, which is located between *xylR* and the transcriptional regulator genes in *S. coelicolor* A3, did not exist in *S. lividans* TK24 [2]. The overall organization of *xyl* genes and mode of regulation are quite similar to those of the *Streptomyces* spp. and *Bacillus* spp. *xyl* operons [12, 20, 29, 30, 40] but differ from those of *S. typhimurium* and *E. coli* [13, 22, 33, 34].

Deletion of *xylR* Gene in *S. lividans*

We have obtained 114 colonies resistant to neomycin by the transformation with the *xylR* deletion plasmid pKCTR20. Among the 114 colonies, 38 colonies were analyzed using their chromosomal DNA digested with EcoRI and KpnI by the Southern hybridization using *neo* and partial *xylR* genes as probes. The chromosomal DNA with double crossover (crossover events I and II in Fig. 3A) showed a signal at 13.1 kb in Southern hybridization with both probes. An EcoRI restriction site exists 9.7 kb upstream from the *xylR* gene. In the case of a single crossover at the 5'-region of the *xylR* gene (event I in Fig. 3A), two signals were detected at 14.5 kb and 0.5 kb with the partial *xylR* gene probe. In the case of a single crossover at the 3'-region of the *xylR* gene (event II in Fig. 3A), a signal band was detected at 3.2 kb with the *neo* gene probe and two

signals at 3.2 and 10.1 kb with the partial *xylR* gene probe. Twenty two strains out of 38 transformants showed integration of the plasmid by the homologous recombination through the sequence to the left (event I) of *xylR'*::*neo*, and 15 strains showed integration through the sequence to the right of *xylR'*::*neo* (event II). Just one strain, designated *S. lividans* XRD47, out of 38 colonies showed integration through both sides (events I and II) by the homologous recombination (Fig. 3B).

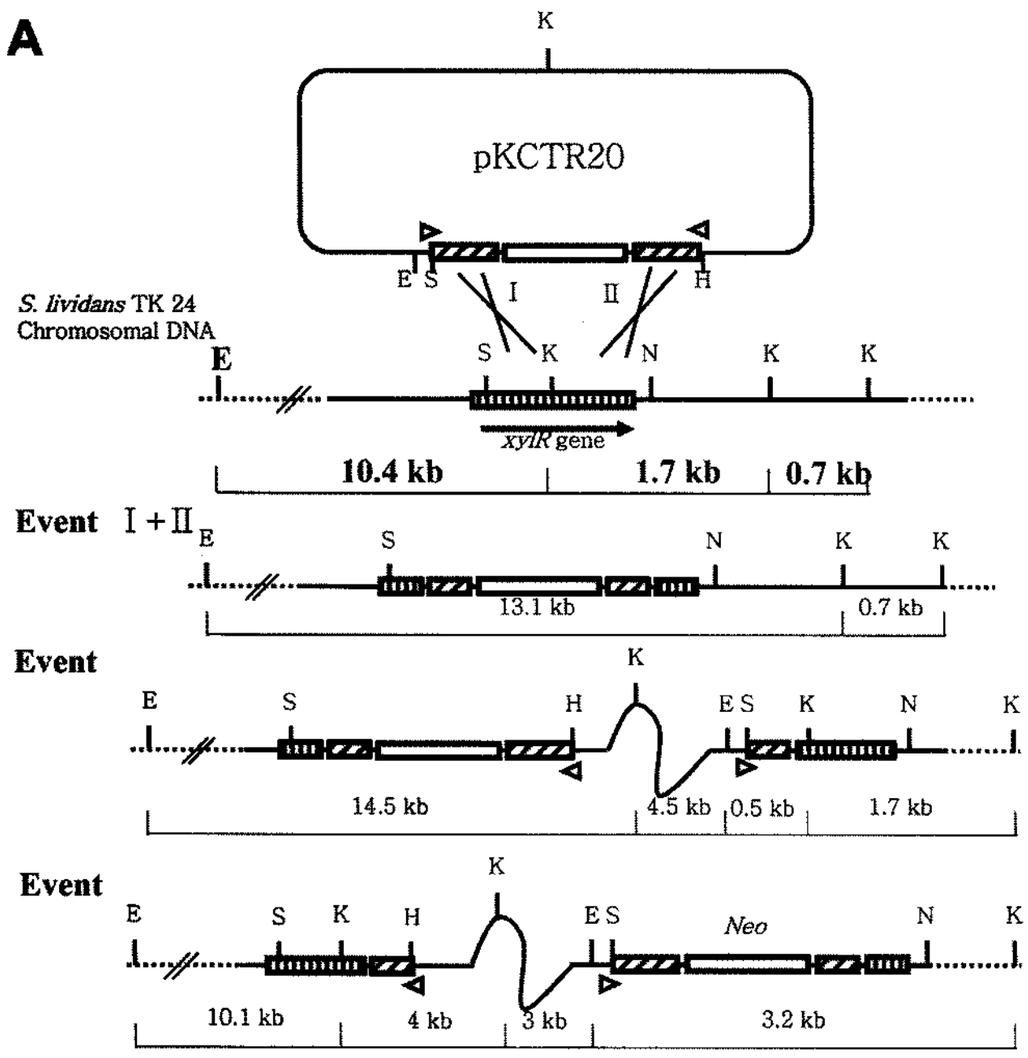

Fig. 3. Disruption of *xylR* by double crossover.
A. Schematic representation of disruption events. Dashed boxes represent the *xylR* gene at both ends of the 5' and 3'-regions (*xylR*) and each arrowhead indicates the truncated region of the *xylR* gene. Striped boxes represent a part or complete *xylR* fragment originated from chromosomal DNA. *Neo* indicates the neomycin resistance gene. **B**. Southern hybridization analysis of the recombinant strains. Genomic DNA from each strain was digested with EcoRI and KpnI. Southern hybridization was performed with the *neo* gene (left panel of **B**) and the 500-bp SacI-KpnI fragment of the *xylR* gene (right panel of **B**) as DNA probe. Lanes: 1, *S. lividans* XRD47 (double crossover at events I and II); 2, *S. lividans* XRD2801 (single crossover event II); 3, *S. lividans* XRD2802 (single crossover event II); 4, *S. lividans* XRD21002 (single crossover event II); 5, *S. lividans* XRD21003 (single crossover event II). Lamda/HindIII DNA size marker positions (kbp) are presented at the left side of each panel.

Effect of *xylR* Deletion on Xylose Isomerase Activity in *S. lividans*

Xylose isomerase has been well known to be an inducible enzyme in several *Streptomyces* sp. strains [13, 22, 33, 34]. As per the summary of xylose isomerase activities in Table 2, wild-type *S. lividans* TK24 produced xylose isomerase only in the presence of xylose. However, *S. lividans* XRD47, the *xylR* deletion mutant, produced xylose isomerase regardless of xylose, and its production level was around 3–5-fold higher than the wild type. In the *xylR* mutant, there are no significant differences of the xylose isomerase activity in the presence of xylose in comparison with the absence of xylose. These results suggest that the XylR protein would act as a repressor in the *xyl* operon in *S. lividans* TK24. However, the *xyl* promoter regulation switch was turned off in the *xylR* mutant strain *S. lividans* XRD47, and therefore, xylose utilization proteins like xylose isomerase were expressed constitutively.

In order to test the complementation of the *xylR* gene in the *xylR* deletion strain, a recombinant plasmid, pWLR25, containing a *xylR* gene was constructed using an *E. coli/Streptomyces* shuttle vector pWHM3 [37]. The production of xylose isomerase in *S. lividans* XRD47/pWLR25 was back to wild-type level, but the constitutive expression level in absence of xylose was higher than in the wild type (Table 2). This result might be due to differences of the expression level of XylR protein in the wild type and plasmid *xylR* in the *xylR* mutant. The expression level of plasmid *xylR* might be lower than those of the chromosomal copy because there is no significant promoter signal upstream of *xylR* (data now shown). Therefore, the plasmid copy of *xylR* may not transcript enough to produce XylR repressor protein on the *xyl* promoter on the chromosome. Moreover, it is well known that the specificity and activity of the transcriptional enhancer often depend on the distance to

Table 2. Xylose isomerase activities in *S. lividans*.

Strain	Activity (Units/mg protein)	
	Uninduced	Induced
S. lividans TK24 (W.T.)	0	0.384
S. lividans XRD47 (*xylR::neo*)	1.121	1.775
S. lividans XRD47/pWLR25	0.168	0.526

Induction of xylose isomerase was carried out in the presence of 0.4% xylose. The enzyme unit for the xylose isomerase activity was defined as the amount of enzyme converting 1 μmol of xylose to xylulose per minute under the conditions of this study.

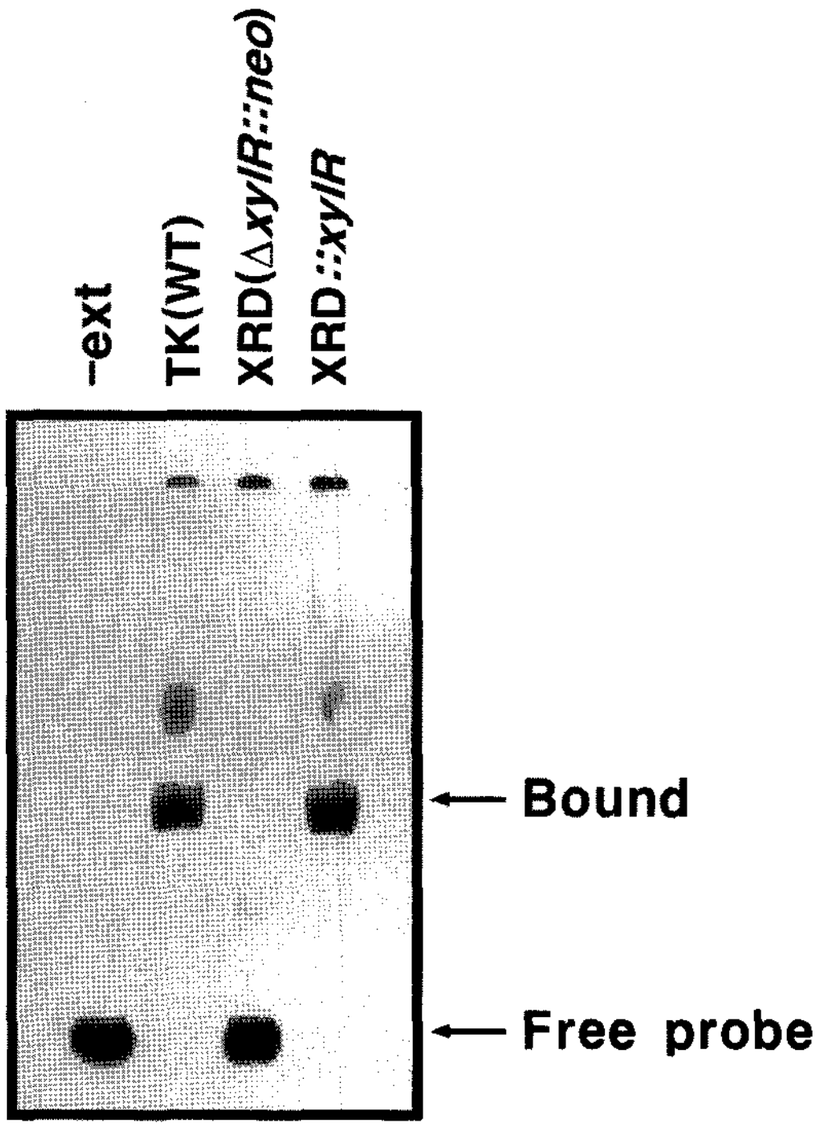

Fig. 4. Gel retardation assay of crude cell extracts of *S. lividans* with the DNA fragments containing the *xylAB* intergenic region.
^{32}P-labeled 195-bp DNA fragments containing the *xylAB* intergenic region were used as a hot probe. The labeled probe DNA fragments mixed with cell extracts, prepared from the *S. lividans* wild-type strain (TK24), *xylR* deletion mutant (XRD47), and *xylR* deletion mutant harboring the recombinant plasmid pWLR25 containing the *S. lividans* TK24 *xylR* gene, were resolved in a 6% polyacrylamide gel.

the promoter [38]. This explanation may extend to the repressor protein, XylR, in this study.

Interaction of XylR Protein with *xylAB* Promoter Region

Gel mobility shift assay was performed to examine the interaction of XylR and promoter region (158 bp) between the *xylA* and *xylB* genes. The mobility of the *xyl* promoter fragment was retarded on 6% polyacrylamide gel electrophoresis by formation of a complex with the crude cell extract of *S. lividans* TK24, but not with the extract of the *xylR* deletion mutant strain *S. lividans* XRD47. With the crude cell extract of *S. lividans* XRD47/pWLR25, the band was shifted at the same position of the gel as that of *S. lividans* TK24 (Fig. 4). The shifted band disappeared with the addition of the excess amount of the cold probe (data not shown). The retardation of the band resulted in the formation of a complex with the *xyl* promoter and XylR protein in the crude cell extracts of *S. lividans* TK24 and *S. lividans* XRD47/pWLR25. The binding affinity of XylR protein to the *xyl* promoter was dramatically decreased by the addition of xylose to the reaction mixture, but did not diminish completely even in the presence of 10 mM

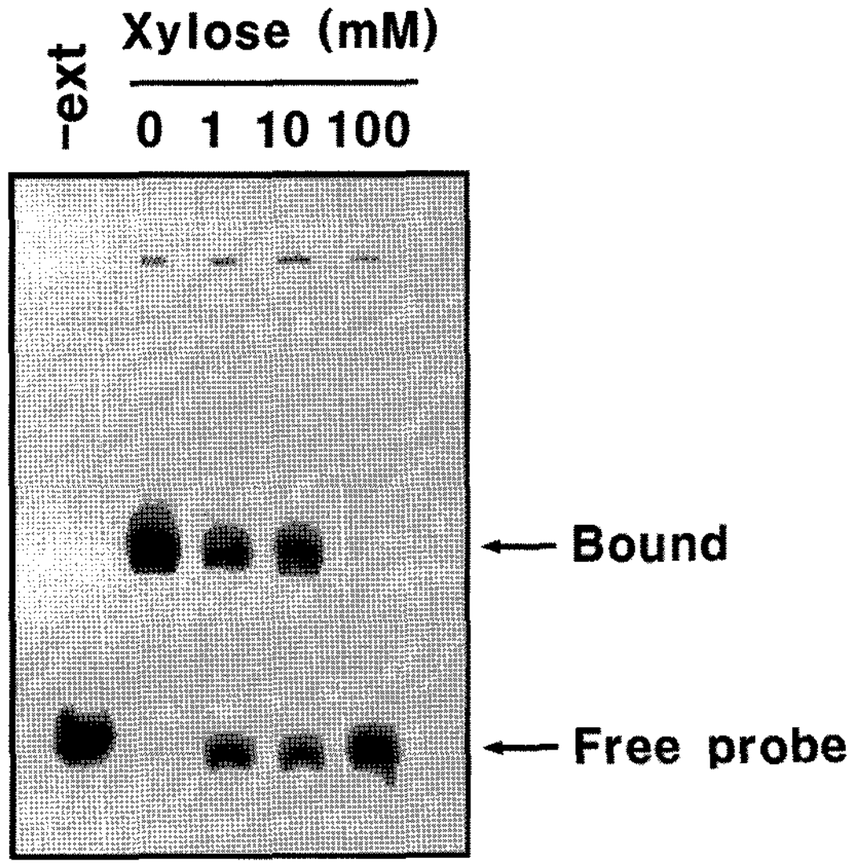

Fig. 5. Effects of xylose concentration for the binding of XylR to *xyl* promoter *in vitro*.
^{32}P-labeled 195 bp DNA fragments containing the *xylAB* intergenic region were used as a hot probe. The labeled probe DNA fragments mixed with cell extracts prepared from the *S. lividans* TK24 in the presence of 1, 10, and 100 mM xylose were resolved in a 6% polyacrylamide gel.

xylose (Fig. 5). This result may explain why the *xylR* mutant strain showed a higher xylose isomerase activity than wild type (Table 2). If the xylose level is not high enough to release XylR repressor *in vivo*, the transcription level could be reduced even in the present of xylose, comparing with the *xylR* mutant strain, which has no XylR repressor *in vivo*.

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References

1. Bejar, S. H., K. Belghith, R. Gargouri, and R. Ellouz. 1994. Construction of a new strain of *Streptomyces violaceoniger*, having strong, constitutive and stable glucose-isomerase activity. *Biotechnol. Lett.* **16:** 1259–1264.
2. Bhosale, S. H., M. B. Rao, and V. V. Deshpande. 1996. Molecular and industrial aspects of glucose isomerase. *Microbiol. Rev.* **60:** 280–300.
3. Bhosale, S. H., M. S. Ghatge, and V. V. Deshpande. 1996. Molecular cloning and expression of the glucose/xylose isomerase gene from *Streptomyces* sp. NCIM 2730 in *Escherichia coli*. *FEMS Microbiol. Lett.* **145:** 95–100.
4. Bierman, M., R. Logan, K. O'Brien, E. T. Seno, R. N. Rao, and B. E. Schoner. 1992. Plasmid cloning vectors for the conjugal transfer of DNA from *Escherichia coli* to *Streptomyces* spp. *Gene* **116:** 43–49.

5. Blackwood, E. M. and T. K. James. 1998. Going the distance: A current view of enhancer action. *Science* **281:** 60–64.
6. Choi, J. H., O. S. Lee, J. H. Shin, Y. Y. Kwak, Y. M. Kim, and I. K. Rhee. 2006. Thermostable xylanase encoded by *xynA* of *Streptomyces thermocyaneoviolaceus*: Cloning, purification, characterization and production of xylooligosaccharides. *J. Microbiol. Biotechnol.* **16:** 57–63.
7. Choi, J. H., T. K. Kim, Y. M. Kim, W. C. Kim, K. Park, and I.-K. Rhee. 2006. Cloning and characterization of a gene cluster for cyclohexanone oxidation in *Rhodococcus* sp. TK6. *J. Microbiol. Biotechnol.* **16:** 511–518.
8. Chou, C. C., M. S. Ladisch, and G. T. Tsao. 1976. Studies on glucose isomerase from a *Streptomyces* species. *Appl. Environ. Microbiol.* **32:** 489–493.
9. Dahl, M. K., D. Schmiedel, and W. Hillen. 1995. Glucose and glucose-6-phosphate interaction with Xyl repressor protein from *Bacillus* spp. may contribute to regulation of xylose utilization. *J. Bacteriol.* **177:** 5467–5472.
10. Denis, F. and R. Brzezinski. 1991. An improved aminoglycoside resistance gene cassette for use in Gram-negative bacteria and *Streptomyces*. *FEMS Microbiol. Lett.* **81:** 261–264.
11. Dische, Z. and E. Borenfreund. 1951. A new spectrophotometric method for the detection and determination of keto sugars and trioses. *J. Biol. Chem.* **192:** 583–587.
12. Gärtner, D., M. Geissendörfer, and W. Hillen. 1988. Expression of the *Bacillus subtilis xyl* operon is repressed at the level of transcription and is induced by xylose. *J. Bacteriol.* **170:** 3102–3109.
13. Ghangas, G. S. and D. B. Wilson. 1984. Isolation and characterization of the *Salmonella typhymurium* LT2 xylose regulon. *J. Bacteriol.* **157:** 158–164.
14. Hopwood, D. A., M. J. Bibb, K. F. Chater, T. Kieser, C. J. Bruton, H. M. Kieser, *et al.* 1985. *Genetic Manipulation Of Streptomyces: A Laboratory Manual*. The John Innes Foundation, Norwich, England.
15. Hur, J. M., S. H. Kwon, J. H. So, M. Jun, Y. H. Kang, Y. M. Lee, *et al.* 2007. Changes in aurantio-obtusin and glucoaurantio-obtusin content in Cassiae Semen *via* treatment with a crude enzyme extract from *Aspergillus usamii*. *J. Microbiol. Biotechnol.* **17:** 1894–1897.
16. Jacob, S., D. Gärtner, R. Allmansberger, 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.
17. Joo, G.-J., J.-H. Shin, G.-Y. Heo, Y.-M. Kim, and I.-K. Rhee. 2005. Molecular cloning and expression of a thermostable xylose (glucose) isomerase gene, *xylA*, from *Streptomyces chibaensis* J-59. *J. Microbiol.* **43:** 34–37.
18. Joo, G.-J., J.-H. Shin, G.-Y. Heo, Y.-Y. Kwak, J.-H. Choi, and I.-K. Rhee. 2001. Purification and characterization of a thermostable xylose (glucose) isomerase from *Streptomyces chibaensis* J-59. *Agric. Chem. Biotechnol.* **44:** 113–118.
19. Kibuchi, T., Y. Itoh, T. Kasumi, and C. Fukazawa. 1990. Molecular cloning of the *xylA* gene encoding xylose isomerase from *Streptomyces griseofuscus* S-41: Primary structure of the gene and its product. *Agric. Biol. Chem.* **54:** 2469–2472.
20. Kraus, A., C. Hueck, D. Gärtner, and W. Hillen. 1994. Catabolite repression of the *Bacillus subtilis xyl* operon involves a *cis* element functional in the context of an unrelated sequence, and glucose exerts additional *xylR*-dependent repression. *J. Bacteriol.* **176:** 1738–1745.
21. Kreuzer, P., D. Gärtner, R. Allmansberger, and W. Hillen. 1989. Identification and sequence analysis of the *Bacillus subtilis* W23 *xylR* gene and *xyl* operator. *J. Bacteriol.* **171:** 3840–3845.
22. Lawlis, V. B., M. S. Dennis, E. Y. Chen, D. H. Smith, and D. J. Henner. 1984. Cloning and sequencing of xylose isomerase and xylulose kinase gene of *Escherichia coli*. *Appl. Environ. Microbiol.* **47:** 15–21.
23. Lokman, B. C., M. Heerikhuisen, R. J. Leer, A. V. D. Broek, Y. Borsboom, S. Chaillou, P. W. Postma, and P. H. Pouwels. 1997. Regulation of expression of the *Lactobacillus pentosus xylAB* operon. *J. Bacteriol.* **179:** 5391–5397.
24. Marcel, T., D. Drocourt, and G. Tiraby. 1987. Cloning of the glucose isomerase (D-xylose isomerase) and xylulose kinase gene of *Streptomyces violaceoniger*. *Mol. Gen. Genet.* **208:** 121–126.
25. Nguyen, J. 1999. The regulatory protein Reg1 of *Streptomyces lividans* binds the promoter region of several genes repressed by glucose. *FEMS Microbiol. Lett.* **175:** 51–58.
26. Rygus, T. and W. Hillen. 1992. Catabolite repression of the *xyl* operon in *Bacillus megaterium*. *J. Bacteriol.* **174:** 3049–3055.
27. Sambrook, J. and D. W. Russell. 2001. *Molecular Cloning: A Laboratory Manual*, 3rd Ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
28. Sambrook, J., E. F. Fritsch, and T. Maniatis. 1989. *Molecular Cloning: A Laboratory Manual*, 2nd Ed. Cold Spring Harber Laboratory Press, Cold Spring Harbor, N.Y.
29. Scheler, A. and W. Hillen. 1994. Regulation of xylose utilization in *Bacillus licheniformis*: Xyl repressor-*xyl*-operator interaction studied by DNA modification protection and interference. *Mol. Microbiol.* **13:** 505–512.
30. Scheler, A., T. Rygus, R. Allmansberger, and W. Hillen. 1991. Molecular cloning, structure, promoters and regulatory elements for transcription of *Bacillus licheniformis* encoded regulon for xylose utilization. *Arch. Microbiol.* **155:** 526–534.
31. 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.
32. Schmiedel, D., M. Kintrup, E. Küster, and W. Hillen. 1997. Regulation of expression, genetic organization and substrate specificity of xylose uptake in *Bacillus megaterium*. *Mol. Microbiol.* **23:** 1053–1062.
33. Shamanna, D. K. and K. E. Sanderson. 1979. Genetics and regulation of D-xylose utilization in *Salmonella typhymurium* LT2. *J. Bacteriol.* **139:** 71–79.
34. Song, S. and C. Park. 1997. Organization and regulation of the D-xylose operons in *Escherichia coli* K-12: XylR acts as a transcriptional activator. *J. Bacteriol.* **179:** 7025–7032.
35. Späth, C., A. Kraus, and W. Hillen. 1997. Contribution of glucose kinase to glucose repression of xylose utilization in *Bacillus megaterium*. *J. Bacteriol.* **179:** 7603–7605.
36. Takasaki, Y. 1996. Studies on sugar isomerization enzyme: Production and utilization of glucose isomerase from *Streptomyces* spp. *Agric. Biol. Chem.* **30:** 1247–1253.
37. Tiraby, G., S. Bejar, D. Drocourt, J. P. Reynes, P. J. Sicard, G. K. Farber, A. Glasfeld, D. Ringe, and G. A. Petsko. 1989.

Genetic, enzymatic, and crystallographic studies of the glucose isomerase of two *Streptomyces* species, pp. 119–126. *In* L. Hershberger (ed.), *Genetic and Molecular Biology of Industrial Microorganisms*. American Society for Microbiolgy, Washington, D. C.

38. Vara, J., M. L. Skarbek, Y. Wang, S. Donadio, and C. R. Hutchinson. 1989. Cloning of genes governing the deoxysugar portion of the erythromycin biosynthesis pathway in *Saccharopolyspora erythraea* (*Streptomyces erythreus*). *J. Bacteriol.* **171:** 5872–5881.
39. Whitaker, R. D., Y. Cho, J. Cha, H. L. Carrell, J. P. Glusker, P. A. Karplus, and C. A. Batt. 1995. Probing the roles of active site residues in D-xylose isomerase. *J. Biol. Chem.* **270:** 22895–22906.
40. Wong, H. C., Y. Ting, H. C. Lin, F. Reichert, K. Myambo, K. W. K. Watt, P. L. Toy, and R. J. Drummond. 1991. Genetic organization and regulation of the xylose degradation genes in *Streptomyces rubuginosus*. *J. Bacteriol.* **173:** 6849–6858.
41. Worcha, M. G., D. L. Steuerwald, and K. E. Brooks. 1983. Amplification of D-xylose and D-glucose isomerase activities in *Escherichia coli* by gene cloning. *Appl. Environ. Microbiol.* **45:** 1402–1404.