The Journal of Microbiology, February 2005, p.34-37 Vol. 43, No. 1

NOTE

Molecular Cloning and Expression of a Thermostable Xylose (Glucose) Isomerase Gene, *xylA*, from *Streptomyces chibaensis* J-59

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(Received May 4, 2004 / Accepted December 16, 2004)

In the present study, the *xylA* gene encoding a thermostable xylose (glucose) isomerase was cloned from *Streptomyces chibaensis* J-59. The open reading frame of *xylA* (1167 bp) encoded a protein of 388 amino acids with a calculated molecular mass of about 43 kDa. The XylA showed high sequence homology (92% identity) with that of *S. olivochromogenes*. The xylose (glucose) isomerase was expressed in *Escherichia coli* and purified. The purified recombinant XylA had an apparent molecular mass of 45 kDa, which corresponds to the molecular mass calculated from the deduced amino acid and that of the purified wild-type enzyme. The N-terminal sequences (14 amino acid residues) of the purified protein revealed that the sequences were identical to that deduced from the DNA sequence of the *xylA* gene. The optimum temperature of the purified enzyme was 85°C and the enzyme exhibited a high level of heat stability.

Key words: *Streptomyces chibaensis*, xylose (glucose) isomerase, *xylA*

D-Xylose (glucose) isomerase (D-xylose ketol-isomerase; E.C. 5.3.1.5) catalyzes the isomerization of D-xylose to xylulose and the conversion of D-glucose to fructose. Thus, this enzyme is an extremely important enzyme in the industrial manufacture of high-fructose corn syrups (Jensen and Rugh, 1987). Immobilized xylose (glucose) isomerase is practically used for industrial purposes (Tsumura *et al.*, 1965; Bhosale *et al.*, 1996). The currently used process is operated at 58°C with non-thermostable xylose isomerases and produces 40~42% fructose syrup. Therefore, an additional chromatographic step is necessary to obtain a 55% syrup concentration. Isomerization at 95°C would achieve 55% syrup without the additional concentration step (Zeikus, 1995) as equilibrium for the isomerization reaction is shifted to the production of fructose at high temperature. Therefore, researchers have sought to isolate the microorganisms which are capable of producing more thermostable enzyme than the one currently used in industry.

In the present study, we isolated *Streptomyces chibaensis* J-59 from a compost (Joo and Rhee, 1997) producing a thermostable xylose (glucose) isomerase, which was purified and characterized as stated previously (Joo *et al.*, 2001). The xylose isomerase of *S. chibaensis* J-59 was more thermostable than any other xylose isomerases of *Streptomyces* spp. (Joo *et al.*, 2001).

To clone a *xylA* gene encoding the xylose (glucose) isomerase from *S. chibaensis* J-59, a probe was prepared by PCR using two primers, GA (5'-TTTGGATCCATGAAC-TACCAGCCCACCCCCGA-3') and NA (5'-TATAAGC-TTTCAGCCGCGGGCGCCCAGCAG-3'), which were designed based on the N-terminal sequence of the xylose (glucose) isomerase of *S. chibaensis* J-59 (Joo *et al.*, 2001) and published sequences of *xylA* of *S. rubiginosus* (Wong *et al.*, 1991) and *S. violaceoniger* (Drocourt *et al.*, 1988). A 5-kb DNA fragment of *Bam*HI-digested chromosomal DNA was hybridized with the 1.2-kb PCR products. The fragment was cloned into the *Bam*HI site of pBR322 and transformed into *Escherichia coli* HB101. A recombinant plasmid, pCHX12, was selected by colony hybridization using the PCR product as a probe. Nucleotide sequence of the 1.95-kb DNA fragment of pCHX12 digested with *Sma*I containing a structural gene of the xylose (glucose) isomerase and promoter region of the gene were determined.

The DNA sequences of the *xylA* and the deduced amino acid sequences are shown in Fig. 1. The open reading frame of *xylA* (1167 bp) encoded a protein of 388 amino acids with a molecular mass of 42,989 kDa. The N-terminal

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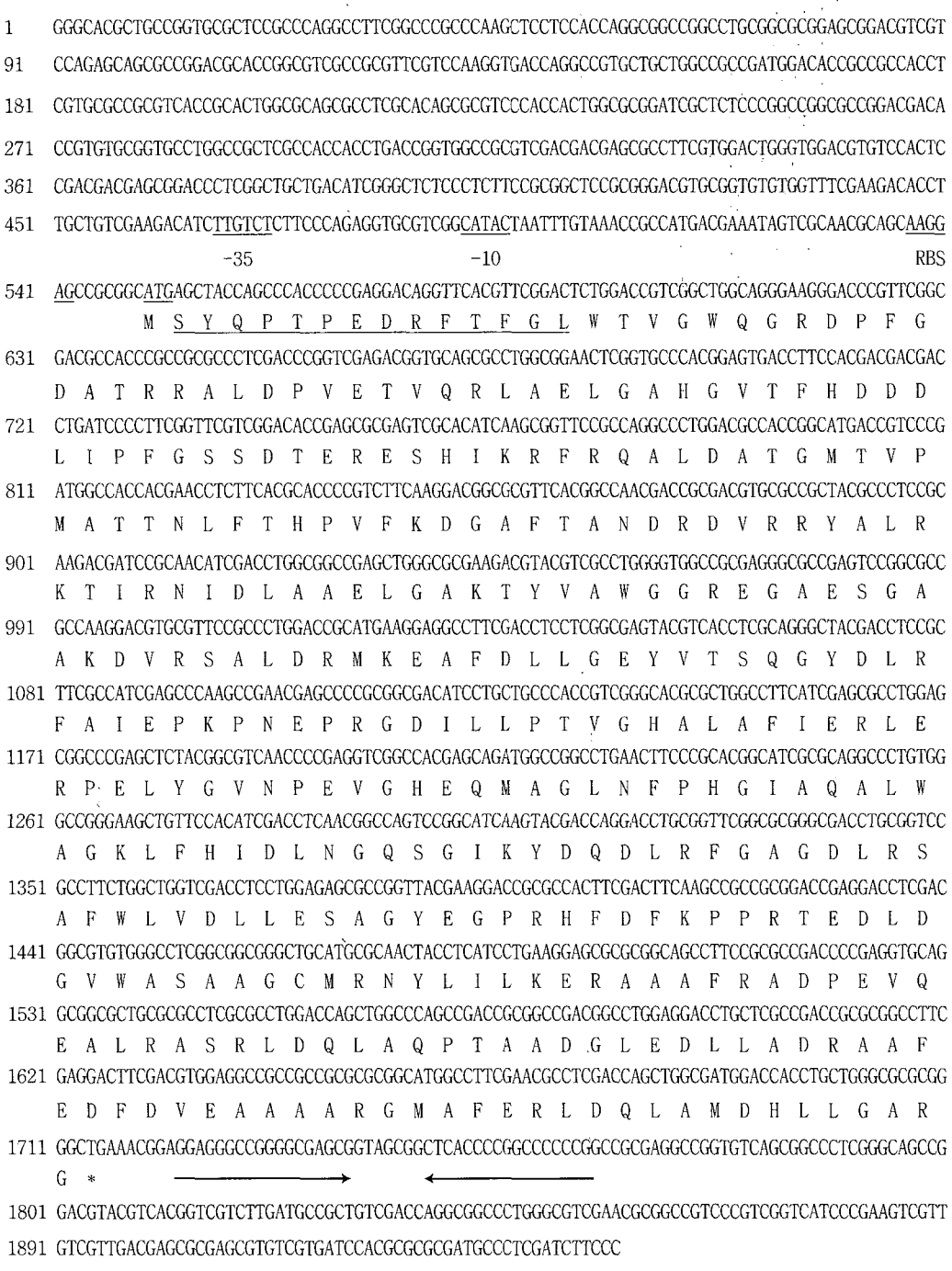

Fig. 1. DNA sequence and deduced amino acid sequence of *Streptomyces chibaensis* J-59 *xyl*A gene. The amino acid residues identified by protein sequencing are underlined. Putative -35 and -10 sequences and ribosomal binding site (RBS) are also underlined. The DNA sequence is numbered on the left. The inverted repeat sequence (IRS) is indicated by the converging arrows.

amino acid sequence (MSYQPTPEDRFTFGL) deduced from the *xylA* gene matched the N-terminal sequence of the purified xylose isomerase, in which the initiation amino acid, methionine was excised (Joo *et al.*, 2001). A possible ribosome binding sequence (AAGGAG) was found 7-bp upstream from the ATG start codon. Although the initiation site for transcription has not yet been determined, two hexanucleotide consensus sequences for -10 and -30 region, CATACT and TTGTCT existed 52-bp upstream from the ATG start codon, spaced by 19-bp. A pair of inverted repeat sequences (IRS) was also found in the region downstream to the translation termination codon, TGA.

Comparison of the deduced amino acid sequence of the XylA with those of proteins in the GenBank database indicated that the greatest similarity was with the XylA of

S. olivochromogenes (Lavie *et al.*, 1994), 92% identity and 94% similarity (data not shown). Many of the bacterial XylA registered in the GenBank database also showed high homology to the XylA of *S. chibaensis* J-59 (data not shown).

In *E. coli* HB101 cells harboring pCHX12, the level of xylose (glucose) isomerase activity was not detected (data not shown), suggesting that the *xylA* gene cloned from *S. chibaensis* J-59 was not expressed in *E. coli* from its own promoter. In order to overexpress the protein in *E. coli*, the *xylA* gene was cloned in pET21a(+) under the control of T7 promoter. PCR was performed using two primers, 5'-CGCCATATGAGCTACCAGCCCACC-3' and 5'-TGCACCGTCTCGACCGGGTCG-3', with pCHX12 as a template. The 107-bp PCR product of the 5' partial *xylA* gene digested with *Nde*I and *Tth*III, and 1.3-kb DNA fragment carrying the rest of the gene on pCHX12 digested with *Tth*III and *Hind*III were cloned into a expression vector, pET21a(+) between *Nde*I and *Hind*III sites. The resulting recombinant plasmid was named pETX23. *E. coli* BLR (DE3) harboring pETX23 was cultured at 37°C in Luria-Bertani (LB) media. After overnight culture, cells were diluted 50-fold into a fresh medium, grown to A_{600} of 0.8, at which point XylA expression was induced by the addition of 1 mM isopropylthiogalactoside (IPTG), and incubated for additional 4 h. After harvesting, cells were resuspended in 100 mM potassium phosphate buffer (pH 7.0), and disrupted by sonication. Unbroken cells were removed by centrifugation (15,000×*g*, 15 min, 4°C) and the supernatant was then heated at 70°C for 20 min. The precipitate was removed by centrifugation (15,000×*g*, 15 min, 4°C) and the supernatant was obtained as partially purified enzyme. Production of the enzyme was monitored by SDS-PAGE analysis and by measuring the enzyme activity. The activity of xylose (glucose) isomerase was measured using the colorimetric assay as described previously (Joo *et al.*, 2001). Protein concentration was measured by Bradford's method (1976).

Coomassie-blue staining of SDS-PAGE gel revealed major recombinant xylose (glucose) isomerase bands in the induced samples (Fig. 2). The protein band of partially purified xylose (glucose) isomerase had an apparent molecular mass near 45 kDa, which corresponds to the molecular mass calculated from the deduced amino acid and that of the previously purified wild-type enzyme (Joo *et al.*, 2001).

The temperature for maximum activity was determined by measuring enzyme activity at different temperatures

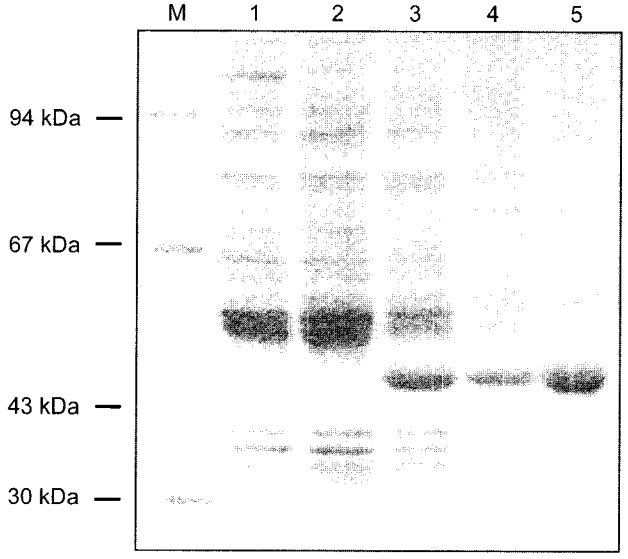

Fig. 2. Expression and purification of XylA. *Escherichia coli* BLR (DE3) harboring pETX23 was cultured at 37°C in LB media. After harvesting, cells were resuspended in 100 mM potassium phosphate buffer (pH 7.0) and disrupted by sonication. Lanes: 1, *E. coli* BLR (DE3); 2, BLR (DE3)/pETX23; 3, BLR (DE3)/pETX23 after 4 h induction with 1 mM IPTG at A_{600} of 0.8; 4, BLR (DE3)/pETX23 after 8 h induction with 1 mM IPTG; 5, partially purified recombinant xylose (glucose) isomerase by heat treating at 70°C for 20 min. The molecular weights of marker protein (lane M) are indicated.

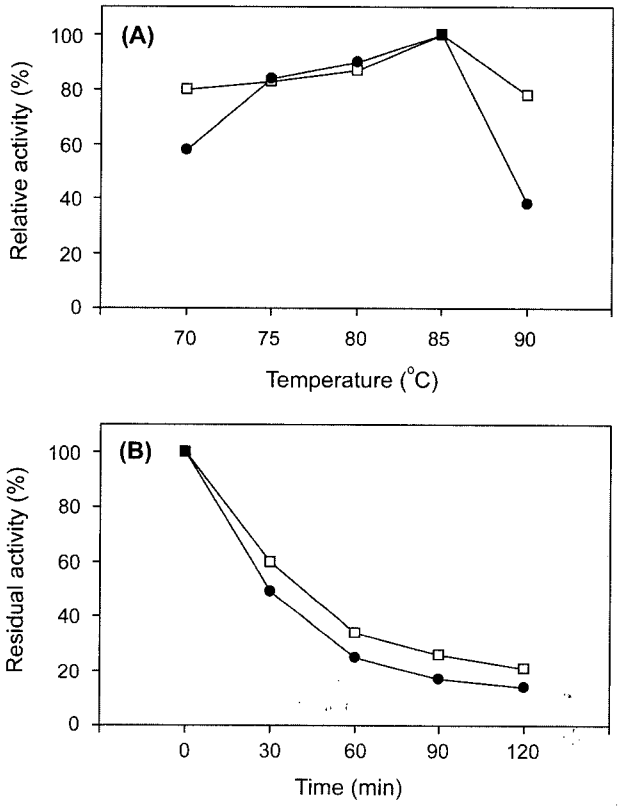

Fig. 3. Influence of temperature on the activity of recombinant xylose (glucose) isomerase and its thermal stability. A, enzyme activity was measured according to the standard assay (Joo *et al.*, 2001) at 70, 75, 80, 85, and 90°C. B, enzyme was preincubated for 30, 60, 90, and 120 min at 90°C prior to assay. The residual activities were measured according to the standard assay. The highest level of activity was set as 100% in each experiment. ●, recombinant enzyme; □, wild-type enzyme.

according to a standard assay (Joo *et al.*, 2001). As shown in Fig. 3A, the optimum temperature of the recombinant enzyme was 85°C. Thermal stability was also investigated by incubating the enzyme at 90°C prior to enzyme assay. After 1 h incubation at 90°C, the recombinant and wild-type enzymes retained 25 and 34% of their activity, respectively (Fig. 3B). No significant differences, including optimum pH and pH stability, were found between native xylose (glucose) isomerase from *S. chibaensis* J-59 and the recombinant enzyme (data not shown).

The results presented in this paper indicate that the recombinant xylose (glucose) isomerase is an attractive candidate for industrial applications because of its thermostability.

This work was supported by Ministry of Science & Technology (MOST) and the Korea Science and Engineering Foundation (KOSEF) through the Center for Traditional Microorganism Resources (TMR) at Keimyung University.

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