Agric. Chem. Biotechnol. **44**(3), 113-118 (2001)

Article

Purification and Characterization of a Thermostable Xylose (Glucose) Isomerase from *Streptomyces chibaensis* J-59

Gil-Jae Joo, Jae-Ho Shin, Gun-Young Heo, Yun-Young Kwak, Jun-Ho Choi and In-Koo Rhee*

Department of Agricultural Chemistry, Kyungpook National University, Daegu 702-701, Korea

Received May 19, 2001

Xylose (glucose) isomerase was purified to homogeneity from cell-extracts of *Streptomyces chibaensis* J-59 via ammonium sulfate precipitation followed by chromatography on DEAE-cellulose, and gel filtration on Sephacryl S-300. The purified enzyme is a homotetramer with a native molecular mass of 180 kDa and a subunit molecular mass of 44 kDa. The amino acid N-terminal sequence of glucose isomerase from *S. chibaensis* J-59 was determined to be Ser-Tyr-Gln-Pro-Thr-Pro-Glu-Asp-Arg-Phe-Thr-Phe-Gly-Leu. The first 14 amino acids of the N-terminal sequence of the enzyme showed strong analogies with N-terminal sequences of glucose isomerase produced by other *Streptomyces* spp. The optimum pH and temperature for activity were 7.5 and 85, respectively. The purified enzyme required Mg^{2+}, Co^{2+}, and Mn^{2+} for the activity, Mg^{2+} being the most effective. The enzyme was not inhibited by Ca^{2+}, but was inhibited by Hg^{2+}, Ag^{+}, and Cu^{2+}. The K_m, V_{max}, and k_{cat} values of *S. chibaensis* J-59 isomerase for glucose were 83 mM, 40.9 U/mg, and 1,843 min^{-1}, respectively. In the presence of Co^{2+}, cell-free enzymes retained 100% without loss of activities by the heat-treatment at 70°C for 7 days. The enzyme retained 50% residual activity after heating at 85°C for 13.5 h, at 90°C for 126 min. The enzyme is more thermostable than any other glucose isomerases of *Streptomyces* spp.

Key words: Streptomyces chibaensis, *thermostable enzyme, xylose (glucose) isomerase.*

D-Xylose (glucose) isomerase (D-xylose ketol-isomerase; E.C. 5.3.1.5) catalyzes the reversible isomerization of D-xylose to D-xylulose at the first step of xylose metabolism following the pentose phosphate pathway, and the isomerization of glucose to fructose. It is thus used industrially for the production of high fructose corn syrup (HFCS) known as glucose isomerase.[1] In particular, immobilized glucose isomerase is used for industrial purposes.[2,3] Although the enzyme has been isolated from many microorganisms,[4] commercial enzyme products are available from only a relatively small number of genera, notably *Streptomyces* species,[5] *Actinoplanes missouriensis,*[6] and *Bacillus subtilis.*[7]

The equilibrium conversion of glucose to fructose under industrial process conditions is around 50%, and the enthalpy of the reaction is 5 kJ/mol. Commercial application of HFCS requires the use of high fructose concentrations. The desired concentration of fructose for many applications by the industry is higher than 50%, at which sugar is easily dissolved in water at high temperature.[8] Higher isomerization yields may be achieved by increasing the reaction temperature. The current process is operated at 58°C with non-thermostable glucose isomerases, resulting in 40-42% fructose syrup. This necessitates an additional chromatographic step to obtain 55% syrup. Performing the isomerization at temperature higher than 90°C would achieve 55% syrup without the chromatographic concentration step.[8] In view of the fact that microorganisms have been isolated for higher thermostable enzyme production than any other strains, continual screening should be done to discover even more thermostable glucose isomerases.

We, therefore, isolated *Streptomyces chibaensis* J-59 from compost producing a thermostable glucose isomerase.[9] *S. chibaensis* J-59 did not grow in the culture medium containing only xylose or xylan as the carbon source, because it was defective in xylulokinase production; *xylB* mutant.[10] It was able to produce xylanase and β-xylosidase as well as glucose isomerase. The glucose isomerase in *S. chibaensis* J-59 was induced in the medium containing xylan or xylose that could be utilized as inducer but not as carbon and energy source.

In this paper, we report on the purification and some physicochemical properties of the glucose isomerase from *S. chibaensis* J-59.

Materials and Methods

Strain and growth conditions. *S. chibaensis* J-59 was maintained either frozen at –20°C in 25% glycerol or at 4°C as 1-week-old slant cultures on YM agar (Difco Co.). It was cultured in 100 m*l* medium containing 0.3% yeast extract, 0.3% malt extract, 0.5% Bacto peptone, and 1% dextrose (pH 6.5) for

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

Abbreviations: GIU, international unit of D-glucose isomerase; U, unit; PAGE, polyacrylamide gel electrophoresis.

three days at 30°C with reciprocal shaking (120 strokes/min) for the seed culture. For large scale enzyme preparation, it was grown for 72 h at 30°C in a 2.5-*l* jar fermentor containing 1 *l* of the liquid medium composed of 0.15% glucose, 1.5% corn steep liquor, 0.1% $MgSO_4 \cdot 7H_2O$, 0.12% $CoCl_2 \cdot 6H_2O$, and 1% xylan as an inducer for enzyme production, and the culture pH was maintained at 7.0 using a pH controller.

Enzyme activity and protein assays. The formation of D-fructose from D-glucose was measured using the colorimetric assay as described previously.[10] A test mixture of 1 m*l*, containing 400 mM glucose (or 100 mM xylose), 20 mM magnesium sulfate, and 100 mM potassium phosphate buffer (pH 7.0), was incubated with 0.2 m*l* of the enzyme solution and 0.8 m*l* of distilled water for 30 min at 70°C. The reaction was stopped by adding 2 m*l* of 500 mM perchloric acid, and the fructose formed was measured using the cysteine-carbazole method.[11] After color development was left to proceed for 60 min at 4°C, the absorbance at 560 nm was determined. One unit of D-glucose isomerase activity (GIU) is defined as the amount of enzyme converting 1.0 μmol D-glucose to D-fructose in 1 min through the above assay conditions. The protein contents were measured by the procedure of Bradford[12] using bovine serum albumin (Sigma Co.) as a standard.

Enzyme purification. Unless otherwise mentioned, all purification steps were carried out at 4°C. Cells were harvested in late exponential growth phase via centrifugation (10,000 × g, 10 min, 4°C). The cell pellet was washed twice and suspended with 50 mM potassium phosphate buffer (pH 7.0). The suspension was centrifuged and the pellet was resuspended in the same buffer and sonicated with a sonicator (90 μA, Ultrasonic Ltd. Co.) for 10 min (five times for 2 min each) at 4°C. Using the method of Chen,[13,14] 0.2% cetyltrimethylammonium bromide (CTAB) was added to the sonicated suspension, and the suspension was incubated for 2 h at 37°C. A clear supernatant fluid was obtained through centrifugation (15,000 × g, 30 min, 4°C). The suspension was heated at 70°C for 20 min, and the precipitate was removed via centrifugation (15,000 × g, 20 min, 4°C). The supernatant was used as the crude enzyme solution. Solid ammonium sulfate was slowly added with continuous stirring to the cell crude enzyme solution to 40% saturation. After incubation for 1 h at 4°C with gentle mixing, the precipitate was removed by the centrifugation (15,000 × g, 30 min, 4°C). Ammonium sulfate concentration was increased stepwise to 80% saturation. The pH of the enzyme solution was adjusted to pH 7.0 with ammonia water. The precipitate was collected via centrifugation, dissolved in 50 mM potassium phosphate buffer (pH 7.0), and dialyzed overnight against the same buffer. After dialysis and centrifugation (15,000 × g, 30 min, 4°C), the supernatant was applied to a column of DEAE-cellulose (ϕ 2.0 by 18 cm), previously equilibrated with 10 mM potassium phosphate buffer (pH 7.0). The enzyme was eluted using the same buffer with a linear gradient of 0 to 50 0mM NaCl at a flow rate of 2 0m*l* · h^{-1}. The active fractions were pooled and dialyzed overnight with the same buffer. The dialyzed enzyme solution was concentrated via ultrafiltration with YM-10 membrane (Amicon Inc.). The concentrated enzyme solution was applied to a column of Sephacryl S-300 (ϕ 1.3 by 80 cm), previously equilibrated with 10 mM potassium phosphate buffer (pH 7.0). The enzyme was eluted with the same buffer at a flow rate of 5 m*l* · h^{-1}, and the active fractions were collected. The enzyme was stored at −20°C until use.

Electrophoresis and molecular mass determination. Purity and size of protein at each purification step were analyzed through the sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) described by Laemmli[15] and native PAGE described by Davis.[16] Molecular mass of glucose isomerase was determined via gel filtration on a Sephadex G-200 column (ϕ 1.6 by 90 cm) using the method of Andrews.[17]

Determination of amino acid sequence. N-Terminal sequence of the purified glucose isomerase was determined through the automated Edman degradation[18] using an Applied Biosystem model 476 A gas-phase protein sequencer at Korea Basic Science Institute.

Calculation of K_m and k_{cat}. Estimations were carried out at substrate concentrations over a range of 1 to 10 mM for xylose and 50 to 200 mM for glucose in 50 mM phosphate buffer (pH 7.5) at 85°C. K_m and k_{cat} values were estimated using Lineweaver-Burk plots.

Results and Discussion

Purification and physical properties. Results of the purification are summarized in Table 1. More than 70% pro-

Table 1. Purification of glucose isomerase from *S. chibaensis* J-59.

	Protein (mg)	Activity* (GIU)	Specific activity (GIU/mg)	Yield (%)	Purification fold (%)
Crude enzyme	1360	3237	2.38	100	1.00
Heat treatment (70, 20 min)	350	3070	8.77	95	3.68
$(NH_4)_2SO_4$ fractionation (40% 80%)	224	2142	9.56	64	4.02
DEAE-cellulose	86	1658	19.27	51	8.10
Sephacryl S-300	53	1060	20.00	33	8.40

*One unit of enzyme activity (GIU) was defined as the amount of enzyme that produced 1 of D-fructose per min under the assay conditions described in Materials and Methods.

Fig. 1. Electrophoresis profile of purified glucose isomerase through SDS-PAGE. SDS-PAGE was done on 10% polyacrylamide gels using a Tris-HCl buffer (pH 8.3). The protein was stained with 2.5% Coomassie blue. Lane 1, purified glucose isomerase; lane 2, low molecular size marker proteins (sizes in kDa).

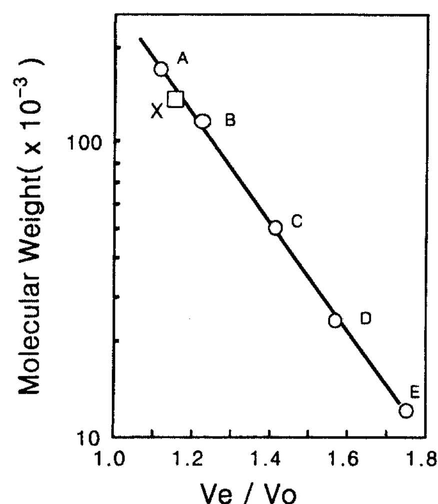

Fig. 2. Molecular weight estimation of purified glucose isomerase through gel filtration on Sephadex G-200. Standard proteins were the molecular weight markers from Sigma Co. (MW-GW-200 kit). A, β-amylase (200 kDa); B, alcohol dehydrogenase (150 kDa); C, bovine serum albumin (67 kDa); D, carbonic anhydrase (30 kDa); E, cytochrome c (12.4 kDa); X, purified glucose isomerase.

teins were removed by the heat treatment at 70°C for 20 min without loss of activity. The enzyme was purified to homogeneity from the cell extract of *S. chibaensis* J-59 via ammonium sulfate fractionation, chromatography on DEAE-cellulose, and gel filtration on Sephacryl S-300. The overall activity recovery was 33% for glucose isomerase.

Homogeneity of the purified enzyme was examined via SDS-PAGE. The purified enzyme exhibited single protein band, and the apparent molecular mass was estimated to be 180 kDa via gel filtration on Sephadex G-200 and 44 kDa via SDS-PAGE (Figs. 1 and 2). These results suggest that the enzyme has four identical subunits with the same apparent molecular mass of 44 kDa.

Molecular mass of glucose isomerase from various microbial sources were in the range of 110 to 230 kDa, and the enzyme was composed of two or four identical subunits.[19,20] *S. chibaensis* J-59 glucose isomerase was similar to the *S. flavogriseus,*[14] *S. albus,*[21] and *Bacillus coagulans*[22] enzymes, but different from those of the *S. olivochromogenes,*[23] *B. stearothermophilius,*[23] and *Candida boidinii*[24] enzymes, which have molecular mass of 130 kDa with two subunits.

N-Terminal amino acid sequences of the purified enzyme from *S. chibaensis* J-59 were determined, from which only one was observed: H_2N-Ser-Tyr-Gln- Pro-Thr-Pro-Glu-Asp-Arg-Phe-Thr-Phe-Gly-Leu. This sequence was aligned in comparison with the five other sequences of glucose isomerase (Table 2). *S. chibaensis* J-59 N-terminal amino acid sequence showed 64, 93, 86, 92, and 100% identity with glucose isomerases produced by *Amullariella* sp.,[25] *S. rubiginosus,*[26] *S. violaceoniger,*[27] *S. violaceoruber,*[19] *S. olivochromogenes,*[23] respectively. In addition, the reported sequences of the first 14 amino acid residues of glucose isomerase from *S. olivochromogenes* were identical, while the molecular mass was different from that of *S. chibaensis* J-59.

Properties of glucose isomerase. Optimum pH for glucose isomerase was determined using 100 mM acetate buffer (pH 3~5.5), 100 mM potassium phosphate buffer (pH 5~8),

Table 2. N-Terminal amino acids of glucose isomerases from various Actinomycetes and *S. chibaensis* J-59.

Strains	N-Terminal amino acid sequences	Ref.
Ampullariella sp.	Ser-Leu-Gln-Ala-The-Pro-Asp-Asp-Lys-Phe-Ser-Phe-Gly-Leu	25
S. rubiginosus	Asn-Tyr-Gln-Pr-Thr-Pro-Gln-Asp-Arg-Phe-Thr-Phe-Gly-Leu	26
S. violaceoniger	Ser-Phe-Gln-Pro-Thr-Pro-Glu-Asp-Lys-Phe-Thr-Phe-Gly-Leu	33
S. violaceoruber	Asp-Tyr-Gln-Pro-Thr-Pro-Glu-Asp-Arg-Phe-Thr-Phe	19
S. olivochromogenes	Ser-Tyr-Gln-Pro-Thr-Pro-Glu-Asp-Arg-Phe-Thr-Phe-Gly-Leu	23
S. chibaensis J-59	Ser-Tyr-Gln-Pro-Thr-Pro-Glu-Asp-Arg-Phe-Thr-Phe-Gly-Leu	This work

Amino acid residues different in sequences compared with those of *S. chibaensis* J-59 are underlined. N-Terminal amino acid sequence of *S. chibaensis* J-59 shows 64, 93, 86, 92, and 100% identity with glucose isomerases produced by *Amullariella* sp., *S. rubiginosus, S. violaceoniger, S. violaceoruber,* and *S. olivochromogenes,* respectively.

Table 3. Kinetic constants for substrate from *S. chibaensis* J-59 glucose isomerase.

Substrate	V_{max} (U/mg)	k_{cat}* (min^{-1})	K_m (mM)	k_{cat}/K_m ($mM^{-1} \cdot min^{-1}$)
Glucose	40.9	1,843	83.3	22.1
Xylose	62.9	2,836	20.2	140.3

*k_{cat} is defined as the number of substrate molecules reacted per active site per min. The reactions were carried out in the presence of 10 mM Mg^{2+} at 70°C for 30 min.

Table 4. Effect of metal ions on the activity of the purified glucose isomerase.

Metal ion (10 mM)	Relative activity (%)
None	100*
$MgSO_4$	126
$CoCl_2$	121
$FeSO_4$	100
$MnCl_2$	100
$BaCl_2$	100
$CaCl_2$	100
$HgCl_2$	11
$CuSO_4$	11
$AgNO_3$	37

*Activity in the absence of metal ions was referred to 100%.

and 100 mM glycine-NaCl-NaOH buffer (pH 8~12) under the assay conditions. The enzyme was most active at pH 7.5. More than 95% of the maximal activity appeared at the pH value of 7.0 and 8.0. About 80% of the maximal activity was maintained at the broad pH range from 6.0 to 9.0. When the enzyme was incubated at various pH values at 70 for 1 h in the absence of substrate, and the residual activity measured, the enzyme was found to be stable at pH 7.5, but showed a 5% loss in activity at the pH value of 6.5 and 8.5. At pH values of 5.5 and 9.5, the enzyme retained 70°C to 80% of its activity (data not shown).

The purified enzyme was able to isomerize D-xylose, D-glucose, and D-ribose, but showed no affinity for L-arabinose, D-galactose, D-mannose, L-rhamnose, and D-allose.[28] Affinity for D-xylose was much higher than those for D-glucose and D-ribose. Substrate specificity of the enzyme was similar to those of enzymes from *S. griseofuscus,*[29] *Lactobacillus xylosus,*[30] and *B. coagulans*[22] in which ketose were formed from D-glucose, D-xylose, and D-ribose.

The K_m, V_{max}, and k_{cat} values of *S. chibaensis* J-59 isomerase for glucose were 83 mM, 40.9 U/mg, and 1,843 min^{-1}, respectively. The K_m, V_{max}, and k_{cat} values of the isomerase for xylose were 20 mM, 62.9 U/mg, and 2,836 min^{-1}, respectively (Table 3). The catalytic efficiency (k_{cat}/K_m) for the isomerization of glucose and xylose was higher than those of other glucose isomerases.[31] Kinetic data of the enzyme for glucose was very similar to those of *S. albus*[21] but different from those of other *Streptomyces* spp.

Most glucose isomerase requires bivalent cation for activity. Although, in general, glucose isomerase from *Streptomyces* spp. requires Mg^{2+} and Co^{2+}, that of *S. chibaensis* J-59 typically requires Mg^{2+}, Co^{2+}, and Mn^{2+} (Table 4). Affinity of the enzyme for glucose was remarkably increased by the addition of Mg^{2+} and Co^{2+}. V_{max} and catalytic efficiency (k_{cat}/K_m) were also increased two- and tenfold by the addition of Mg^{2+}, respectively (data not shown). Requirements for metal ions for enzyme activity were similar to those of enzymes produced by *S. griseofuscus,*[29] *S. phaeochromogenus,*[2] and *S. albus.*[21] In addition, the enzyme was completely inhibited by Hg^{2+}, Ag^{+}, and Cu^{2+}. On the other hand, Ca^{2+}, required in the hydrolysis of corn starch by amylase, did not inhibit the isomerase (Table 4).

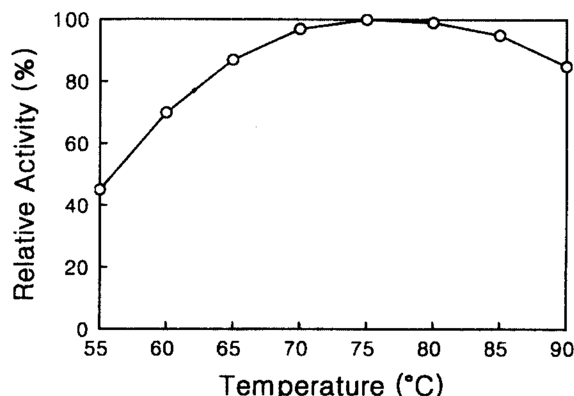

Fig. 3. Optimal temperature of the purified glucose isomerase. The enzyme activity was measured in the standard reaction mixture at the indicated temperature for 30 min at pH 7.5.

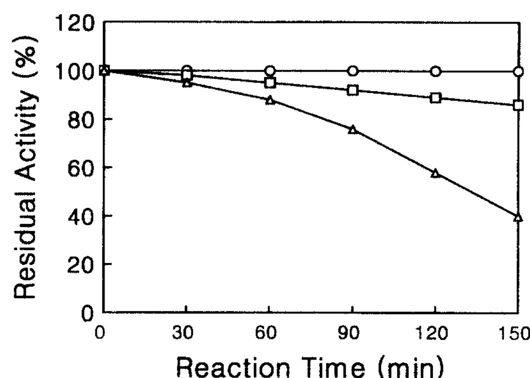

Fig. 4. Thermostability of glucose isomerase in the presence of Co^{2+} at 70°C (○), 85°C (□), and 90°C (△) for 150 min.

Thermostability of *S. chibaensis* J-59 glucose isomerase. Optimum temperature for the glucose isomerase activity was 75°C (Fig. 3). At 65 and 85°C, the enzyme exhibited approximately 90 and 95% of the maximal activity, respectively. These properties of the purified enzyme were similar to the glucose isomerases from *S. flavogriseus*[14] and *S. griseofuscus.*[29]

Table 5. Comparison of thermostability with *Thermotoga neapolitana*, *Streptomyces* sp. (PLC strain), and *S. chibaensis* J-59 glucose isomerase.

Strains \ Temperature (°C)	Thermostability of glucose isomerase [Half time ($T_{1/2}$) in the presence of Co^{2+}]			Ref.
	70°C	85°C	90°C	
Thermotoga neapolitana	-	83 min	64 min	31
Streptomyces sp. (PLC strain)	4.5 days (in presence of Mn^{2+})	-	-	32
S. chibaensis J-59	> 7 days*	810 min	126 min	This work

*After heat treatment at 70°C for 7 days, the residual activity was 100%. -, data not available.

Thermostability was examined by incubating the enzyme solution (1 unit/ml) at pH 7.5 in the presence of 2.5 mM $CoCl_2$ at various temperatures for 150 min and measuring the residual activity. The enzyme retained 100% of its activity at 70°C and rapidly lost its activity above 90°C. The thermostability of *S. chibaensis* J-59 glucose isomerase in the presence of Co^{2+} at 70, 85, and 90 is shown in Fig. 4. The enzyme was highly thermostable, so it was not denatured via heat-treatment for 2 weeks at 70°C in a buffer containing Co^{2+}, and approximately 50% of the activity was retained upon the heat-treatment at 90°C for 126 min. The enzyme of *S. chibaensis* J-59 was more thermostable than those of *Thermotoga neapolitana,*[31] *S. olivochromogenes,*[23] and *Streptomyces* sp. PLC strain,[32] one of the thermostable glucose isomerase producer in *Streptomyces* species (Table 5). Cobalt ions considerably increased the thermostability of the glucose isomerase from *Streptomyces,*[2,23] whereas this metal ion had no effect on the heat stability of the *S. violaceoniger* enzyme.[33] The ideal glucose isomerase should possess a low pH optimum, a higher temperature optimum, a resistance to inhibition by Ca^{2+}, and a higher affinity for glucose than the presently used enzymes. Introduction of all these properties into a single protein is a Herculean task, which has been an obstacle in the development of an economically feasible commercial process for enzymatic isomerization of glucose into fructose.[3,8,31]

In this paper, several advantages of *S. chibaensis* J-59 glucose isomerase were emphasized for biotechnological application. Furthermore, the glucose isomerase from *S. chibaensis* J-59 is remarkably stable and resistant to high temperature. Since the equilibrium between glucose and fructose shifts more toward fructose with increasing temperature, larger fructose yields can be obtained when the reaction temperature is enhanced, as is possible with the thermostable enzyme.

Acknowledgments. This work was supported by a grant from the Korea Science and Engineering Foundation (No. 951-0605-071-2).

References

1. Jensen, V. J. and Rugh, S. (1987) Industrial scale application of immobilized glucose isomerase. *Methods Enzymol.* **136**, 356-370.
2. Tsumura, N., Hagi, M. and Sato, T. (1965) Enzymatic conversion of D-glucose to D-fructose. Part II. Properties of the enzyme from *Streptomyces phaeochromogenes. Agric. Biol. Chem.* **29**, 1129-1134.
3. Bhosale, S. H., Rao, M. B. and Deshpande, V. V. (1996) Molecular and industrial aspects of glucose isomerase. *Microbiol. Rev.* **60**, 280-300.
4. Lehmacher, A. and Bisswanger, H. (1990) Isolation and characterization of an extremely thermostable D-xylose isomerase from *Thermus aquaticus* HB8. *J. Gen. Microbiol.* **136**, 697-686.
5. Jergensen, U. B., Karlsen, L. G., Nielsen, N. B., Pedersen, S. and Rugh, S. (1988) A new immobilized glucose isomerase with high productivity produced by a strain of *Streptomyces murinus. Starch* **40**, 307-313.
6. Gong, C. S., Chen, L. F. and Tsao, G. T. (1980) Purification and properties of glucose isomerase of *Actinoplnes missourienis. Biotechnol. Bioeng.* **22**, 833-845.
7. Wilhelm, M. and Hollenberg, C. P. (1985) Nucleotide sequence of the *Bacillus subtilis* xylose isomerase gene: extensive homology between the *Bacillus* and *Escherichia coli* enzyme. *Nucleic Acids Res.* **13**, 5717-5722.
8. Zeikus, J. G. (1995) Molecular determinants of thermoenzyme activity and stability: Analysis of xylose isomerase and amylopullulanase. In *Enzymes for Carbohydrate Engineering II, 95' Agricultural Biotechology Symposium*, pp. 29-51, The Research Center for New Bio-Materials in Agriculture, Korea.
9. Joo, G.-J., Kwon, G.-S. and Rhee, I.-K. (1997) Isolation and identification of a *Streptomyces* sp. J-59 producing thermostable glucose isomerase. *Kor. J. Appl. Microbiol. Biotechnol.* **25**, 15-22.
10. Joo, G.-J. and Rhee, I.-K. (1997) Production of glucose isomerase from *xylB* mutant *Streptomyces chibaensis* J-59. *Kor. J. Appl. Microbiol. Biotechnol.* **25**, 75-81.
11. Takasaki, Y. (1966) Studies on sugar isomerization enzyme. Production and utilization of glucose isomerase from *Streptomyces* spp. *Agric. Biol. Chem.* **30**, 1247-1253.
12. Bradford, M. M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding. *Anal. Biochem.* **72**, 248-254.
13. Chen, W. P., Anderson, A. W. and Han, Y. W. (1979)

Extraction of glucose isomerase from *Streptomyces flavogriseus. Appl. Environ. Microbiol.* **37**, 785-787.
14. Chen, W. P., Anderson, A. W. and Han, Y. W. (1979) Production of glucose isomerase by *Streptomyces flavogriseus. Appl. Environ. Microbiol.* **37**, 324-331.
15. Laemmi, U. K. (1970) Cleavages of structural proteins during the assembly of the head of bacteriophage 74. *Nature* **227**, 680-685.
16. Davis, B. J. (1964) Disc gel electrophoresis, II. Method and application to human serum protein. *Am. N. Y. Acad. Si.* **121**, 404-427.
17. Andrews, P. (1964) Estimation of the molecular weights of proteins by Sephadex gel filtration. *Biochem. J.* **91**, 222-234.
18. Edman, P. and Begg, G. (1969) A protein sequenator. *Eur. J. Biochem.* **1**, 80-91.
19. Callens, M., Kersters-Hilderson, H., Vandekerckhore, van Opstal, O. and De Bruyne, C. K. (1985) Purification and some physiochemical properties of D-xylose isomerase from *Streptomyces violaceoruber. Biochem. Intl.* **11**, 467-475.
20. Yamanaka, K. (1968) Purification, crystallization and properties of the D-xylose isomerase from *Lactobacillus brevis. Biochim. Biophys. Acta.* **151**, 670-680.
21. Takasaki, Y., Kosugi, Y. and Kanbayashi, A. (1969) *Streptomyces* glucose isomerase. In *Fermentation Advances*, Perlman, D. (ed.) pp. 561-589, Academic Press, New York.
22. Danno, G. (1970) Studies on D-glucose isomerizing enzyme from *Bacillus coagulans* strain HN-68. Part IV. Purification, crystallization and some physiochemical properties. *Agric. Biol. Chem.* **34**, 1795-1804.
23. Suekane, M., Tamura, M. C. and Tomomura, J. (1978) Physicochemical and enzymatic properties of purified glucose isomerase from *Streptomyces olivochromogenes* and *Bacillus stearothermophilis. Agric. Biol. Chem.* **42**, 909-917.
24. Vongsuvanlert, V. and Tani, Y. (1988) Purification and characterization of xylose isomerase of a methanol yeast, *Candida boidinii*, which is involved in sorbitol production from glucose. *Agric Biol. Chem.* **52**, 1817-1824.
25. Saari, G. C., Kumar, A. A., Kawasaki, G. H., Insley, M. Y. and O'Hara, P. J. (1987) Sequencing of *Ampullariella* sp. strain 3876 gene coding for xylose isomerase. *J. Bacteriol.* **169**, 612-618.
26. Wong, H. C., Ting, Y., Lin, H. C., Reichert, F., Myambo, K., Watt, K. W., Toy, P. L. and Drummond, R. J. (1991) Genetic organization and regulation of the xylose degradation genes in *Streptomyces rubiginosus. J. Bacteriol.* **173**, 6849-6858.
27. Drocourt, D., Bejar, S., Calmels, T., Reynes, J. P. and Tiraby, G. (1988) Nucleotide sequence of the xylose isomerase gene from *Streptomyces violaceoniger. Nucleic Acids Res.* **16**, 9337.
28. Joo, G.-J. (1996) Production of thermostable D-glucose isomerase and analysis of *xylA* gene in *Streptomyces chibaensis* J-59. Ph. D. Thesis, Kyungpook National University, Daegu, Korea.
29. Kasumi, T., Hayashi, K. and Tsumura. N. (1982) Role of cobalt in stabilizing the molecular structure of glucose isomerase from *Streptomyces griseofuscus* S-41. *Agric. Biol. Chem.* **46**, 2130
30. Yamanaka, K. and Takahara, N. (1977) Purification and properties of D-xylose isomerase from *Lactobacillus xylosus. Agric. Biol. Chem.* **41**, 1909-1915.
31. Vieille, C., Hess, J. M., Kelly, R. M. and Zeikus, J. G. (1995) *xyl*A cloning, sequencing and biochemical characterization of xylose isomerase from *Thermotoga neapolitana. Appl. Environ Microbiol.* **61**, 1867-1875.
32. Inyang, C. U., Gebhart, U., Obi, K. C. and Bisswanger, H. (1995) Isolation and characterization of D-glucose/xylose isomerase from a new thermophilic strain *Streptomyces* sp. (PLC). *Appl. Microbiol. Biotcehnol.* **43**, 632-638.
33. Marcel, T., Drocourt, D. and Tiraby, G. (1987) Cloning of the glucose isomerase (xylose isomerase) and xylulose kinase gene of *Streptomyces violaceoniger. Mol. Gen. Genet.* **208**, 121-126.