Journal of Life Science **2004**, Vol. 14. No. 4. 636~643 ©JLS

Purification and Properties of D-Xylose Isomerase from *Lactococcus* sp. JK-8

Jun, Hong-Ki*, Suk-Young Kim and Hyung-Suk Baik

Division of Biological Science, Pusan National University, Busan 609-735, Korea

Received July 24, 2004 / Accepted August 8, 2004

D-Xylose isomerase produced by *Lactococcus* sp. JK-8, isolated from kimchi, was purified 17-fold of homogeneity, and its physicochemical properties were determined. Although the N-terminal amino acid sequence of D-xylose isomerase was analysed to Ala-Tyr-Phe-Asn-Asp-Ile-Ala-Pro-Ile-Lys, it was not similar to that of *Lactobacillus* enzyme. The molecular weight of the purified enzyme was estimated to be 180 kDa by gel filtration, 45 kDa by SDS-PAGE and the enzyme was homotetramer. The optimum pH of the enzyme was around 7 and stable between pH 6 and 8. The optimum reaction temperature was 70℃ and stable up to 70℃ in the presence of 1 mM Mn^{2+}. Like other D-xylose isomerases, this enzyme required divalent cation, such as Mg^{2+}, Co^{2+}, or Mn^{2+} for the activity and thermostability. Mn^{2+} was the best activator. Substrate specificity studies showed that this enzyme was highly active on D-xylose. The enzyme had an isoelectric point of 4.8, and K_m values for D-xylose was 5.9 mM.

Key words – D-xylose isomerase, *Lactococcus* sp. JK-8, kimchi

D-Xylose isomerase (D-xylose ketol-isomerase, EC 5.3.1.5), commonly referred to as glucose isomerase, catalyzes the conversion of D-glucose to D-fructose and D-xylose to D-xylulose. Both reactions are industrial importance because the former being involved in the production of high-fructose corn syrup (HFCS) and the latter playing a key role in biomass utilization. For these applications, the immobilized enzyme or whole cell preparation are used (1,3).

D-Xylose isomerase generally requires divalent metal cations such as Mg^{2+}, Mn^{2+}, or Co^{2+} for activity and stability (3). For homologues of this enzyme from *Actinomycetaceas* such as *Streptomyces* (2,6), *Arthrobacter* (15), Mg^{2+} is most effective for these activities. In the case of other D-xylose isomerase, Co^{2+} and Mn^{2+} is more effective to isomerize D-glucose and D-xylose respectively (7-9,11,17). Homologues of D-xylose isomerase have been found in various microorganisms. D-xylose isomerses from *Streptomyces* species have been widely studied and used for commercial HFCS processes due to their high activities and thermostabilities. Among the lactic acid bacteria, *Lactobacillus brevis* produced the highest yield of D-xylose isomerase, but this enzyme could not be used commercially because of its low thermostability (1,3,5).

However, enzymatic and physicochemical properties of the D-xylose isomerase from *Lactococcus* species among the lactic acid bacteria have not yet been reported (14). In the present paper, we describe the purification and several properties of D-xylose isomerase from *Lactococcus sp.* JK-8. This strain was isolated from Kimchi in our laboratory.

***Corresponding author**
Tel : +82-51-510-2270, Fax : +82-51-513-4532
E-mail : hkjun@pusan.ac.kr

Materials and Methods

Materials

D-Xylose was obtained from Junsei Chemical Co. D-Xylitol, carbazole, cysteine hydrochloride, DEAE-cellulose, Sephacryl S-200HR, and hydroxyapatite were purchased from Sigma Chemical Co. DEAE-Sephadex A-50 was produced from Pharmacia Fine Chemicals. All other chemicals were used to analytical grade.

Microorganism and medium

Lactococcus sp. JK-8 was isolated from Kimchi. The bacterial cells were grown in cultivation medium containing 1.5% D-xylose, 1.0% tryptone, 0.5% yeast extract, 0.1% K_2HPO_4, 0.04% $MgSO_4 \cdot 7H_2O$, 0.014% $MnSO_4 \cdot 4H_2O$, 0.004% $FeSO_4 \cdot 7H_2O$ (pH 7.0) for 28 hr at 30℃ in static culture. D-Xylose was autoclaved separately and added just before inoculation.

Determination of protein cincentration and enzyme assay

Preparated protein concentration was determined by the method of Lowry (13), usingbovine serum albumin as the

standard. The protein concentration of column fractions was determined by measuring the absorbance at 280 nM. The specific activity of the enzyme was expressed as units per mg of protein. For assay of D-xylose isomerase activity, a reaction mixture (1 ml) contained 5 mM D-xylose, 1 mM $MnCl_2 \cdot 4H_2O$ and a enzyme preparation in 50 mM potassium phosphate buffer (pH 7.0). After 10 min incubation at 37℃, 0.05 ml of 50% trichloroacetic acid was added to stop the reaction. The amount of D-xylulose produced was determined by the cysteine/carbazole method (4). One unit of the enzyme activity was defined as the amount of the enzyme that produced 1.0 mol of D-xylulose per minute under the assay conditions.

Preparation of crude enzyme

All treatments were carried out at 4℃ unless otherwise stated. Incubated cells (35 g, wet weight, obtained from 12 liter medium) were harvested by centrifugation at 10,000× *g* for 15 min, and washed twice with cold 50 mM potassium buffer (pH 7.0). The washed cells were suspended in the same buffer, and sonicated in an ice bath. The cell debris was removed by centrifugation at 20,000×*g* for 30 min. Unbroken cells were resuspended in the same buffer and the sonication repeated in the same manner. The supernatants obtained ware used as a crude extract. The crude extract was added by dropwise 5% to final 5% concentration with 1 M $MnCl_2 \cdot 4H_2O$. Value of pH was maintained at 7.0 with 1 N NaOH. After standing for 30 min, the precipitate was removed by centrifugation at 20,000×*g* for 30 min. The $MnCl_2$ treated supernatant was heated for 10 min at 65℃, and cooled to 4℃. The soluble fraction was recovered after centrifugation at 20,000×*g* for 30 min. Solid $(NH_4)_2SO_4$ was added to the heat-treated extract to give 35 % saturation, and its solution was removed to be precipitated by centrifugation at 20,000×*g* for 30 min. More $(NH_4)_2SO_4$ was added to the supernatant to give 80% saturation. This solution was centrifuged a precipitate collected, dissolved in 50 mM potassium phosphate buffer (pH 7.0), and dialyzed overnight against the same buffer.

Column chromatography

The crude enzyme was applied to a DEAE-cellulose column, equilibrated with 50 mM potassium phosphate buffer (pH 7.0). The column was washed with the same buffer and eluted with a liner KCl salt gradient (0~0.6 M) in the same buffer. The activity-peak fractions were pooled and concentrated by ultrafiltration (ZM 50 membrane ; Amicon Co.) The concentrated enzyme was applied to a DEAE-Sephadex A-50 column, previously equilibrated with 50 mM potassium phosphate buffer (pH 7.0). The column was washed with the same buffer and eluted with a liner KCl salt gradient (0~1.0 M) in the same buffer. The fractions containing enzyme activity were concentrated by ultrafiltraion and further purified by gel filtration on a column of Sephacryl S-200HR that had been equilibrated in 50 mM potassium phosphate buffer (pH 7.0). The activity-peak fractions were pooled and concentrated by ultrafiltration. The concentrated enzyme was dialyzed against 1 mM potassium phosphate buffer (pH 7.0). The dialyzed solution was applied to a hydroxyapatite column, equilibrated with 1 mM potassium phosphate buffer (pH 7.0). The enzyme was eluted stepwise with 8, 20, 40 mM potassium phosphate buffer (pH 7.0).

Electrophoresis

SDS-PAGE was performed as described by Laemmli (10). Native PAGE was performed without SDS and Tris/HCl buffer (pH 8.8) was used during polyacrylamide gel preparation. Isoelectric focusing was done with a 7.5% polyacrylamide gel containing 2.4% Ampholine (pH 3.0~10.0) for 5 hr at a constant voltage of 200 V. Protein bands were deterimined by Coomassie Brilliant Blue G-250 staining.

Determination of molecular weight

The molecular weight of D-xylose isomerase was determined by gel filtration on a Sephacryl S-200HR column, and Blue dextran (M.W. 2,000 kDa), β-amylase from sweet potato (M.W. 200 kDa), alcohol dehydrogenase from yeast (M.W. 150 kDa), and bovine serum albumin (M.W. 66 kDa) were used as standard proteins. The subunit molecular weight was estimated by SDS-PAGE using 10% polyacrylamide gel. β-Galactosidase (M.W. 116 kDa), phosphorylase (M.W. 97 kDa), fructose-6-phosphate (M.W. 84 kDa), bovine serum albumin (M.W. 66 kDa), glutamic dehydrogenase (M.W. 55 kDa), ovalbumin (M.W. 45 kDa), and glyceraldehyde-3-phosphate dehydrogenase (M.W. 36 kDa) were used as standard markers (Sigma Chemical Co.).

Analysis of N-terminal amino acid sequence

The N-terminal sequence of D-xylose isomerase was determined by automated Edman degradation with a Miligen/Biosearch 6600 Prosequencer system (Millipore, Milford, U.S.A.).

Results and Discussions

Purification of D-xylose isomerase from *Lactococcus* sp. JK-8

The purification of the enzyme is summarized in Table 1. The enzyme was purified from the culture cells of *Lactococcus* sp. JK-8 by $MnCl_2$ treatment, heat treatment, $(NH_4)_2SO_4$ fractionation, DEAE-Cellulose, DEAE-Sephadex A-50, Sephacryl S-200HR, and hydroxyapatite column chromatographies. The elution patterns on the DEAE-cellulose, DEAE-Sephadex A-50, and Sephacryl S-200HR column chromatographies are shown in Fig. 1. A, B, C,

Table 1. Purification of D-xylose isomerase from *Lactococcus* sp. JK-8

Step	Total activity (units)	Total protein (mg)	Specific activity (units/mg)	Yield (%)
Cell-free extract	196,907	2,661	74	100
$MnCl_2$ treatment	191,150	1,916	100	97
Heat treatment (65℃, 10 min)	186,682	1,294	144	95
$(NH_4)_2SO_4$ fractionation (35~80%)	174,193	387	450	88
DEAE-cellulose	103,631	155	669	52
DEAE-Sephadex A-50	74,558	92	810	38
Sephacryl S-200HR	36,529	37	987	19
Hydroxyapatite	15,469	12	1,289	8

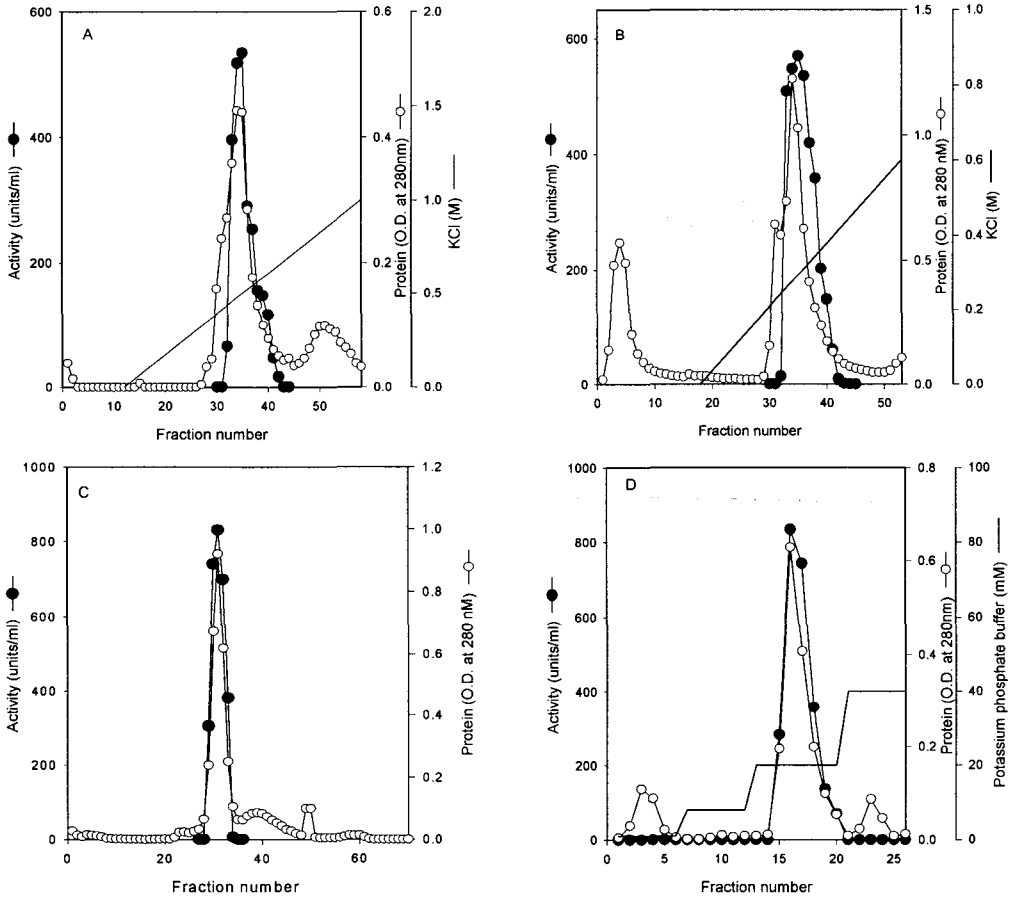

Fig. 1. Purification of D-xylose isomerase produced by *Lactococcus* sp. JK-8 using various chromatographic methods.
(A) DEAE-cellulose column chromatography.
(B) DEAE-Sephadex A-50 column chromatography.
(C) Sephacryl S-200 HR column chromatography.
(D) Hydroxyapatite column chromatography.

respectively. The elution profile on the final hydroxyapatite column chromatography is shown in Fig. 1.D, and the enzyme protein was eluted with 20 mM potassium phosphate buffer (pH 7.0). The purified enzyme was considered to be homogeneous by the detection of a single band on SDS/PAGE and native PAGE (Fig. 2). By these procedures, the enzyme was purified by 17-fold to compare cell-free extract with a yield of 8%.

Comparison of N-terminal amino acid sequence

The N-terminal sequence up to 10 residues was analysed by automated Edman degradation of the enzyme. The N-terminal sequence of the residues from *Lactococcus* sp. JK-8 enzyme (Ala-Tyr-Phe-Asn-Asp-Ile-Ala-Pro-Ile-Lys) is identical with that of *Lactococcus lactis* subsp. *lactis* (NCBI data), but it is different from those of *Lactobacillus brevis* (Met-Thr-Glu-Glu-Tyr-Trp-Lys-Gly-Val-Asp-Lys ; (1) or *Lactobacillus xylosus* (Met-Thr-Asn-Glu-Tyr-Trp-Gln-Gly-Val-Asp-Gln ; (1).

Determination of molecular weight and Isoelectric point

The molecular weights of D-xylose isomerases from various microbial sources were reported to varying in the range of 52 to 191 kDa and the enzymes to be composed of two or four identical subunits (1). The molecular weight of D-xylose isomerase from *Lactococcus* sp. JK-8 was determined by gel filtration on Sephacryl S- 200HR to 180 kDa (Fig. 3). SDS-PAGE analysis showed a single band for the enzyme with the molecular weight of 45 kDa, indicating that the enzyme was composed of homotetrameric subunits (Fig. 2). This value is very closed to those of *Lactobacillus brevis* (191 kDa with 4 subunits of 47 kDa) (16) and *Lactobacillus xylosus* (183 kDa with 4 subunits of 45 kDa) (17).

The isoelectric point of the enzyme was about 4.8 by isoelectric focusing, which is similar to those of the thermostable enzyme from *Thermus aquaticus* (pI 4.4) (12), *Clostridium thermosulfurogenes* (pI 4.9) (11), and *Thermoanaerobacter* (pI 4.8) (11).

Effect of pH on activity and stability

The optimum pH for D-xylose isomerase was determined by using acetate buffer (pH 3 to 5), potassium phosphate buffer (pH 5 to 8), glycine-NaOH buffer (pH 8 to 10), and Na_2HPO_4-NaOH buffer (pH 10 to 12) under the standard assay conditions. As shown in Fig. 4. A, the enzyme was the highest active at pH 7.0. The effects of pH stability of the enzyme are shown in Fig. 4. B. After incubation at various pH values at 30℃ for 1 hr, the residual activity was assayed at pH 7.0. The enzyme was stable between pH 6.0 and 8.0. Although, D-xylose isomerase is generally stable over a wide pH range of 4.0 to 11.0 (2, 11, 18), that from *Lactococcus* sp. JK-8 was stable in only a narrow pH range.

Effect of temperature on activity and stability

The optimum temperature was found to approximately

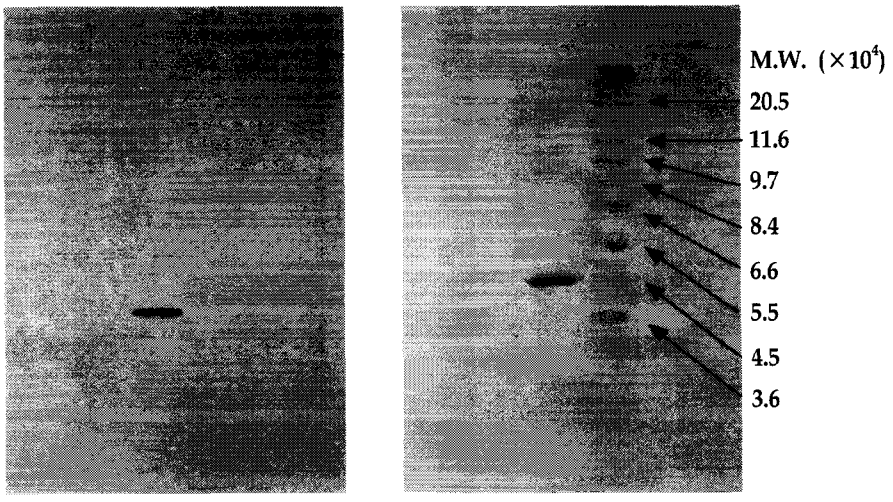

Fig. 2. Electrophoretic analysis of the purified D-xylose isomerase from *Lactococcus* sp. JK-8.
(A) Native PAGE (7% gel), (B) SDS-PAGE (10% gel)

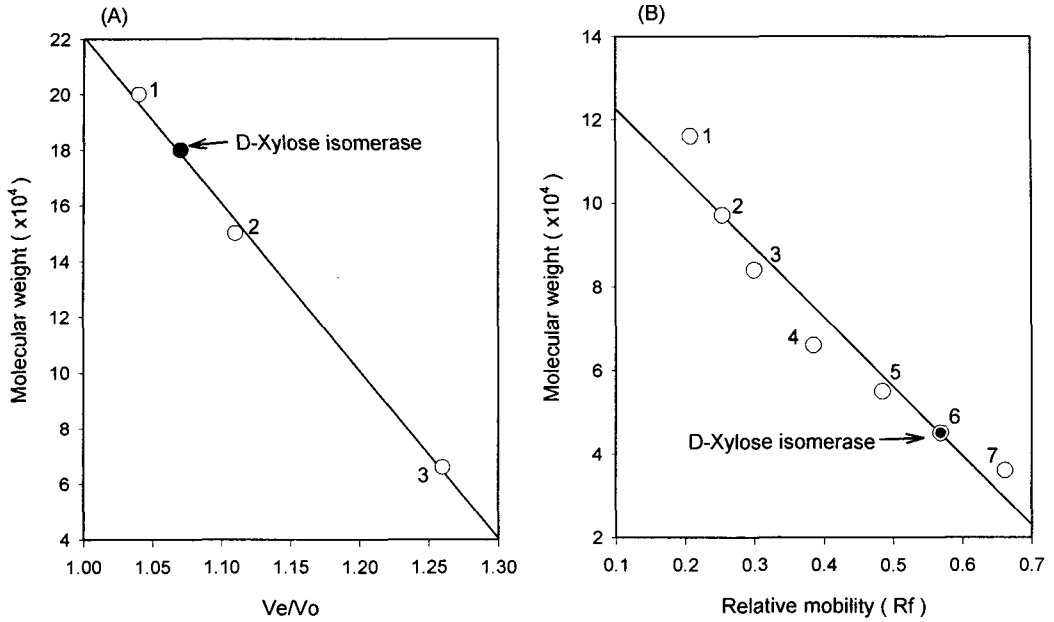

Fig. 3. Estimation of molecular weight of D-xylose isomerase from *Lactococcus* sp. JK-8 by Sephacryl S-200HR gel filtration (A) and SDS-PAGE (B).
(A) : 1, β-amylase from sweet potato (M.W. 200 kDa); 2, alcohol dehydrogenase (M.W. 150 kDa); 3, bovine serum albumin (M.W. 66 kDa); Vo (Void volume), Blue dextran (M.W. 2,000 kDa) elution volume; Ve (Elution volume), sample elution volume.
(B) : 1, β-galactosidase (M.W. 116 kDa); 2, phosphorylase (M.W. 97 kDa); 3, fructose-6-phosphate (M.W. 84 kDa); 4, bovine serum albumin (M.W. 66 kDa); 5, glutamic dehydrogenase (M.W. 55 kDa); 6, ovalbumin (M.W. 45 kDa); 7, Glyceraldehyde-3-phosphate dehydrogenase (M.W. 36 kDa).

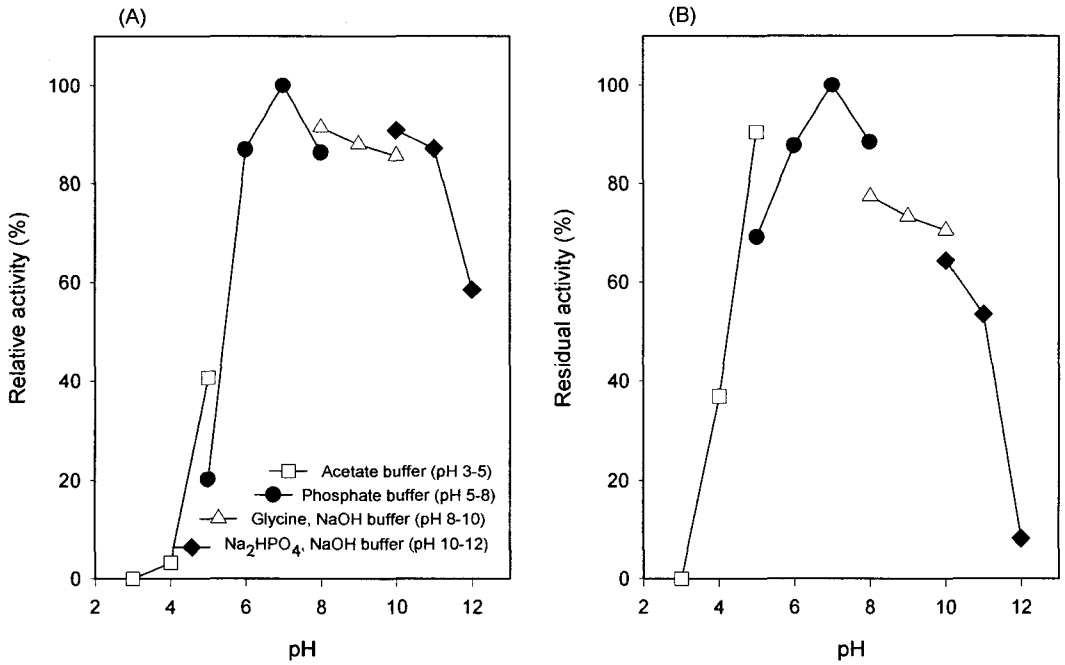

Fig. 4. Effect of pH on D-xylose isomerase activity (A) and stability (B).
(A) The enzyme activity was measured in the standard reaction mixture at indicated pH for 10 min at 37℃.
(B) The residual activity was measured. After the enzyme was incubated at 30℃ for 1 hr in each buffer.

70℃ under the standard assay conditions (Fig 5. A) while the temperature was varied over a range of 20℃ to 80℃ (Fig 5. B). Thermostability was examined by incubating the enzyme solution at pH 7.0 in the presence of 1 mM $MnCl_2$

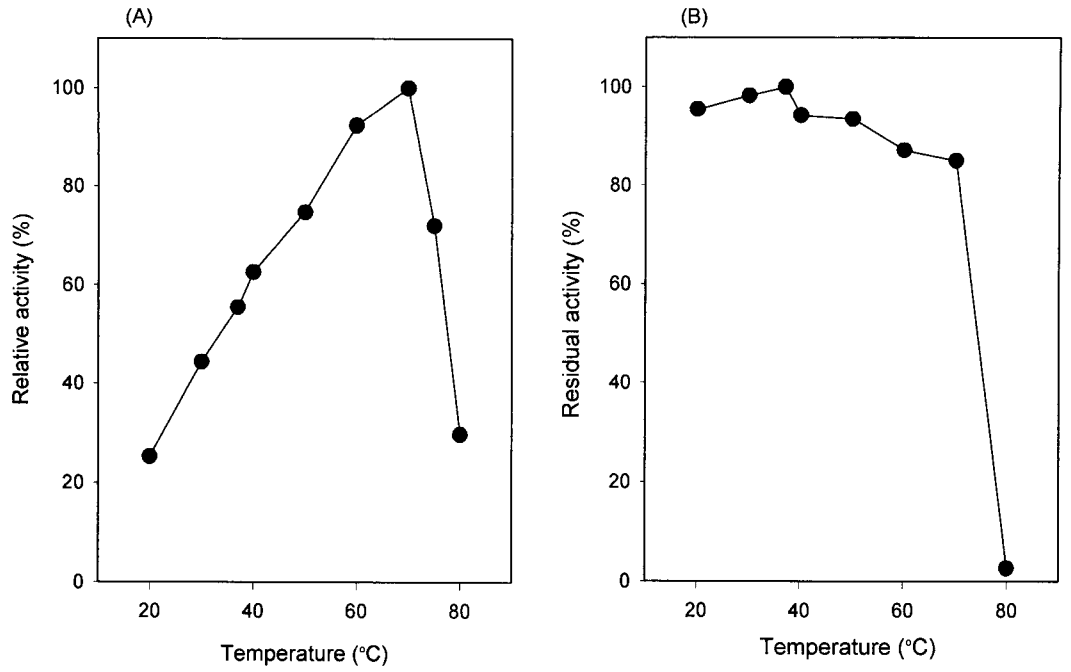

Fig. 5. Effect of temperature on D-xylose isomerase activity (A) and stability (B).
(A) The enzyme activity was measured in the standard reaction mixture at indicated temperature for 10 min at pH 7.0.
(B) The enzyme was incubated at pH 7.0 with 1 mM $MnCl_2$ at various temperature for 30 min. The residual activity was assayed with 0.1 ml of heat-treated enzyme.

at various temperatures for 30 min and measuring the residual activity. As shown in Fig. 5. B, the enzyme was stable up to 70℃ and completely inactivated at 80℃. Thermostability of *Lactococcus* sp. JK-8 enzyme was higher than that of *Lactobacillus* enzymes (3,16,17).

Substrate specificity

The substrate specificity of D-xylose isomerase is shown in Table 2. The enzyme had the highest activity to D-xylose, but showed no activity to L-arabinose, D-galactose, D-glucose, D-ribose, D-maltose, or D-mannose under the standard assay conditions. However, the enzyme showed a little activity for D-glucose under unusual conditons such as a high concentration of D-glucose and high reaction temperature (data not shown). The K_m of the enzyme for D-xylose was determined for D-xylose from Lineweaver-Burk plot, and calculated to be 5.9 mM (data not shown). The K_m of the enzyme from *Lactococcus* sp. JK-8 was very close to that of *Lactobacillus* enzymes (*Lactobacillus brevis* : 5.0 mM, *Latobacillus xylosus* : 5.3 mM (16,17).

Effect of metal ions

D-Xylose isomerase requires divalent metal cations such as Mg^{2+}, Mn^{2+}, and Co^{2+} for activity and stability (3). The effects of metal ions on isomerizing activity of *Lactococcus* sp. JK-8 were shown in Table 3. The enzyme activity was stimulated by the addition of 1 mM Mn^{2+}, Co^{2+}, or Mg^{2+}. When the enzyme was treated with EDTA, it showed no activity in the absence of metal ions. However the activity was restored by the addition of Mn^{2+}, Co^{2+}, or Mg^{2+}. Mn^{2+} was the best activator. The activity of enzyme was inhibited by Cu^{2+} and Fe^{2+}.

Table 2. Substrate specificity of D-xylose isomerase from *Lactococcus* sp. JK-8

Substrate (10 mM)	Relative activity (%)
D-xylose	100
L-arbinose	0
D-galactose	0
D-glucose	0
D-maltose	0
D-mannose	0
D-ribose	0
D-glucose (200 mM)	Trace

The enzyme (about 9 μg of protein) was used for each assay, except for D-glucose (about 168 of protein).
The enzyme activity was measured in standard assay conditions for 10 min at 37℃.
The reaction mixture was incubated at 55℃ for 30 min. Color was developed at 55℃ for 30 min and measured at 560 nM.

Table 3. Effects of metals ions on the activity of D-xylose isomerase from *Lactococcus* sp JK-8

Metal ions (1 mM)	Relative activity (%)	
	Dialysis without EDTA	Dialysis with EDTA
None	100	0
$MnCl_2$	584	575
$MgCl_2$	403	225
$CoCl_2$	457	419
$CuSO_4$	16	0
$CaCl_2$	84	0
$FeSO_4$	49	0
$BaCl_2$	124	19
$ZnCl_2$	99	25
$NiCl_2$	81	0
$CdCl_2$	109	0

The enzyme was dialyzed against 50 mM potassium phosphate buffer (pH 7.0) containing 20 mM EDTA for 12 hr at 4℃, and then dialyzed against the same buffer without EDTA for 12 hr at 4℃. The enzyme activity was measured in the standard reaction mixture in the presence of 1 mM metal ion for 10 min at 37℃.

Table 4. Effects of known inhibitors to the activity of D-xylose isomerase from *Lactococcus* sp. JK-8

Reagents	Concentration (mM)	Relative activity (%)
None		100
Ammonium persulfate	10	89
L-Cysteine hydrochloride	10	102
N-Ethylmaleimide	10	90
β-Mercaptoethanol	10	67
SDS	10	91
NaN_3	10	84
Na_2HAsO_4	10	85
NaF	10	95
Sodium thioglycolate	10	111
Pentachlorophenol	10	102
1,10-Phenanthroline	10	47
Potassium permanganate	10	0
PMSF	10	94
Tris	10	23
Xylitol	5	70
Mannitol	5	80
Ribitol	5	84
Sorbitol	5	81
Inositol	5	95

The reaction was performed after preincubation of the enzyme solution with various inhibitors at 37℃ for 5 min.

Effects of known inhibitors for D-xylose isomerase activity

The enzyme solution was mixed with 50 mM potassium phosphate buffer (pH 7.0) containing a test reagent and preincubated at 37℃ for 5 min. Then the substrate (D-xylose) was added. As shown in Table 4, the enzyme was inhibited by 1,10-phenanthroline, potassium permanganate, Tris, and xylitol. Unlike D-xylose isomerase of *Lactobacillus brevis* (16), *Lactobacillus xylosus* (17), the enzyme of *Lactococcus* sp. JK-8 was inhibited by ribitol.

References

1. Bhosale, S. H., M. B. Rao and V. V. Deshpande. 1996. Molecular and industrial aspects of glucose isomerase. *Microbiol. Rev.* **60**, 280-300.
2. Callens, M., H. Kersters-Hilderson, O. V. Opstal and C. K. De Bruyne. 1986. Catalytic properties of D-xylose isomerase from *Streptomyces violaceoruber*. *Enzyme Microb. Technol.* **8**, 696-700.
3. Chen, W. P. 1980. Glucose isomerase. *Process Biochem.* **15**, 30-35, 36-41.
4. 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.
5. Erlandson, K. A., J. H. Park, W. El Khal, H. H. Kao, P. Basaran, S. Brydges and C. A. Batt. 2000. Dissolution of xylose metabolism in *Lactococcus lactis*. *Appl. Environ. Microbiol.* **66**, 3974-3980.
6. Inyang, C. U., U. Gebhart, S. K. C. Obi and H. Bisswanger. 1995. Isolation and characterization of a D-glucose/xylose isomerase from a new thermophilic strain *Streptomyces* sp. (PLC). *Appl. Microbiol. Biotechnol.* **43**, 632-638.
7. Kawai, Y., H. Konishi, H. Horitsu, H. Sakurai, K. Takamizawa, T. Suzuki and K. Kawai. 1994. Purification and Characterization of D-xylose isomerase from *Bifidobacterium adolescentis*. *Biosci. Biotech. Biochem.* **58**, 691-694.
8. Kitada, M., Y. Dobashi and K. Horikoshi. 1989. Enzymatic properties of purified D-xylose isomerase from a thermophilic alkalophile, *Bacillus* TX-3. *Agric. Biol. Chem.* **53**, 1461-1468.
9. Kwon, H.-J., M. Kitada and K. Horikoshi. 1987. Purification and properties of D-xylose isomerase from alkalophilic *Bacillus* No. KX-6. *Agric. Biol. Chem.* **51**, 1983-1989.
10. Laemmli U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature.* **227**, 680-685.
11. Lee, C. and J. G. Zeikus. 1991. Purification and characterization of thermostable glucose isomerase from *Clostridium thermosulfurogenes* and *Thermoanaerobacter* strain B6A. *Biochem. J.* **274**, 565-571.
12. Lehmacher, A. and H. Bisswanger. 1990. Isolation and characterization of an extremely thermostable D-xylose isomerase from *Thermus aquaticus* HB 8. *J. Gen. Microbiol.* **136**, 679-686.
13. Lowry, O. H., N. J. Rosebrough, A. S. Farr and R. J. Randall. 1951. Protein measurement with the folin phenol

reagent. *J. Biol. Chem.* **193**, 265-275.
14. Park, H. D., G. J. Joo and I. K. Rhee. 1997. Overproduction of *Escherichia coli* D-xylose isomerase using ëPL promoter. *J. Microbial. Biotechnol.* **7(1)**, 8-12.
15. Smith, C. A., M. Rangarajan and B. S. Hartley. 1991. D-Xylose (D-glucose) isomerase from Arthrobacter strain N.R.R.L.B3728-Purification and properties. *Biochem. J.* **277**, 255-261.
16. Yamanaka, K. 1975 D-Xylose isomerase from *Lactobacillus brevis*. pp. 466-471. *Methods in Enzymol.* vol. XLI. Academic Press Inc., New York, N. Y.
17. Yamanaka, K. and N. Takahara. 1977. Purification and properties of D-xylose isomerase from *Lactobacillus xylosus*. *Agric. Biol. Chem.* **41**, 1909-1915.
18. Wong, D. W. S. 1995. Xylose isomerase. pp. 358-383. In Food enzymes (Structure and Mechanism). Chapman & Hall.

초록 : *Lactococcus* sp. JK-8에서 생산된 D-Xylose isomerase의 정제와 특성에 관한 연구

전홍기* · 김숙영 · 백형석
(부산대학교 생명과학부)

김치에서 분리된 *Lactococcus* sp. JK-8 균주에 의해 생산되는 D-xylose isomerase를 17배 정제하여 물리 화학적인 특성을 조사하였다. D-Xylose isomerase의 N 말단 아미노산 서열을 결정한 결과 Ala-Tyr-Phe-Asn-Asp-Ile-Ala-Pro-Ile-Lys로 확인되었고 이것은 *Lactococcus* 속의 D-xylose isomerase와는 유사하지 않았다. 정제된 효소의 분자량은 gel filtration의 경우 180 kDa, subunit의 분자량은 SDS-PAGE에서 45 kDa으로 측정되었고 이 효소는 4개의 subunit으로 구성된 homotetramer였다. 최적 반응 pH는 pH 7 부근이었고 pH 6~8 사이에서 안정하였다. 최적 반응온도는 70℃였고 1 mM Mn^{2+}의 존재 하에서는 70℃ 이상에서도 안정하였다. 이 효소도 다른 D-xylose isomerase처럼 활성과 열 안정성을 위해서 Mg^{2+}, Co^{2+} 또는 Mn^{2+}와 같은 2가의 양이온을 필요로 하였으며, 이중 Mn^{2+}가 효소 활성에 가장 효과적이었다. 기질 특이성에 관한 연구에서는 D-xylose를 사용했을 때 높은 활성을 가짐을 알 수 있었다. 이 효소의 pI 값은 4.8이었고, D-xylose에 대한 K_m 값은 5.9 mM이었다.