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# Overexpression, crystallization and preliminary X-ray crystallographic analysis of a putative xylose isomerase from *Bacteroides thetaiotaomicron*

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*Bacteroides thetaiotaomicron* BT0793, a putative xylose isomerase, was overexpressed in *Escherichia coli*, purified and crystallized using polyethylene glycol monomethyl ether 550 as the precipitant. X-ray diffraction data were collected to 2.10 Å resolution at 100 K using synchrotron X-rays. The crystal was found to belong to space group *P*1, with unit-cell parameters $a = 96.3$, $b = 101.7$, $c = 108.3$ Å, $\alpha = 82.8$, $\beta = 68.2$, $\gamma = 83.0^\circ$. The asymmetric unit contained eight subunits of xylose isomerase with a crystal volume per protein weight ($V_M$) of 2.38 Å$^3$ Da$^{-1}$ and a solvent content of 48.3%.

## 1. Introduction

*Bacteroides thetaiotaomicron* is a Gram-negative anaerobe that dominantly resides in the human intestinal tract. Complete genome sequencing of this microbe has already been performed (Xu *et al.*, 2003). Analysis of the genome suggests that the microbe possesses various proteins related to harvesting dietary polysaccharides and metabolizing the liberated sugars, which confers a necessary advantage for survival in the intestinal ecosystem (Xu *et al.*, 2003). Therefore, studies of sugar-metabolizing enzymes in this microbe might be useful for understanding the symbiotic host–bacteria relationship.

Xylose isomerase (XI; EC 5.3.1.5) catalyzes the isomerization of aldose and ketose sugars such as the conversion between D-xylose and D-xylulose and between D-glucose and D-fructose (Fig. 1; Takasaki *et al.*, 1969). Isomerization of D-glucose to produce D-fructose is commercially important in the production of high-fructose corn syrup (HFCS; Antrim *et al.*, 1979). Moreover, because the fructose:glucose ratio increases according to the temperature, XIs have been used as a representative model protein for studies of enzyme thermostability (Hartley *et al.*, 2000). XIs can be classified into two groups. Class 1 includes XIs from *Streptomyces*, *Arthrobacter* and *Thermus* species and *Actinoplanes missouriensis*, while class 2 includes XIs from *Bacillus* and *Thermotoga* species, *Escherichia coli* and *Clostridium thermosulfurogenes* (Hartley *et al.*, 2000; Zeikus, 1996). These two classes of XIs share similar three-dimensional structures except that class 2 XIs are found to have about 50 extra residues at the N-terminus (Hartley *et al.*, 2000). The functional role of the 50 extra residues of class 2 XIs is not clear. Crystal structures of XIs have been extensively studied. For the class 1 XIs, many XI structures such as those from *Streptomyces rubiginosus*, *Arthrobacter* sp. NRRL B3728, *Actinoplanes missouriensis*, *Streptomyces olivochromogenes* and *Thermus* species have been reported (Carrell *et al.*, 1984; Collyer & Blow, 1990; Jenkins *et al.*, 1992; Lavie *et al.*, 1994; Chang *et al.*, 1999). For the class 2 XIs, XIs from *Thermotoga neapolitana*, *Thermoanaerobacterium thermosulfurigenes* and *Geobacillus stearothermophilus* have been deposited in the Protein Data Bank (PDB entries 1a0e, 1a0c and 1a0d; O. Gallay, R. Chopra, E. Conti, P. Brick, R. Jackson, B. Hartley, C. Vieille, J. G. Zeikus & D. Blow, unpublished work). The typical structure of XIs showed a tetramer of four identical subunits, with each subunit consisting of $(\beta/\alpha)_8$-barrel and C-terminal helical domains.

Recently, a class 2 XI from *Prevotella ruminicola* TC2-24, a microbe isolated from the bovine rumen, was used for the fermentation of D-xylose by *Saccharomyces cerevisiae* and resulted in good

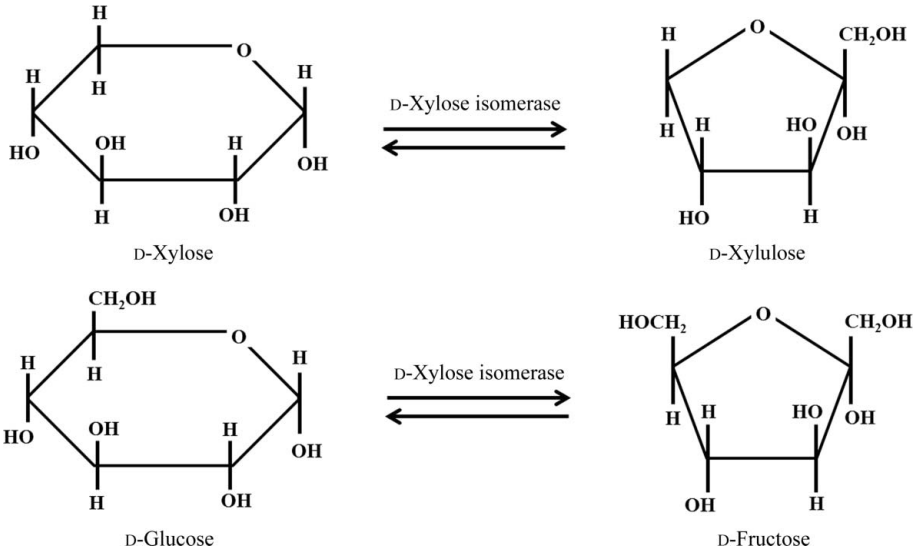


**Figure 1**
The aldose/ketose chemical reactions catalyzed by xylose isomerase.

yields in ethanol production (Hector *et al.*, 2013). This XI shows high similarity to *B. thetaiotaomicron* XI (76.6% amino-acid sequence identity). This implies that XIs from gut or intestinal tract microbes can be useful in the food industry, and detailed structural analyses of XIs from gut or intestinal tract microbes will provide guidance for enzyme-engineering research.

In order to deepen the understanding of class 2 XIs from intestinal tract microbes, we have initiated the structural determination of a class 2 XI from *B. thetaiotaomicron*. As the first step towards structural determination, the recombinant protein was overexpressed in *E. coli*, purified and crystallized. Well diffracting crystals were obtained by the sitting-drop vapour-diffusion method and X-ray diffraction data were collected to 2.1 Å resolution using synchrotron radiation.

## 2. Materials and methods

### 2.1. Overexpression and purification

The full-length *B. thetaiotaomicron* BT0793 gene (*xylA*; UniProtKB entry Q8A9M2) was amplified from genomic DNA (strain DSM2079) by the polymerase chain reaction (PCR) and cloned into pET28b(+) vector (Novagen) using the *Nde*I and *Xho*I restriction sites. This construct adds 20 residues, including a poly-histidine tag, to the N-terminus of the recombinant protein. The protein was overexpressed in *E. coli* Rosetta2 (DE3)pLysS cells (Novagen). The cells were grown at 310 K in 4 l Luria–Bertani broth medium to an $OD_{600}$ of 0.6; expression of the recombinant protein was then induced by 0.1 m*M* isopropyl *β*-D-1-thiogalactopyranoside (IPTG) at 291 K. Cell growth continued at 291 K for 20 h after IPTG induction and the cells were harvested by centrifugation at 5600*g* for 10 min at 277 K. The cell pellets were stored frozen at 203 K. Recombinant XI was highly expressed as a soluble form at various IPTG concentrations (Fig. 2). The cell pellet was suspended in ice-cold lysis buffer [25 m*M* Tris–HCl pH 7.4, 138 m*M* NaCl, 2 m*M* KCl, 10%(*v*/*v*) glycerol, 1 m*M* phenylmethylsulfonyl fluoride] containing 0.8 m*M* lysozyme and was homogenized by sonication on ice. The crude lysate was centrifuged at 36 000*g* for 1 h at 277 K and the supernatant was collected. The first purification step utilized the N-terminal hexahistidine tag by affinity chromatography on an Ni–nitrilotriacetic acid (Ni–NTA) column (Qiagen). The supernatant was applied onto an Ni–NTA column and washed with wash buffer [20 m*M* Tris–HCl pH 7.9, 60 m*M* imidazole, 500 m*M* NaCl, 10%(*v*/*v*) glycerol], after which the protein was eluted with elution buffer [20 m*M* Tris–HCl pH 7.9, 500 m*M* imidazole, 500 m*M* NaCl, 10%(*v*/*v*) glycerol]. The purified protein fractions from affinity chromatography were pooled and concentrated in an Amicon stirred cell using a YM-10 membrane. The final purification step was performed by size-exclusion chromatography. Concentrated sample was injected onto a HiLoad 16/60 Superdex 200 prep-grade column (GE Healthcare) which had previously been equilibrated with 20 m*M* Tris–HCl pH 7.5, 200 m*M* NaCl, 1 m*M* dithiothreitol (DTT).

The protein concentration was estimated by measuring the absorbance at 280 nm, employing the molar extinction coefficient of 65 445 $M^{-1}$ $cm^{-1}$ (1.337 mg $ml^{-1}$) calculated using the ExPASy Proteomics Server (http://www.expasy.org). The purified protein solution was concentrated using a YM-10 membrane (Millipore). The

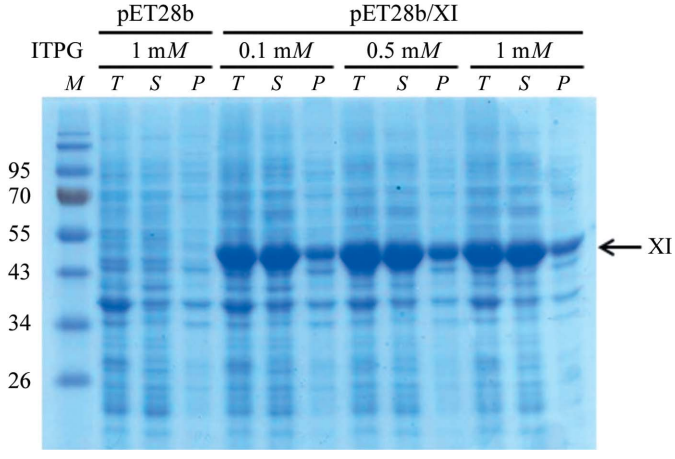


**Figure 2**
SDS–PAGE analysis of recombinant protein expression in *E. coli*. pET28b, *E. coli* Rosetta 2(DE3)pLysS harbouring pET28b(+) empty vector; pET28b/XI, *E. coli* Rosetta 2(DE3)pLysS harbouring XI cloned pET28b(+); IPTG, IPTG concentration for protein expression; lane *M*, size markers (labelled in kDa); lanes *T*, total cell extract; lanes *S*, supernatant after cell lysis; lanes *P*, pellet after cell lysis.

**Table 1**
Data-collection statistics.

Values in parentheses are for the outer shell.

| | |
|---|---|
| Diffraction source | Beamline 7A, PLS |
| Wavelength (Å) | 0.97935 |
| Temperature (K) | 100 |
| Space group | *P*1 |
| Resolution range (Å) | 50.0–2.10 (2.14–2.10) |
| No. of unique reflections | 214399 |
| No. of observed reflections | 845230 |
| Completeness (%) | 98.3 (97.2) |
| Multiplicity | 3.9 (3.9) |
| $\langle I/\sigma(I)\rangle$ | 19.4 (3.6) |
| $R_{\rm merge}$† (%) | 7.0 (39.0) |
| Average *B* factor (Å$^2$) | 1.8 |
| Mosaicity (°) | 0.60 |
| Data-processing software | *HKL*-2000 |

† $R_{\rm merge} = \sum_{hkl}\sum_i |I_i(hkl) - \langle I(hkl)\rangle| / \sum_{hkl}\sum_i I_i(hkl)$, where $I_i(hkl)$ is the intensity of the *i*th measurement of reflection *hkl* and $\langle I(hkl)\rangle$ is the mean value of $I_i(hkl)$ for all *i* measurements.

final protein concentration was 22 mg ml$^{-1}$ and the purified protein was stored at 203 K for use in the subsequent experiments.

### 2.2. Crystallization, X-ray data collection and phasing

Initial crystallization conditions were explored by employing the sitting-drop crystallization method using 96-well plates (Axygen Biosciences) at 288 K. The crystallization drop was prepared by mixing 1 µl reservoir solution and the same amount of protein solution (22 mg ml$^{-1}$ protein in 20 m*M* Tris–HCl pH 7.5, 200 m*M* NaCl, 1 m*M* DTT). The Crystal Screen, Crystal Screen 2, PEG/Ion and MembFac (Hampton Research) kits were employed. For data collection, the crystal of XI was flash-cooled using a cryoprotectant solution consisting of 100 m*M* sodium chloride, 100 m*M* bicine pH 9.0, 20%(*v*/*v*) polyethylene glycol monomethyl ether 550 (PEG MME 550). Crystals were soaked in 20 µl cryoprotectant solution for a few seconds before being flash-cooled in liquid nitrogen. X-ray diffraction data were collected at 100 K using an ADSC Quantum 270 CCD detector system (Area Detector Systems Corporation, Poway, California, USA) on beamline 7A of the Pohang Light Source (PLS), Republic of Korea. The crystal was rotated through a total of 360° with 1.0° oscillation range per frame. The raw data were processed and scaled using the *HKL*-2000 program package (Otwinowski & Minor, 1997). The program *PHENIX* was used for molecular-replacement phasing methods (Adams *et al.*, 2010).

## 3. Results and discussion

The recombinant XI was overexpressed in *E. coli* as a soluble form and purified to give a yield of ∼80 mg purified protein per litre of culture. Crystals of XI were grown in several crystallization conditions. The best XI crystal was obtained using a reservoir solution consisting of 100 m*M* sodium chloride, 100 m*M* bicine pH 9.0, 20%(*v*/*v*) PEG MME 550. Crystals grew to suitable sizes for X-ray diffraction experiments within 3 weeks (Fig. 3). The approximate dimensions of the crystals were 0.5 × 0.1 × 0.1 mm (Fig. 3). Crystals diffracted to at least 2.0 Å resolution with synchrotron radiation, and the diffraction data were collected to 2.10 Å resolution (Fig. 3). A total of 845 230 measured reflections were merged into 214 399 unique reflections, with an $R_{\rm merge}$ of 7.0%. The merged data set was 98.3% complete to 2.10 Å resolution. The native crystals of XI belonged to space group *P*1, with unit-cell parameters $a$ = 96.3, $b$ = 101.7, $c$ = 108.3 Å, $\alpha$ = 82.8, $\beta$ = 68.2, $\gamma$ = 83.0°. The presence of

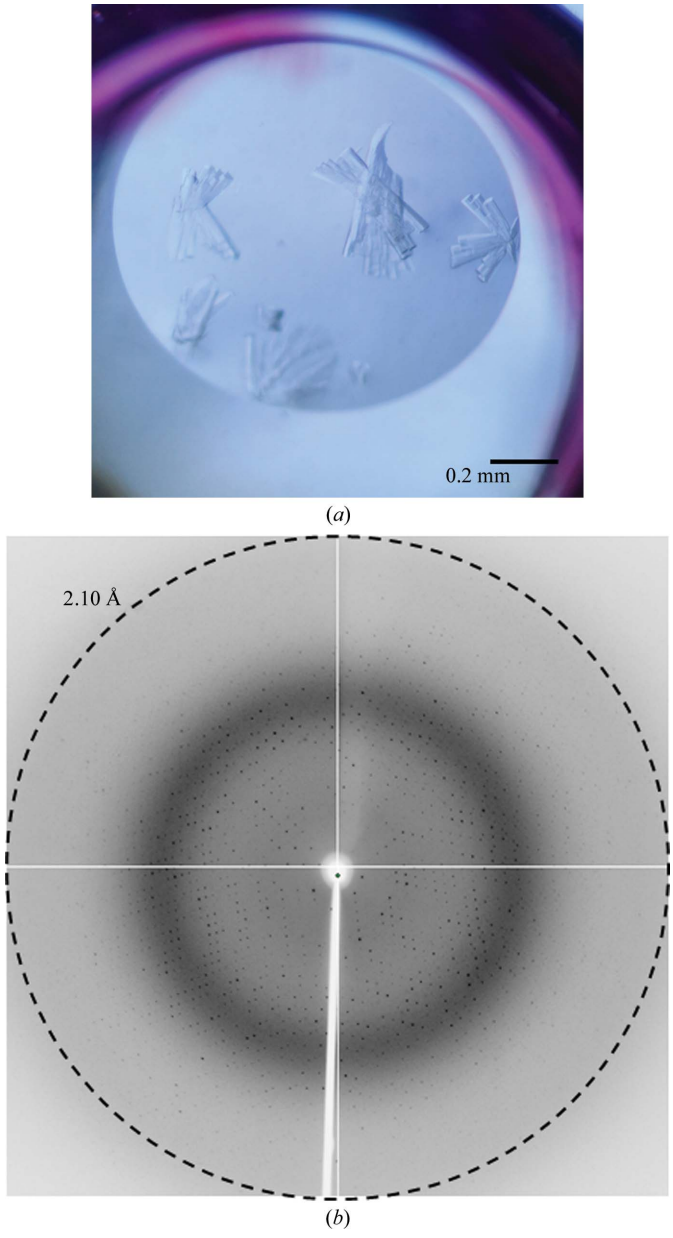


**Figure 3**
(*a*) Crystals of *B. thetaiotaomicron* XI. (*b*) Diffraction pattern of the XI crystal.

eight subunits of XI in an asymmetric unit gave a crystal volume per protein mass ($V_{\rm M}$) of 2.38 Å$^3$ Da$^{-1}$ and a corresponding solvent content of 48.3% (Matthews, 1968). The statistics of data collection are summarized in Table 1. Molecular replacement using one subunit structure of *T. neapolitana* XI (PDB entry 1a0e) as a search model was performed to solve the phase problem. The program *PHENIX* gave eight molecular-replacement solutions and the resulting electron-density map from the model was interpretable.

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