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# Thermostable Glucose Isomerase Produced by *Thermoanaerobacter Yonseiensis* Kb-1

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In the last two decades, our understanding of the biology of thermophilic and other extremophilic microorganisms has been greatly advanced. Thermophiles, which are found in both the bacterial and the archaeal domain, have been detected in association with all types of thermal habitats, including hot springs, volcanos, solfataric fields, and deep-sea hydrothermal vents (1). Due to the biotechnological potential of anaerobic microbes, many research groups have focused on the themophilic anaerobes which are able to utilize monomeric and polymeric carbohydrates.

Xylose isomerase (EC 5.3.1.5) is an intracellular enzyme which catalyzes the reversible isomerization of D-xylose to D-xylulose. This enzyme also catalyzes the isomerization of glucose to the much sweeter fructose. This conversion used in industry for the production of high fructose corn syrup (HFCS) from corn starch (2). Because of the equilibrium of the isomerization reaction, generally only 42% of glucose is converted into fructose. However, at higher temparature, the equilibrium between glucose and fructose shifts toward fructose (3). The desire for higher process temperatures has stimulated the screening of extreme thermophiles for more thermostable xylose isomerase (4).

Our group has tried to isolate new thermophilic and xylose utilizing anaerobes and finally isolated one novel species of *Thermoanaerobacter* (5). The new isolated strain KB-1 was strictly anaerobic, extremely thermophilic, spore forming and xylose utilizing bacterium isolated from a geothermally hot stream in Sileri on Java island, Indonesia. The temperature optimum for growth was 75 , and growth occurred in the range of 50-85 . The pH range for growth was 4.5-9.0, with an optimum at pH 6.5. The results of 16S rDNA sequence comparisons revealed that strain KB-1 belongs to the clostridial cluster V showing highest identities (92.7 %) to the members of the genus *Thermoanaerobacter.* Taking into account the physiological and molecular properties of the new isolate, we propose that strain KB-1 should be classified as a new species of the genus *Thermoanaerobacter*, designated *Thermoanaerobacter yonseiensis* KB-1.

In order to clone the thermostable glucose isomerase gene from the strain KB-1, the genomic DNA library of *T. yonseiensis* was constructed by ZAP express vector and positive clones were selected by plaque hybridization. Selected clones were used to infect *E. coli* XL1-Blue MRF', and were subjected to *in vivo* excision with the ExAssist helper phage, producing plasmid pBK-CMVXYL harboring the KB-1 glucose isomerase gene. The insert of pBK-CMVXYL was sequenced. The genes encoding glucose(xylose) isomerase (*xylA*) and part of xylulose kinase(*xylB*) from *T. yonseiensis* were found to constitute an operon. The KB-1 glucose isomerase gene encoded a polypeptide of 438 residues with a calculated molecular weight of 50,154 Da. Comparison of the deduced amino acid sequence with sequences of other xylose isomerases showed that the enzyme had 92% identity with a xylose isomerase from *Thermoanaerobacter ethanolicus.* The recombinant enzyme expressed in *Escherichia*

*coli* BL21(DE3) was easily purified by heat treatment and gel filtration. The wild type glucose isomerase was also purified by ammonium sulfate precipitation, DEAE CL6B anion exchange column and gel filtration to compare the characteristics between recombinant and native KB-1 glucose isomerases. The characteristics between the recombinant and wild type enzymes were identical. The enzyme was themostable (stable for 1h at 85 in the absence of substrate) and showed the highest activity between 90 and 95. Like other xylose isomerases, this enzyme required $Co^{2+}$ and $Mg^{2+}$ for thermostability and maximal enzyme activity. 57% fructose solution was formed with the KB-1 glucose isomerase when reacted at temperature of 85. The tertiary structure of the KB-1 glucose isomerase was drawn using the Swiss-Pdb Viewer (version 3.6b) program and the active site (His101, Lys233) and the metal binding site (Glu231, Glu267, Asp295, Asp338) were shown around the putative active site pocket in KB-1 glucose isomerase. It is speculated that the C-term loop play an important role to stabilize the tertiary structure of the glucose isomerase. The activity of Val185Thr mutant KB-1 glucose isomerase was increased over 50% compared with the native KB-1 glucose isomerase. The development of KB-1 glucose isomerase for industrial application has been studied by site-directed mutagenesis and direct laboratory evolution methods.

## References


1. Stetter, K. O. 1996. Hyperthermophilic procaryotes. *FEMS Microbiol Rev* **18**, 149-158.
2. Bhosale, S. H., Rao, M. B. and Deshpande, V. V. 1996. Molecular and industrial aspects of glucose isomerase. *Microbiol. Rev.* **60**, 280-300.
3. Tawari, Y. B. and Goldberg, R. N. 1984. Thermodynamics of the conversion of aqueous glucose to fructose. *Appl. Biochem. Biotechnol.* **11**, 17-24.
4. Brown, S. H., Sjoholm, C. and Kelly, R. M. 1992 Purification and characterization of a highly thermostable glucose isomerase produced by the extremely thermophilic eubacterium, *Thermotoga martima. Biotch & Bioeng.* **41**, 878-886.
5. Kim, B. C., Grote, R., Lee D.W., Antranikian, G. and Pyun, Y. R. In press (2001). *Thermoanaero-bacter yonseiensis* sp. nov., a novel extreme thermophilic, xylose-utilizing bacterium that grows at up to 85. *Int J Syst Bacteriol.*