

*Bacillus subtilis*에서 L-arabinose isomerase의 클로닝 및 특성규명

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Cloning and characterization of L-arabinose isomerase from *Bacillus subtilis*

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Objectives

To screen for an enzyme L-arabinose isomerase which can isomerize L-arabinose to L-ribulose with high turnover rate in order to produce more amount of L-ribulose, a rare sugar.

Materials and Methods

Bacillus subtilis str. 168 was obtained from Korea Research Institute of Bioscience and Biotechnology, South Korea. Protein concentrations were determined by the Bradford method using Bovine serum albumin as a standard protein. The molecular mass of the native enzyme was determined by gel filtration chromatography. The generated L-ribulose was determined by cysteine carbazole sulfuric-acid method and the absorbance was measured at 560 nm. Kinetic parameters of BSAI were determined in 100 mM phosphate buffer (pH 7.5), 1 mM Mn²⁺ and 1-600 mM substrate (L-arabinose).

Results

The *araA* gene encoding an L-AI has been cloned from *B. subtilis* and overexpressed in soluble form. Biochemical and kinetic properties of L-arabinose isomerase from *Bacillus subtilis* (BSAI) was characterized completely. Addition of Mn²⁺ increased the activity by 42 fold and appeared to be required for catalytic activity. BSAI is highly specific towards the substrate L-arabinose and exhibits the highest k_{cat} ever reported among L-AIs. Compared to other known L-AIs, BSAI catalyzes the isomerization of L-arabinose with the highest turnover rate ever reported.

시험성적

Bacteria	M_r , subunit (kDa)	Optimum pH	k_{cat} (min ⁻¹)	k_{cat}/K_m (min ⁻¹ mM ⁻¹)	References
<i>E. coli</i>	55	8	NR	NR	Yoon et al.(2003)
<i>Bacillus halodurans</i>	57	7.5 – 8	1864	51	Lee et al.(2005)
<i>Lactobacillus plantarum</i>	55	7.5	667	15	Chouayekh et al.(2007)
<i>G. stearothermophilus</i>	57	7 – 7.5	4100	61	Kim et al. (2006)
<i>G. thermodenitrificans</i>	55	8.5	6960	48	Kim and Oh(2005)
<i>Bacillus stearothermophilus</i>	56	7.5	1988	71	Rhimi (2006)
<i>Thermatoga neopolitana</i>	56	7.0	6740	58	Kim et al.(2002)
<i>Thermatoga maritima</i>	57	7.5	2340	75	Lee et al.(2004)
<i>Bacillus subtilis</i>	56	7.5	14504	121	This study

Table 1. Biochemical and kinetic properties of L-AIs from various organisms