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Cloning of *araA* Gene Encoding L-Arabinose Isomerase from Marine *Geobacillus stearothermophilus* Isolated from Tanjung Api, Poso, Indonesia

DEWI FITRIANI, BUDI SAKSONO*

Research Center for Biotechnology, Indonesia Institute of Science, Jalan Raya Bogor Km. 46, Cibinong 16911, Indonesia

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L-arabinose isomerase is an enzyme converting D-galactose to D-tagatose. D-tagatose is a potential sweetener-sucrose substitute which has low calorie. This research was to clone and sequence *araA* gene from marine bacterial strain *Geobacillus stearothermophilus* isolated from Tanjung Api Poso Indonesia. The amplified *araA* gene consisted of 1494 bp nucleotides encoding 497 amino acids. DNA alignment analysis showed that the gene had high homology with that of *G. stearothermophilus* T6. The enzyme had optimum activity at high temperature and alkalin condition.

Key words: cloning, *araA* gene, marine bacterium, *Geobacillus stearothermophilus*

INTRODUCTION

L-Arabinose isomerase (L-AI) (EC 5.3.1.4) is an intracelluler enzyme that catalyzes the reversible isomerization of L-arabinose to L-ribulose (Izumori *et al*. 1978). It also can convert D-galactose to D-tagatose (Cheetam & Wootton 1993).

Although the main conversion of L-arabinose isomerase is L-arabinose to L-ribulose, production of D-tagatose is very important as a-low calorie sweeteners. D-tagatose has sucrose-like taste, but does not contribute to calorie production (Levin 2002). In addition, D-tagatose is an anti-hyperglicemial factor with a very low calorie carbohydrate and bulking agent (Levin *et al*. 1995). It is also an efficient antibiofilm which can be used as a cytoprotectants for storage of organs to reduce the referfusion injury (Levin *et al*. 2000). D-tagatose is interested as material for food and drug industry (Rhimi & Bejar 2005).

Thermophilic L-AI has been reported possessing a catalytic activity for conversion of D-galactose to D-tagatose. Generally, isomerization process performed at high temperature (> 70 °C) offers several advantages, such as higher conversion yield, faster reaction rate, and lower viscosity of the substrate in the product stream. However, higher-temperature process introduces undesired effect like browning and unwanted by-product formation (Liu *et al*. 1996). In order to overcome these problems a thermostable L-AI with acidic pH optimum (pHopt) would be desirable and crucial for industrial application (Lee *et al*. 2005).

Many researches have been reported the thermophile L-AIs bacteria i.e. L-arabinose isomerase *B. stearothermophilus* US100 (Rhimi 2005), *Geobacillus stearothermophilus* (Jung *et al*. 2005), *G. thermodenitrificans* (Kim *et al*. 2005), *Thermus* sp. (Kim *et al*. 2003b), *Thermoanaerobacter mathranii* (Jorgensen *et al*. 2004) and the acidic L-AI from *Alicyclobacillus acidocaldarius* (Lee *et al*. 2005). Moreover, those of L- AI had been purified and characterized.

L-arabinose isomerase from *G. stearothermophilus* (GSAI) has the highest level of tagatose production and productivity. The production of tagatose is about 230 g/l (Kim *et al*. 2003a) and the productivity is about 54 g/l/h (Ryu *et al*. 2003) using a bioreactor containing immobilized *G. stearothermophilus* L-AI. These results approach commercial production criteria.

The GSAI had been cloned, expressed and characterized. This paper reported the cloning sequencing and amino acid sequence analysis of L-arabinose isomerase from marine bacterium *G. stearothermophilus* isolated from Tanjung Api, Poso, Indonesia.

MATERIALS AND METHODS

Bacterial Strain and Culture Condition. Marine bacterium *G. stearothermophilus* was isolated from Tanjung Api, Poso, Indonesia. It was grown in Nutrient Broth (NB) on an incubator shaker at 55 °C. pGEM-T Easy was used as a cloning vector and *Escherichia coli* DH5á was used as a host for cloning. This strain was grown in Luria Bertani (LB) medium on an incubator shaker at 37 °C.

Cloning Gene. Genomic DNA of *G. stearothermophilus* was isolated from cells by using Genomic DNA purification Kit (Fermentas) according to the manufacturer's instruction. A modification was performed by adding lysozyme for 5 hours to lyse the cell wall of bacteria. To amplify the *araA* gene, we designed two primers from *G. stearothermophilus* T6 (GeneBank accession number AF160811). The oligonucleotides primers were AraA-F

*Corresponding author. Phone: +62-21-8754587, Fax: +62-21-8754588, E-mail: budisaksono@yahoo.com

(GAACGGGATCCAGCAATGATGCTG) and AraA-R (ATCATACCGCCCCCGCCAAA) with a restriction site of *Bam*H1 in it (underlined). Dreamtaq DNA polymerase (Fermentas) was used to amplify this gene. The PCR conditions were set up as follow; the DNA amplification in 35 cycles, denaturation at 94 °C for 30s, annealing at 52 °C for 30s, elongation at 72 °C for 1 min, and final elongation at 72 °C for 7 min. The PCR product was purified by using DNA Gel Extraction Kit (Fermentas). The purified PCR product was then inserted into pGEM-T Easy vector and transformed into *E. coli* DH5á. Clones were screened using blue-white selection. The positive clone was showed as white colony in LB plate containing amphicillin 100 mg/ml, Isoprophyl-â-D-thiogalactopyranosidase (IPTG) 0,1 mM and 5-bromo-4-chloro-3-indolyl-â-D-galactopyranoside (X-gal) 2 µg/ml. The clones were checked by colony PCR and plasmid isolation. Further confirmation was done by sequencing of the amplified gene from positive clone.

DNA Sequence Analysis. The amplified DNA gene from positive clone was sequenced at 1stBase Singapore. The sequences were analyzed by multiple sequence alignment using the Clustal W Bioedit 7.0.3.5 program. Amino acid homology of the *araA* gene was determined by using BLASTp (http://www.ncbi.nml.nih.gov/blast).

RESULTS

The PCR product of *araA* gene from the marine strain *G. stearothermophilus* isolated from Tanjung Api, Poso, Indonesia was 1512 bp (Figure 1). The sequence of the gene was shown in Figure 2.

Based on amino acid analysis using BLASTp, the amino acid sequence of *araA* gene of the bacterium exhibited a higher degree of similarity to the *araA* gene of thermophilic bacteria i.e. *G. stearothermophilus* T6 (98%), *Alicyclobacillus acidocaldarius* (97%), *Thermus* sp. (96%), and *Geobacillus thermodenitrificans* (95%) than those of hyperthermophiles i.e. *Thermatoga neopolitana* (63%) and *Thermatoga maritima* (62%) or the mesophiles i.e. *Bacillus halodurans* (68%) and *E. coli* (61%) (Table 1).

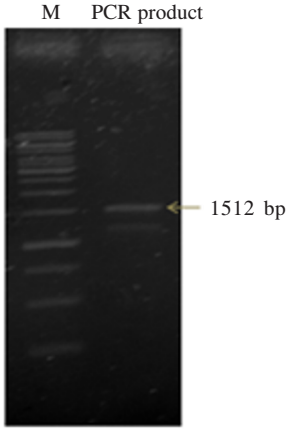

Figure 1. The PCR product of *araA* gene from Indonesian marine bacterial strain *G. stearothermophilus* isolated from Tanjung Api, Poso, Indonesia. Marker 1 kb DNA ladder (lane 1), PCR product (lane 2).

Most of the bacteria having high similarity of their *ara*A gene with the marine strain *G. stearothermophilus* were thermophilic bacteria and active in alkalin condition, only *Alicyclobacillus acidocaldarius* was active in acidic condition (Table 2).

Amino acid sequence analysis using clustalW multiple alignment revealed that 4 amino acids of the marine GSAI different from other thermophiles at the position of R26, S208, K361 and P481. Interestingly, the amino acid of S208 (Serine) only found in this marine GSAI. The catalytic site of the enzyme at E307, E332, H349, H448, D309, E352, and H447 were conserved for L-AIs (Figure 3).

DISCUSSION

This research was important as primary study to screen L-arabinose isomerase as a novel tagatose producer in Indonesia. As a low calorie sweetener, tagatose has potency to substitute sucrose for diabetic and obesity sufferers. The main character of L-AI to convert D-galactose to D-tagatose is at high temperature. The marine bacterial strain *G. stearothermophilus* isolated from Tanjung api, Poso was found in the sea around a mountain. This bacterium lives at high temperature, so that it has potency to produce a thermophile L-AI.

Generally, isomerization is performed at high temperature, so that thermophile L-AI is suitable for this process. Isomerization at high temperature offers several advantages such as higher conversion yield, faster reaction rate, and lower viscosity of the substrate (Liu *et al*. 1996). Previous study of L-AI from *G. stearothermophilus* (GSAI) found that GSAI is suitable for commercial production of D-tagatose because it has high conversion of D-galactose to D-tagatose (Kim *et al*. 2003a; Ryu *et al*. 2003).

This study was succed to clone and sequence *araA* gene from Indonesian marine bacterial strain *G. stearothermophilus*. Based on BLASTp analysis, L-AI from the bacterium has high identity with most thermophile L-AIs which have optimum activity in high temperature and alkalin condition. The sequence analysis found 4 amino acids existed in the L-AI from the bacterium differ from other thermophile L-AIs. The differences of these amino acids were at position of R26, S208, K361, and P481. We found specific amino acid present in our AI at the position of 208, that is Serine residue, whereas others GSAI was Asparagine and Threonine in *B. halodurans*. These amino acids might be related to catalytic activity of L-AI because the position of these amino acids was close to catalytic site of L-AI (Rhimi *et al*. 2007). The three-dimentional (3D) protein structure analysis and enzyme assay may prove the statement.

Rhimi *et al*. (2007) found that the catalytic site of BSAI us100 were at position of E306, E331, H348, and H447. The other amino acids i.e. D308, E351, F329, and H446 also contributed to catalytic activity. This catalytic site was conserved with those of marine local GSAI at position of E307, E332, H349, and H448, as well as at position of D309, E352, F330, and H447.