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Cloning, expression and characterization of L-arabinose isomerise from thermophilic *Anoxybacillus kestanbolensis* AC26Sari strain: Bioconversation of L-arabinose to L-ribulose

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L-Arabinose isomerase (L-AI) is a pivotal enzyme in the microbial pentose phosphate pathway. It is considered as a significant biological catalyst in rare sugar production. This enzyme can isomerize L-arabinose into L-ribulose and also D-galactose into D-tagatose. Here, we cloned the *araA* gene encoding L-arabinose isomerase from *Anoxybacillus kestanbolensis* AC26Sari strain, sequenced and over-expressed in *E. coli* BL21 (DE3): pLysS. This gene is involved in L-arabinose operon in *A. kestanbolensis* AC26Sari. DNA sequence analysis revealed an open reading frame of 1,506 bp, capable of encoding a polypeptide of 502 amino acid residues with calculated molecular weight of 55.6776 kDa. The recombinant was purified by heat treatment and Ni-HisTaq chromatography. The purified enzyme showed maximal activity at pH 8.5 and 65ºC and required divalent cations such as Co^{2+} and Mn^{2+} for its activity and thermostability. The apparent K_m value of the enzyme for L-arabinose was 6.5 mM (V_{max}, 140.1002 U/mg) as determined in the precence of both 1 mM Co^{2+} and Mn^{2+}.

Keywords: Biocatalysis, Microbial pentose phosphate pathway

L-arabinose is one of the most abundant monosaccharide in nature and some organisms have the ability to use arabinose as a carbon source. Therefore, this pathway in bacteria has been extensively investigated[1]. Bacteria are enable to transport L-arabinose (via the unlinked *araE* and *araFGH* gene products) by the arabinose regulon. After that they convert the arabinose to D-xylulose-5-phosphate in three enzymatic steps catalyzed by the *araBAD* gene products. The D-xylulose-5-phosphate is then along metabolized via the pentose phosphate pathway. *araBAD* operon consist of three metabolic genes, *araB*, *araA*, *araD*, encoding, L-ribulokinase (EC 2.7.1.16), L-arabinose isomerase (L-AI, EC 5.3.1.4) and L-ribulose-5-phosphate 4-epimerase (EC 5.1.3.4), respectively.

L-AI is an intracellular enzyme that catalyzes the reversible isomerization of L-arabinose to L-ribulose[2]. L-AI can be used also to produce D-tagatose from D-galactose. Because of the structural similarity between L-arabinose and D-galactose, including the shared L-cis-hydroxyl configuration at C3-C4, L-AI can catalysis the isomerization of D-galactose into D-tagatose[3,4].

Currently, isomerases are becoming increasingly significant as they play a pivotal role in the synthesis of uncommon sugars, simply termed as rare sugars[5]. Because of their scarcity in nature and costly methods of production, rare sugars are available only in limited amounts and at great expense[6]. D-tagatose is a ketohexoserarely found in nature. Recently, there has been increasing industrial interest in D-tagatose as a low-calorie sugar-substituting sweetener (1.5 kcal/g), because it has only 38% of the energy content of sucrose[7].

L-arabinose isomerase (L-AI) activity and the mechanism has been reported in various *viz*., *Escherichia coli*, *Bacillus licheniformis*, *Bacillus subtilis*, *Thermotoga neapolitana*, *Geobacillus stearothermophilus*, *Thermoanaerobacter mathranii*, *Alicyclobacillus acidocaldarius*, *Geobacillus thermodenitrificans* and *Anoxybacillus flavithermus*. However, L-AI has not been reported from *Anoxybacillus kestanbolensis* AC26Sari until now.

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The catalytic activity of *A. kestanbolensis* AC26Sari L-AI is a metal ion dependent; Mn^{2+} binding produces an enzyme with greater intrinsic activity and heat stability. It has been noted that all of the simple sugar isomerases are metallloproteins. In addition, although the L-AI of *A. kestanbolensis* AC26Sari is strict substrate specificity for L-arabinose, it can also convert other aldoses, such as D-lactose, D-xylose, D-ribose, D-mannose, D-allose, into their ketose forms at low rates.

In this study, we attempted cloning, over-expression, purification and as well as the biochemical characterization of a thermoactive L-arabinose isomerase isolated from *Anoxybacillus kestanbolensis* AC26Sari. The enzyme behaviour toward metallic ions for its activity and thermostability is also detailed and discussed in comparison with other L-AIs.

Materials and Methods

Materials

L-Arabinose and D-galactose were purchased from Sigma. D-ribose and D-mannose were obtained from Bacto and D-xylose was obtained from Fisher Bioreagents. Genomic DNA Isolation Kit, pGEM-T Easy Cloning Vector Kit, Taq DNA polymerase, dNTP set, HisLinkTM Protein Purification Kit and the restriction enzymes used in this study were purchased from Promega. pET28a(+) expression vector was obtained from Invitrogen. All other chemicals were acquired from Merck and Applichem.

Bacterial strains and culture conditions

A. kestanbolensis AC26Sari was isolated and identified from Camkoy mud hot spring (Canakkale-TURKEY)[8]. *E. coli* strains used in this study were BL21 (DE3): pLysS (Novagen) and JM101 (NEB). pGEM-T Easy and pET28a(+) vectors were used for cloning and overexpression.

A. kestanbolensis AC26Sari was grown aerobically at 55°C and pH 7.0 in either Luria-Bertani medium (LB) or minimal medium with the addition of 2 g/L casamino acid (M9CA)[9]. All *E. coli* strains containing recombinant plasmids were cultured in LB medium supplemented with 50 µg/mL ampicillin or kanamycin for selection, as appropriate, at 37°C and pH 7.4, unless otherwise stated.

Cloning and expression of *araA* gene from *A. kestanbolensis* AC26Sari

The genomic DNA was isolated from harvested cells from *A. kestanbolensis* AC26Sari using a genomic DNA isolation kit. Primers (AraisoF 5'-GTGTTATTATTACGTCCTT-3' and AraisoR 5'-CGTAAAGGCGGTGAAGTTGCC-3') designated for amplification of *araA* gene, according to the genome sequence of *A. flavithermus* (NCBI GenBank: CP000922). PCR was performed by Taq DNA polymerase. The DNA fragments were denaturated at 95°C for 2 min, and then amplified for 36 cycles with denaturation at 94°C for 1 min, annealing at 50-55°C for 1 min 30 s, and extension at 72°C for 2 min, were held at 72°C for 5 min. After amplification, 1,500 bp DNA fragment was cloned into pGEM-T easy vector, transformed into *E. coli* JM101 and sequenced by Macrogen, South Korea.

According to the sequence results, two expression primers (AraizF -5' CGCTAGC GTG TTA TTA TTA CGT CCT TAT g -3' and AraizR -5' GGGATCC CTA TTA TAT TAA ATC TTA TTT TCC-3') were designed to introduce the *BamH1* and *Nhe1* restriction sites for cloning into expression vector pET28a(+). The *araA* gene was amplified by PCR with the same reaction conditions. The gene was then cloned into the expression vector pET28a(+) to create pET-AI. The recombinant plasmid was transformed into *E. coli* BL21 (DE3): pLysS for expression[10].

Overexpression of *araA* gene

E. coli BL21 (DE3): pLysS cells harboring pET-AI was grown in LB medium containing 50 µg/mL of kanamycin at 37°C until the OD_{600} reached to 0.6-0.8. Isopropyl-beta-D-thiogalactopyranoside (IPTG) was added to the culture medium at 1 mM final concentration to induce the expression for a further 3-4 h at 37ºC. The cells were harvested by centri-fugation at 11,000 rpm for 15 min. The cells were resuspended in 50 mM phosphate buffer (pH 7.0) and disrupted by sonication with Sartorius Labsonic M (80% amplitude, 0.6 cycle for 5 min). The lysates were centrifuged (14,800 rpm, for 15 min at 4ºC) to remove cell debris. The enzymes expressed from *E. coli* BL21/ pET-AI was designated as KstAI.

Purification of KstAI

For purification of KstAI, a crude extract was heated at 65°C for 15 min. After centrifugation at 14,800 rpm for 20 min, the supernatant was loaded onto a Ni-HisTag affinity column chromatography. Purity of KstAI was verified by 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)[11]. Protein concentration was determined according to the Bradford method with a Bovine serum albumin as a standard[12]. The purified enzyme

was dialyzed against 50 mM phosphate buffer (pH 7.0) containing 10 mM EDTA (ethylenediaminetetraacetic acid) for 24 h at 4ºC. Subsequently, the enzyme was dialyzed against 50 mM EDTA-free phosphate buffer (pH 7.5) for 24 h at 4°C before the characterization studies.

Enzyme activity assay

The activity of L-arabinose isomerase was determined by measuring L-ribulose production using L-arabinose as a substrate. The concentration of L-ribulose in the reaction was calibrated based on the standard linear graph prepared using different concentrations of L-ribulose. One unit of enzyme activity was defined as the amount of enzyme that catalyzes the formation of 1µmol of L-ribulose per minute. In standard conditions, the reaction mixture contains 1 mM $MnCl_2$, 1 mM $CoCl_2$, 40 mM L-arabinose (substrate), 100 mM glycine (pH 8.5) and 1,4 U (µmol/min) enzyme. The reaction mixture was incubated at 65ºC for 20 min followed by cooling the samples on ice to stop the reaction. The generated L-ribulose was determined by cysteine carbazole sulfiric acid method, and the absorbance was measured at 560 nm[13].

Effect of temperature on activity and stability

The effects of temperature on KstAI activity was determined spectrophotometrically using L-arabinose as the substrate. The activity assays were performed over the temperature range of 35°C to 99°C, using the standart enzyme activity assay described above, and the results were expressed as relative activity (%).

The effects of temperature on KstAI stability was determined by measuring the residual activity (%) at various temperatures (50-55-60-65-70-75 and 80ºC) for different periods of times (30-210 min). Samples were taken at intervals and the residual activities (%) were measured using the standart enzyme activity assay.

Effect of pH on enzyme activity and stability

The optimum pH of KstAI was determined using the standard assay conditions with two buffer systems, 100 mM MOPS (pH 4.0-8.0), and 100 mM glycine (pH 8.0-11.0) and their relative activities (%) were measured.

The effects of pH on KstAI stability was investigated by incubating the enzyme at 25ºC with 100 mM MOPS (pH 5.0-8.0), and 100 mM glycine (pH 8.0-11.0). At a certain time intervals, samples were withdrawn, and residual activities (%) were measured under standard assay conditions.

Effect of metal ions and inhibitors on enzyme activity

The enzyme was assessed under standard conditions in the presence of several metal ions ($MnCl_2$, $CoCl_2$, $MgCl_2$, $FeSO_4$, $CaCl_2$, $ZnCl_2$, $CuCl_2$, LiCl and KCl) with a final concentration of 1 mM. The activity of the enzyme without metal ions was defined as the 100% level and the residual activity (%) was assayed spectrophotometrically.

In order to determine more exactly the effect of the Mn^{2+} and Co^{2+} concentration on enzyme activity, reactions were performed under the standard reaction conditions with the addition of Mn^{2+} or Co^{2+} in the range of 0-10 mM. The activity of the enzyme without metal ions was defined as the 100% level and the residual activity (%) was assayed.

To investigate the effects of inhibitors, β-mercapto-ethanol, EDTA and different sugar alcohols; L-arabitol, ribitol and mannitol were added to final concentration of 10 mM. Reactions were carried out at 65ºC in 100 mM glycine buffer (pH 8.5) for 20 min. The activity of the enzyme without inhibitor was defined as the 100% level and the residual activity (%) was assayed spectrophotometrically.

Substrate specifty

Spectrophotometric methods were used to determine the effect of KstAI on different aldoses; L-arabinose, D-ribose, D-mannose, D-xylose, D-galactose and D-glucose at a final concentration of 40 mM. The results were expressed as relative activity (%) obtained at standard reaction conditions.

Determination of kinetic parameters

The kinetic parameters of KstAI were determined with different substrate concentrations (1-50 mM substrate) of L-arabinose. K_m (Michaelis constant, mM) and V_{max} (µmolµmin/mg) for L-arabinose were obtained using the Lineweaver-Burk plots (1/S *vs* 1/V) of the Michaelis-Menten equation.

All experiments were carried out in triplicates and datas were represented by vertical error bars in the graphics.

Results

Cloning and overexpression of *araA* gene from *A. kestanbolensis* AC26Sari

The gene of *A. kestanbolensis* AC26Sari was first cloned into the pGEM-T Easy vector system, sequenced and then cloned into pET28a(+) expression vector. The analysis of the whole gene revealed the presence of a 1,506 bp open reading frame (ORF) encoding a hypothetical 502 aminoacid protein with a

molecular mass of 55,6776 kDa. The recombinant protein was over-expressed with the induction of expression by IPTG under the control of T7 promoter in *E.coli* BL21 (DE3): pLysS, which produced an apparent band in SDS-PAGE gel.

Purification of KstAI

KstAI was purified by heat shock treatment and Ni-HisTag affinity column chromatography. Purity of KstAI was verified by SDS-PAGE analysis (Fig. 1). Purification steps were summarized in Table 1.

Effect of temperature on activity and stability

The optimum temperature of KstAI was observed to be 65°C (Fig. 2A). After incubation at different temperautes, the enzyme at 50 and 55°C was determined to be more stable than the other temperatures. After the first half hour, the enzyme lost activity at each temperature (Fig. 2B).

Effect of pH on enzyme activity and stability

The optimum pH for the isomerase activity of the enzyme was observed to be 8.5 (Fig. 2C). When assayed at various pH values, the KstAI showed high activity in alkaline conditions (8.0-10.0). At pH 5.0-7.0, enzyme activity start to decreased after 24 hours (Fig. 2D).

Effect of metal ions and inhibitors on enzyme activity

Experiments have been conducted using L-arabinose as substrate to examine the effect of various metal

Table 1 — Summary of purification steps

Purification steps	Total protein (mg)	Total activity (U)	Spesific activity (U/mg)	Yield (%)	Purification factor
Crude Extract	40.8	1170.4	28.7	100	1
Heat shock treatment	15.2	888.6	58.5	75.9	2.04
Ni-HisTag affinity column chromatography	2.54	330.8	130.2	28.3	4.5

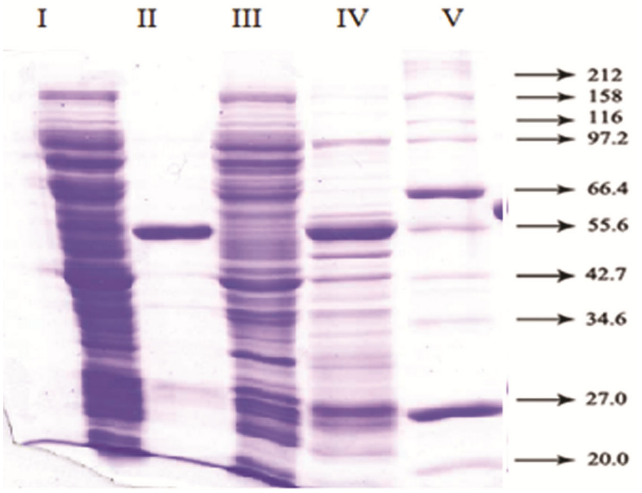

Fig. 1 — SDS-PAGE analysis of purified KstAI. [Lane I, Crude extract of KstAI; Lane II, Purified KstAI after heat shock treatment and Ni-His tag column chromatography; Lane III, Expressed BL21 (DE3):pLysS containing pET 28a(+) expression vector that doesn`t include *araA* gene; Lane IV, KstAI after heat shock treatment; and Lane V, Molecular weight marker]

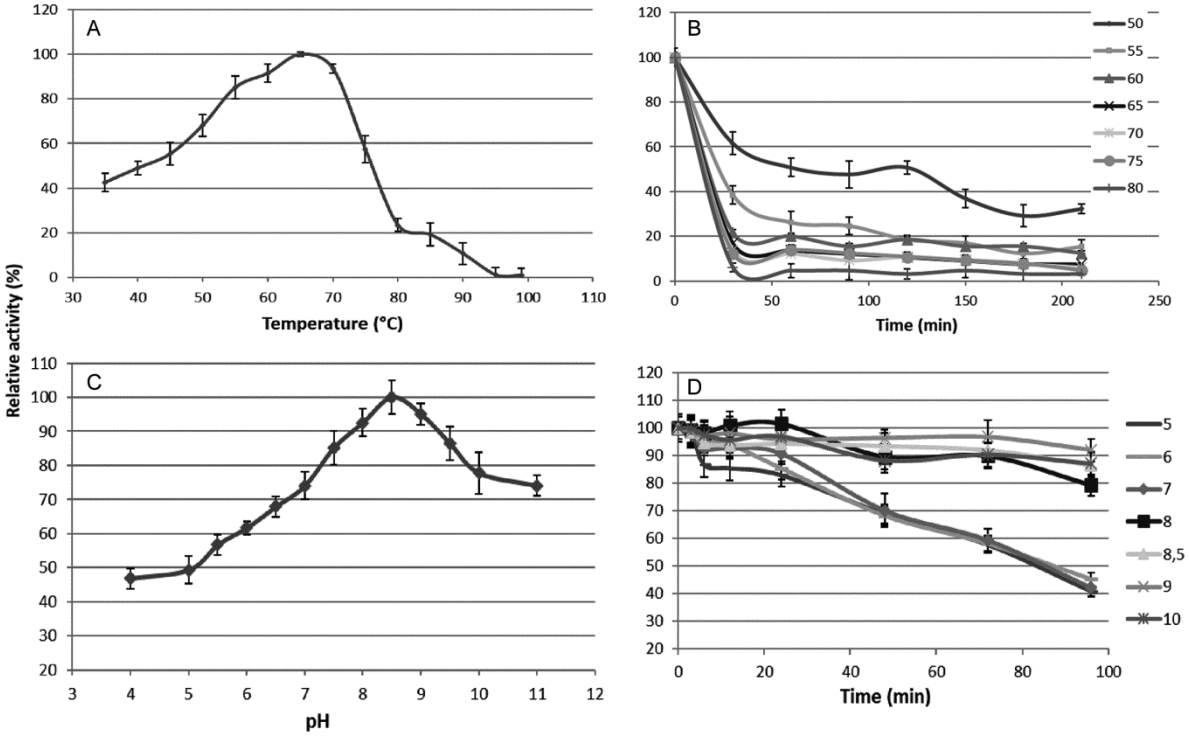

Fig. 2 — Effects of (A & B) temperature; and (C & D) pH on KstAI activity and stability, respectively

ions and chemicals on L-AI activity. $MnCl_2$ was found to increase the activity of the enzyme most. $CoCl_2$, $MgCl_2$, $CaCl_2$, KCl and $MnCl_2$-$CoCl_2$ combination were found to enhance the activity. Other metal ions and chemicals were found to inhibit the activity of the enzyme. Among them, $CuCl_2$ was inhibited the enzymatic activity. EDTA and β-mercaptoethanol were also found to inhibit enzyme activity (Fig. 3).

In the other step of the experiment, the effects of Co^{+2} and Mn^{+2} on the activity of the enzyme at different concentrations (1, 2.5, 5, 10 mM) were investigated. As a result, 1 mM concentrations of both metal salts were determined to give the highest activity value (Fig. 4).

The effects of sugar alcohols on L-AI activity were determined and as a result L-arabitol, ribitol inhibited the enzyme activity and mannitol did not affect the activity (Fig. 5).

Substrate specifty

KstAI was found to be specific only for L-arabinose aldose. It was determined that KstAI had reacted very little with other aldose sugars and did not react with glucose (Fig. 6).

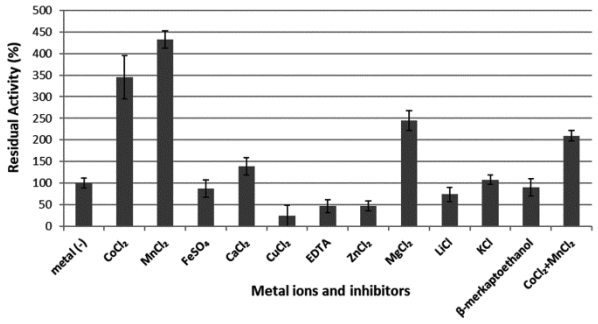

Fig. 3 — Effects of metal ions and other reagents on KstAI activity

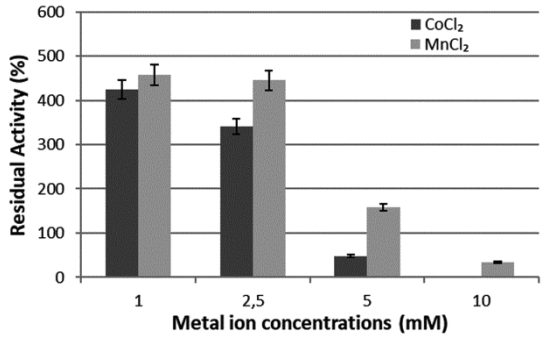

Fig. 4 — Effects of different concentrations of Co^{+2} and Mn^{+2} salts on KstAI activity

Determination of kinetic parameters

Kinetic parameters were measured using a spectrophotometric activity assay with L-arabinose as the substrate. KstAI exhibited a simple Michaelis–Menten kinetics for L-arabinose. The values of K_m and V_{max} were found to be 6.552 mM and 140.1002 U/mg, respectively.

Discussion

In this study, the L-arabinose isomerase gene of *Anoxybacillus kestanbolensis* AC26Sari was cloned and characterized. This study is important because for the first time a L-AI enzyme has been characterized in *Anoxybacillus kestanbolensis* strains. In addition to this, the increasing interest in microbial enzymes and the fact that thermophilic bacteria are the focus of interest increases the importance of this study.

The molecular weight of KstAI was determined as 55.6776 kDa. When other studies were examined the molecular weight of L-AI was 56.658 kDa in *Thermotoga maritima*[4], 56 kDa in *G. stearothermophilus*[14], 57 kDa in *Clostridium*

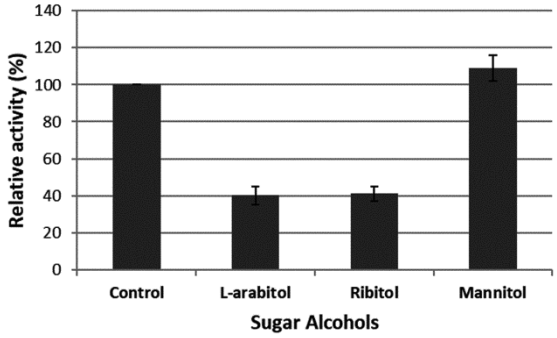

Fig. 5 — Effects of sugar alcohols (L-arabitol, ribitol, mannitol) on KstAI activity

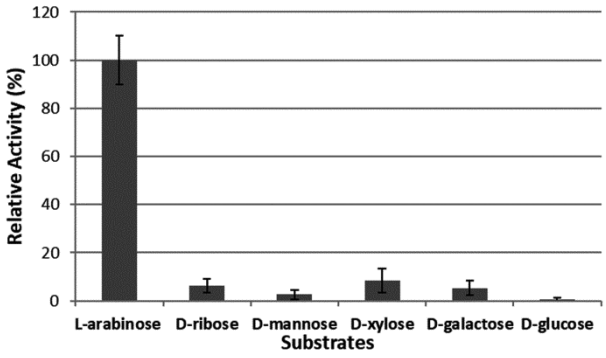

Fig. 6 — Substrate specificity of the purified KstAI towards L-arabinose, D-ribose, D-mannose, D-xylose, D-galactose and D-glucose

hylemonae[15], and 56 kDa in *E. coli*. According to these results, the protein was found to be of the expected molecular weight and similar to other L-AIs.

Industrial enzymatic reactions that take place at high temperatures are more preferred for industrial use than those of mesophilic bacteria, especially because of their resistance to chemical degradation. As a result of the optimum temperature studies, it was found that the KstAI was showed optimum activity at 65ºC. In the literature, the optimum working temperature of this enzyme in other bacteria were, 50ºC in *Enterococcus faecium* DBFIQ E36[16], 60 ºC in *Bacillus coagulans* NL01[17], 42ºC in *Bacillus subtilis*[18], 65ºC in *Lactobacillus brevis*[19].

The pH of the medium in which the reaction takes place affects the primary and secondary structure of the enzyme and thus its activity. The optimum activity of the KstAI was determined at pH 8.5. In contrast, activity under acidic conditions was weaker. The activity decreased approximately 50% when pH dropped to 5.0, as did most of the alkali L-AIs previously, because under this condition it was difficult to ionize some of the side chain groups which possibly near their substrate binding sites[16]. The optimum pH of other L-AIs was determined as pH 6.5 in *Lactobacillus brevis* PC16[20], pH 7.0 in *Thermoanaerobacter mathranii*[7], pH 6.0 in *Pediococcus pentosaceus*[21], and *G. stearothermophilus*[14]. Given these results, it can be said that thermophilic L-AIs generally achieve optimum activity under alkaline conditions.

As a result of KstAI thermal stability studies, it was seen that activity decreases approximately 40% at temperatures below 60ºC in the first half hour in the absence of metal ions. After 50 min, the remaining activity was preserved. It was observed that *G. thermodenitrificans* L-AI remained stable at temperatures below 60ºC, above 65°C the enzyme activity decreases with increasing reaction time, the enzyme has a half-life of 30.5 min at 75°C, 24.9 min at 80°C[22]. *P. pentosaceus* L-AI stability decreases with half-lives of 2 h and 0.8 h at 55°C and 60°C, respectively. The enzyme maintained approximately 80% of its maximal activity after 3 h incubation at 50°C in the presence of 0.6 mM Mn^{2+} or 0.8 mM Co^{2+}[21]. The L-AI of *G.stearothermophilus* was determined to be stable at temperatures below 55ºC, but unstable above 60ºC[14]. It was determined that KstAI was a metal bound enzyme but its thermal stability is not high. This is thought to be due to the absence of buffer and metal ions in the reaction tube to keep the enzyme stable during heat treatment. Because the rate of isomerization reaction increases at high temperatures so the cofactor becomes essential for maintaining the thermal stability of L-AI[16].

The study on the effect of pH on the enzyme stability showed that the KstAI was stable up to 79% at pH 8.0, 86% at pH 8.5, 92% at pH 9.0, 87% at pH 10.0 and approximately 40% at pH 5.0-6.0-7.0 for 96 h. The experimental data showed that the enzyme from *Lactobacillus brevis* was stable up to approximately 80% at pH 8.0 and 9.0 but the stability decrease up to 20% at pH 10.0[19]. The L-AI of *Thermoanaerobacterium saccharolyticum* NTOU1 showed very minimal activity when the pH either drops below 5.0 or increases to above 9.5[23]. The L-AI of *Bacillus coagulans* retains 90% of its maximum activity at pH 6.0 and shows about 75% activity at pH 8.0, below pH 6.0 and above pH 8.0 the activity decreases drastically[24]. According to these results, it can be suggested that KstAI is more stable at higher pHs.

All known L-AIs exhibit high activity against aldoses, L-arabinose and D-galactose[2]. When the KstAI substrate specificity was examined, it was determined that it showed the highest activity against L-arabinose (100%). KstAI isomerize other aldose such as D-xylose, D-galactose, D-glucose, D-mannose and D-ribose with 8, 5, 0.3, 2 and 6% of L-arabinose isomerase activity, respectively. These results showed that KstAI is specific for L-arabinose as substrate. In the studies conducted with *G. stearothermophilus*[22] L-AI, high activity against L-arabinose was observed, whereas activity against D-glucose was not found and low activity against other aldoses. It was found that L-AI of *B. licheniformis* has a high preference only for L-arabinose, D-galactose showed approximately 2% activity while other aldoses did not serve as substrates[25]. The previous studies showed that L-arabinose and D-galactose bind to the active site of the enzyme by hydrogen bonds in their study on the crystal structure of L-AI[5]. The hydrogen bond is formed between the C2 and C5 atoms of these substrates and the amino acids E330 and E305 of the enzyme. In this binding, the distance between C2-C5 atoms and amino acids E330-E305 of L-arabinose is indicated as 2.0Å and 1.9Å. For D-galactose, these values are 5.5Å and 5.3Å. The fact that L-arabinose is closer to the active site explains why it prefers L-arabinose over D-galactose. The interest of *A. flavithermus* L-arabinose isomerase to D-galactose

was 60% higher than that of L-arabinose[26]. Although it is a genus of *Anoxybacillus*, its L-AI differs from KstAI in terms of substrate specificity. Most L-AIs (including *A. flavithermus* L-AI) differ in 4 amino acids in the conserved regions (V226-I226; R295-Q295; G340-S340; I468-V468) of the active site. It was considered that the difference in substrate specificity of Kst-AI was due to these amino acid sequences.

L-arabinose isomerases perform isomerization by the hydride exchange mechanism from C2 to C1, and metal ions play an important role in this reaction. In particular, Mn^{2+} and Co^{2+} metal ions are known to activate L-arabinose isomerase. The highest activity was observed in the presence of Mn^{2+} (432%) and Co^{2+} (344%). Mg^{2+} (244%), Mn^{2+}-Co^{2+} combination (209%), Ca^{2+} (138%) and K^{2+} (107%) ions were increased the activity and Li^{+} (73%), Cu^{2+} (24%), Fe^{2+} (87%) and Zn^{2+} (47%) ions were inhibited the activity. It was thought that the reason for these results was related to the interaction of the enzyme with amino acids in the metal binding sites. In particular, the Mn^{2+} coordinates with the amino acids mediating between the substrate and the active site. Mn^{2+} is known as the cofactor of the enzyme in mesophilic and thermophilic bacteria, which increases the rate of conversion of arabinose to ribulose[2]. Hyperthermophiles need Co^{2+} ions, which are not allowed to be used in food products. Therefore, KstAI can be used in the production of industrial L-ribulose since it does not require Co^{2+} ion for its activity. When the effect of sugar alcohols, which are other inhibitors of this enzyme, is examined, L-arabitol and ribitol were inhited the activity of KstAI. L-arabitol and ribitol are also potent inhibitors of *G.thermodenitrificans*, *G. stearothermophilus*, *E. coli* and *Mycobacterium smegmatis* L-AIs[2,22]. Mannitol was found not to decrease enzyme activity. Mannitol did not also alter activity of *Lactobacillus gayoni* L-Al in parallel with KstAI[2].

The K_m and V_{max} values of KstAI were determined to be 6.552 mM and 140,1002 U/mg toward L-arabinose, respectively. K_m and V_{max} values of *Pseudoalteromonas haloplanktis* L-AI were 111.68 mM and 13.91 U/mg, respectively[27]. K_m values of *G. stearothermophilus*, *T. neapolitana* and *T. maritima* were found as 67 mM, 116 mM and 31 mM, respectively[14]. V_{max} values of *G. stearothermophilus* and *T. neapolitana* were found as 96 U/mg and 119 U/mg, respectively[14]. Compared to these K_m and V_{max} values, it can be said that KstAI presents an advantage over other L-AIs.

Conclusion

L-arabinose isomerase was cloned and characterized the enzymatic properties of this recombinant enzyme and compared these properties with other L-AIs for the first time from *Anoxybacillus kestanbolensis* AC26Sari strain. This isomerase preferably uses L-arabinose as a substrate. This enzyme is stable at high pHs and has a high affinity for the substrate it is used for and it does not require Co^{2+} ion for its activity. Thus, it has potential for industrial applications.

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Conflict of interest

Authors declare no competing interests.

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