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RESEARCH PAPER

Towards efficient enzymatic conversion of D-galactose to D-tagatose: purification and characterization of L-arabinose isomerase from *Lactobacillus brevis*

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Abstract

L-arabinose isomerase (L-AI) (EC 5. 3. 1. 4. L-AI) that mediates the isomerization of D-galactose to D-tagatose was isolated from *Lactobacillus brevis* (MF 465792), and was further purified and characterized. Pure enzyme with molecular weight of 60.1 kDa was successfully obtained after the purification using Native-PAGE gel extraction method, which was a monomer in solution. The L-AI was found to be stable at 45–75 °C, and at pH 7.0–9.0. Its optimum temperature and pH was determined as 65 °C and 7.0, respectively. Besides, we found that Ca^{2+}, Cu^{2+}, and Ba^{2+} ions inhibited the enzyme activity, whereas the enzyme activity was significantly enhanced in the presence of Mg^{2+}, Mn^{2+}, or Co^{2+} ions. The optimum concentration of Mn^{2+} and Co^{2+} was determined to be 1 mM. Furthermore, we characterized the kinetic parameters for L-AI and determined the K_m (129 mM) and the V_{max} (0.045 mM min^{-1}) values. Notably, *L. brevis* L-AI exhibited a high bioconversion yield of 43% from D-galactose to D-tagatose under the optimal condition, and appeared to be a more efficient catalyst compared with other L-AIs from various organisms.

Keywords Purification · Characterization · L-arabinose isomerase · *Lactobacillus brevis* · D-tagatose

Abbreviations

EDTA	Ethylenediaminetetraacetic acid
SDS	Sodium dodecyl sulfate
PAGE	Polyacrylamide gel electrophoresis
L-AI	L-arabinose isomerase
L. brevis	*Lactobacillus brevis*

Introduction

D-tagatose, a natural rare ketohexose, is an isomer of D-galactose and an epimer of D-fructose [1, 2]. As a novel functional sweetener, D-tagatose exhibits similar properties to sucrose (92% sweetness of sucrose), but contains much lower calories and offers additional health benefits, and therefore, has attracted extensive attention. D-tagatose has been widely used in various health foods and health care products, such as antihyperglycemic agent, diabetic food, weight loss food, and others, due to its low calories, minimal glycemic effect, prebiotic function, and dental care applications [3]. Inspired by its previously demonstrated health-promoting functionalities, tagatose is qualified as generally recognized as safe (GRAS) for use in foods under FDA-regulated program [4]. Joint FAO/WHO Expert Committee on Food Additives (JECFA) stated that there is no need to limit the allowed daily intake (ADI) of tagatose [5].

Currently, the two main preparation methods of D-tagatose are chemical isomerization and biosynthesis [6]. As previously demonstrated in several academic reports, chemical isomerization has many disadvantages. The industrial enzymatic reactions of D-galactose isomerization for D-tagatose production require high temperature and a large amount of

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acid to improve the yield of D-tagatose. Unfortunately, these operations can introduce undesired reactions. On the contrary, thanks to the mild reaction conditions, low energy consumption and little environmental pollution, the biosynthesis method is much more competitive for D-tagatose production, in which microbial enzymes in several microorganisms convert D-galactose into D-tagatose using L-arabinose isomerase.

L-arabinose isomerase (L-AI; EC 5. 3. 1. 4) catalyzes the reversible isomerization of L-arabinose to L-ribulose, and can also convert D-galactose to D-tagatose due to similar configuration of the substrates [6–9]. Researchers have investigated various mesophilic, thermophilic, and hyperthermophilic bacteria for D-tagatose production, including *Bacillus stearothermophilus* [10], *Escherichia coli* [11] and *Lactobacillus plantarum* [12], *Lactobacillus brevis* [13], and others.

In this study, a strain of *L. brevis* was obtained from fermented cabbage and explored its potential to produce D-tagatose by L-arabinose isomerase. The L-AI produced by *L. brevis* was isolated, purified, and characterized for the first time.

Materials and methods

Materials

The microorganism, *Lactobacillus brevis* (MF 465792) strain producing L-arabinose isomerase, was laboratory preservation strains. D-tagatose was purchased from Adamas (Shanghai, China). D-galactose and L-arabinose were purchased from Huachang Pharmaceutical (Zhangjiagang, China). Man-1, CBM28-GFP were constructed and purified from our laboratory, and bovine serum albumin (BSA) was purchased from Sigma-Aldrich. Sepharose CL-6B and empty columns were purchased from GE Healthcare (Uppsala, Sweden). All other chemicals were of analytical grade.

Enzyme analysis

L-arabinose isomerase activity was determined by measurement of the amount of D-tagatose generated from D-galactose. L-arabinose isomerase activity was measured using 50 mM D-galactose in 50 mM phosphate buffer (pH 7.0) as the substrate. 0.05 ml of enzyme solution was added to the same volume of D-galactose solution and allowed to react at 65 °C for 30 min. The enzymatic reaction was stopped by cooling the samples at room temperature. The amount of the product, D-tagatose, was determined spectrophotometrically at 400 nm using the resorcinol method. One unit of L-arabinose isomerase activity was defined as the amount of enzyme catalyzing the formation of 1 μmol D-tagatose per min under the specified conditions. The concentration of protein was measured by the Bradford method using bovine serum albumin as a standard protein.

Microorganism incubation and crude enzyme extraction

The *L. brevis* strain was incubated at 37 °C with shaking at 180 rpm in MRS medium [14, 15]. After incubated for 24 h, cells were harvested and resuspended in 10 ml of 50 mM phosphate buffer (pH 7.0), disrupted by sonication (350 W, pulse on, 1 s; pulse off, 1 s) for 40 min, and the cells debris were removed by centrifugation (12,000*g*, 20 min) and the supernatant was used in the enzyme purification procedure.

Ammonium sulfate fractionation

Pellets of $(NH_4)_2SO_4$ were slowly added to the supernatant with shaking to 60% saturation. After incubation at 4 °C for 4 h, the enzymatic extract was centrifuged. A second step of precipitation was performed by adding ammonium sulfate to 80% saturation and incubating at 4 °C for 4 h. The final precipitate was collected by centrifugation and dissolved in 50 mM phosphate buffer (pH 7.0) for purification or dissolved in 50 mM Tris–HCl buffer (pH 7.5) for enzymatic assays.

Purification of L-arabinose isomerase and estimation of molecular weight

The crude protein in 50 mM phosphate buffer after ammonium sulfate fractionation was dialyzed against 50 mM phosphate buffer, pH 7.0. After removing the nondissolved fraction by centrifugation, the enzyme solution was fractionated on a column (φ2.0 × 200 mm) of DEAE-Sepharose CL-6B. The column was eluted stepwise with 50, 100, 150, 200, 250, and 300 mM NaCl in 50 mM Tris–HCl buffer, pH 7.5. Then, the fractions containing L-AI activity were further purified by Native-PAGE gel extraction. The purified enzyme was used for the study of enzymatic properties. The enzyme purity and molecular weight were estimated by the SDS-PAGE method using trypsin inhibitor (20.1 kDa), carbonic anhydrase (29.0 kDa), ovalbumin (44.3 kDa), bovine serum albumin (66.4 kDa), and phosphorylase b (97.2 kDa) as standard proteins [16]. Meanwhile, the oligomerization state of the enzyme was examined by the Native-PAGE using CBM28-GFP (49.1 kDa) and Man-1 (116 kDa) as standard proteins.

The effect of pH and temperature on L-arabinose isomerase activity

The optimal pH of the L-arabinose isomerase was tested at 65 °C with different buffers at 50 mM. For the pH

ranges of 3.0–5.0, 6.0–7.0, 8.0–9.0, and 10.0, citrate, phosphate, Tris–HCl, and sodium carbonate buffers were used, respectively. The pH stability was determined by standard enzyme assay after preincubating the purified enzyme in buffers at different pH at room temperature for 1 h. Optimal temperature was tested between 45 and 95 °C by standard enzyme assay. Thermal stability was evaluated by performing the standard enzymatic assay after a 1-h incubation of the enzyme solution at different temperatures.

The effects of various metallic ions on L-arabinose isomerase activity

Before studying the effects of metal ions on L-AI activity, metallic ions were removed from the purified L-AI by treatment with 10 mM EDTA overnight at 4 °C, dialyzed against a 50 mM Tris–HCl buffer (pH 7.5). Then, the enzymatic activity was assessed in the presence of different metal ions ($MnCl_2$, $CoCl_2$, $MgCl_2$, $CaCl_2$, $CuSO_4$, and $BaCl_2$) with a final concentration of 5 mM. To determine the effect of Mn^{2+} and Co^{2+} concentration on enzyme activity, different cations at concentrations of 0.5–5 mM were added to the dialyzed enzyme, and L-AI activity was measured under standard conditions.

Determination of the kinetic parameters

The kinetic parameters of L-AI were determined in 50 mM phosphate buffer (pH 7.0) using D-galactose (25–600 mM) that was preincubated at 65 °C for 30 min. The K_m (mM) and V_{max} (U/mg protein) of L-AI were calculated by fitting the experimental data to the Michaelis–Menten equation.

Production of D-tagatose by L-AI

For the production of D-tagatose, 2 ml of L-AI was incubated with 4 ml 75 mM D-galactose in 50 mM phosphate buffer (pH 7.0) at 65 °C for 48 h to determine its conversion rate of D-galactose to D-tagatose. The concentrations of D-tagatose and D-galactose were determined by a HPLC system equipped with PSS831115 Waters Spherisorb column (operating temperature, 35 °C; 4.6 × 250 mm; 5 µm) and a UV detector (G4212-60008) using water as the mobile phase at a flow rate of 0.5 mL min^{-1}. 10 mg mL^{-1} of D-tagatose and D-galactose solutions were used as the standards.

Results

Isolation and identification of the *L. brevis*

The *L. brevis* used in this study was screened from fermented cabbage. Based on the morphology, as well as physiological and biochemical tests, the *L. brevis* was classified as the lactic acid bacteria. Besides, a BLAST sequence alignment of the 16S rDNA of *Lactobacillus brevis* ATCC14869 showed 99.67% similarity. The bootstrap analysis of the phylogenetic tree is shown in Fig. 1.

Enzyme purification and molecular weight

The crude enzyme extraction was first precipitated by selective fractionation with ammonium sulfate between 60 and 80% saturation. After centrifugation, the pelleted crude protein was dissolved, dialyzed, and fractionated on a DEAE-Sepharose CL-6B column as illustrated in the chromatogram shown in Fig. 2a. The peak eluted with 200 mM NaCl solution contained active enzyme that exhibited isomerization

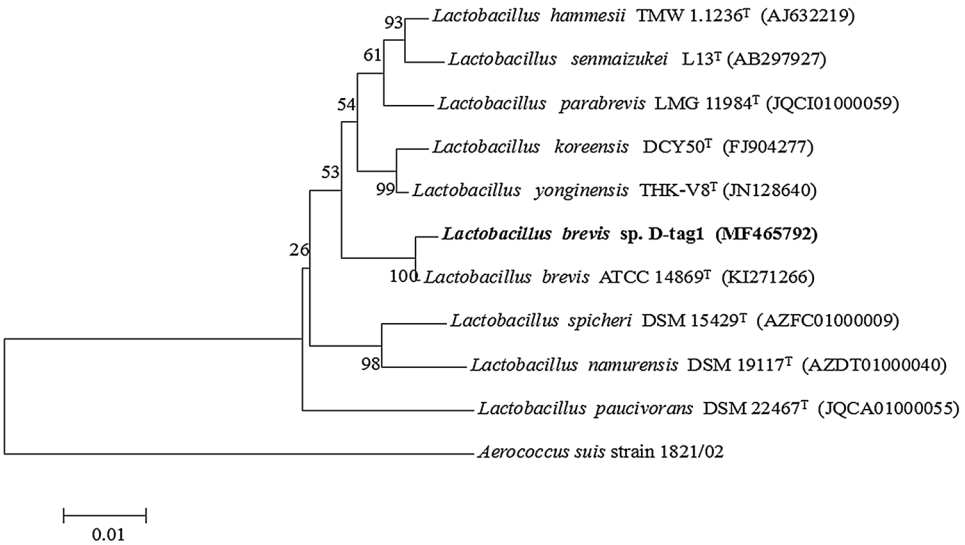

Fig. 1 The phylogenetic tree of *L. brevis*

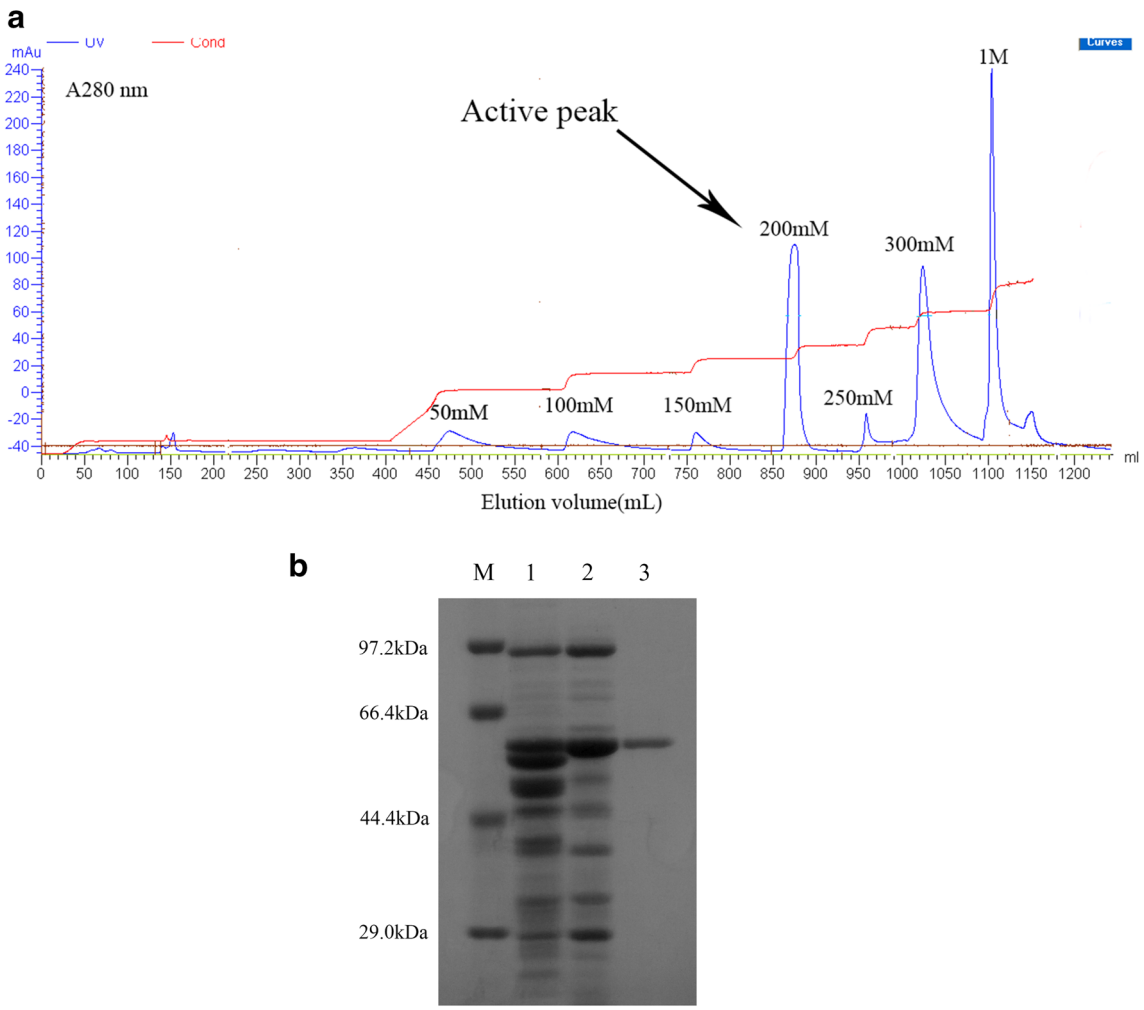

Fig. 2 **a** Purification of L-AI on DEAE-Sepharose CL-6B column, φ2.0×200 mm; elution buffer, 50, 100, 150, 200, 250, and 300 mM NaCl in 50 mM Tris–HCl buffer (pH 7.5). **b** SDS–PAGE analysis of L-AI from each purification step. *Lanes*: M, molecular standard marker; 1, cell extract after (NH4)2SO4; 2, sample from *lanes* 1 after DEAE-Sepharose CL-6B; 3, sample from *lanes* 2 after Native-PAGE

activity of D-galactose to D-tagatose, despite the relatively low purity as shown in Fig. 2b (Lane 2). Purified L-arabinose isomerase was obtained by employing Native-PAGE gel excision, as confirmed by the single band in SDS–PAGE (Lane 3 in Fig. 2b). The activity (yield) of pure enzyme (i.e., L-arabinose isomerase) was 0.1% of the total starting enzyme used in the purification, and the specific activity of the pure enzyme was 8.3 U/mg, which was increased approximately 83 fold (Table 1).

The molecular weight of the L-arabinose isomerase was determined from a standard curve, which was established by linear regression between the logarithm of the molecular weights of the standard protein samples and elution volumes (Fig. 3a). Native polyacrylamide gel electrophoresis showed

Table 1 Purification of the L-AI

Purification step	Total protein (mg)	Total activity (U)	Specific activity (U/mg)	Activity yield (%)	Purification fold
Crude extract	1898	191.7	0.1010	100.0	1.000
$(NH_4)_2SO_4$	415.8	44.08	0.1060	22.99	1.050
DEAE-Sepharose CL-6B	49.20	19.06	0.3874	9.943	3.836
Native-PAGE	1.500	12.50	8.333	6.520	82.50

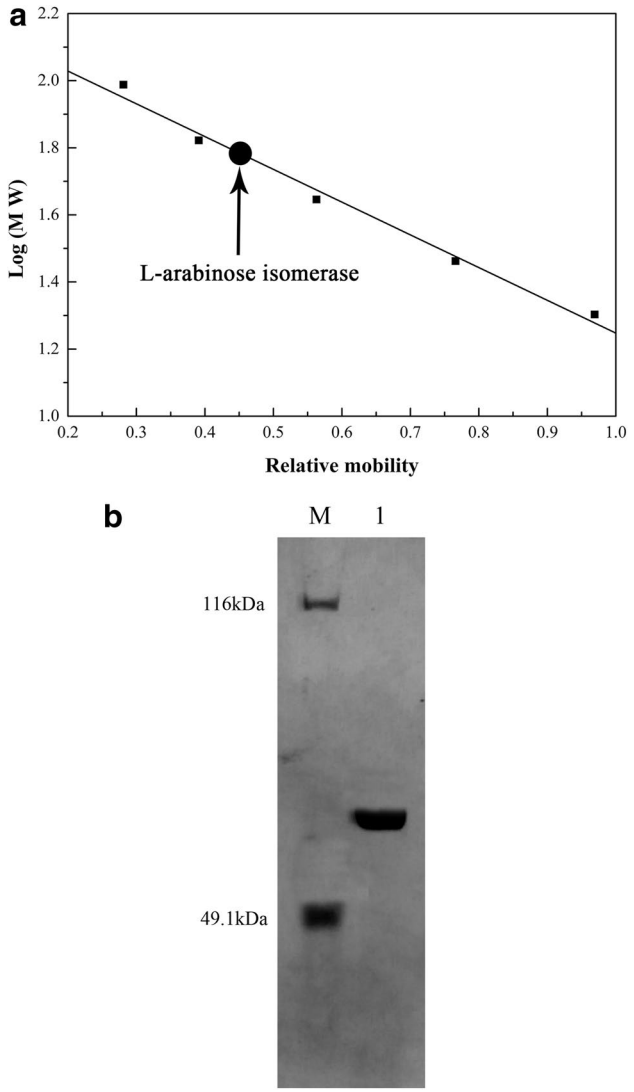

Fig. 3 **a** Subunit molecular weight of L-AI analysis by SDS–PAGE protein markers: trypsin inhibitor (20.1 kDa); carbonic anhydrase (29.0 kDa); ovalbumin (45 kDa); bovine serum albumin (66 kDa); phosphorylase b (97 kDa). **b** Native-PAGE analysis of L-AI after purification. *Lanes*: M, molecular standard protein; 1, the purified L-AI

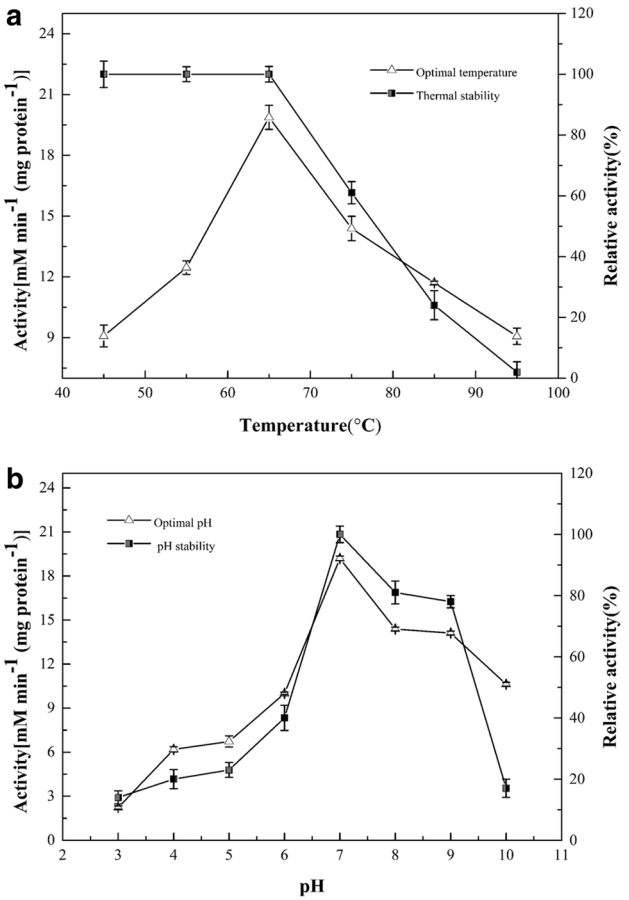

Fig. 4 **a** Optimal temperature (open triangles) and temperature stability (closed squares) of L-AI. Activities at optimal temperature were defined as 100%. Error bars represent the standard deviation from three separate experiments. **b** Optimal pH (open triangles) and pH stability (closed squares) of L-AI. Activities at optimal pH were defined as 100%. Error bars represent the standard deviation from three separate experiments

that the molecular weight of L-arabinose isomerase was larger than that of CBM28-GFP (49.1 kDa) and less than that of Man-1 (116 kDa) (Fig. 3b). Therefore, we conclude that the L-arabinose isomerase exists as a monomer with a molecular weight of 60.1 kDa.

The effect of temperature on activity and thermal stability

To determine the optimal temperature, the enzyme activity was measured by carrying out standard assays at different temperatures. As shown in Fig. 4a, the optimal temperature for the pure enzyme was 65 °C. The thermal stability profile demonstrated that the enzyme possessed high stability at 45–75 °C, whereas a rapid decrease in activity was observed at temperatures higher than 75 °C (Fig. 4a).

The effect of pH on enzyme activity and stability

To determine the optimal pH, the enzyme activity was measured by carrying out standard assays in different buffers with varying pH values, ranging from 3.0 to 10.0. As shown in Fig. 4b, the optimal pH for L-arabinose isomerase is 7.0. Our experimental data showed that the pure enzyme from *L. brevis* strain was stable in a narrow pH range from 8.0 to 9.0, whereas a rapid decline in activity was observed at pH below 6.0 or above 9.0 (Fig. 4b).

The effects of metallic ions on enzyme activity

The effects of various metallic ions, including Mn^{2+}, Co^{2+}, Mg^{2+}, Ca^{2+}, Cu^{2+}, and Ba^{2+}, on the activity of L-arabinose isomerase were investigated. We found that Ca^{2+}, Ba^{2+}, and Cu^{2+} ions inhibited the activity at the concentration of 5 mM, while Mg^{2+}, Mn^{2+}, and Co^{2+} ions strongly enhanced the enzyme activity (Fig. 5a). We further examined the effects of Mn^{2+} and Co^{2+} concentrations on the activity of L-arabinose isomerase. As the concentration of Mn^{2+} or Co^{2+} increased, we observed growing enzyme activity until it reached the maximum at 1 mM Mn^{2+} or Co^{2+}(Fig. 5b).

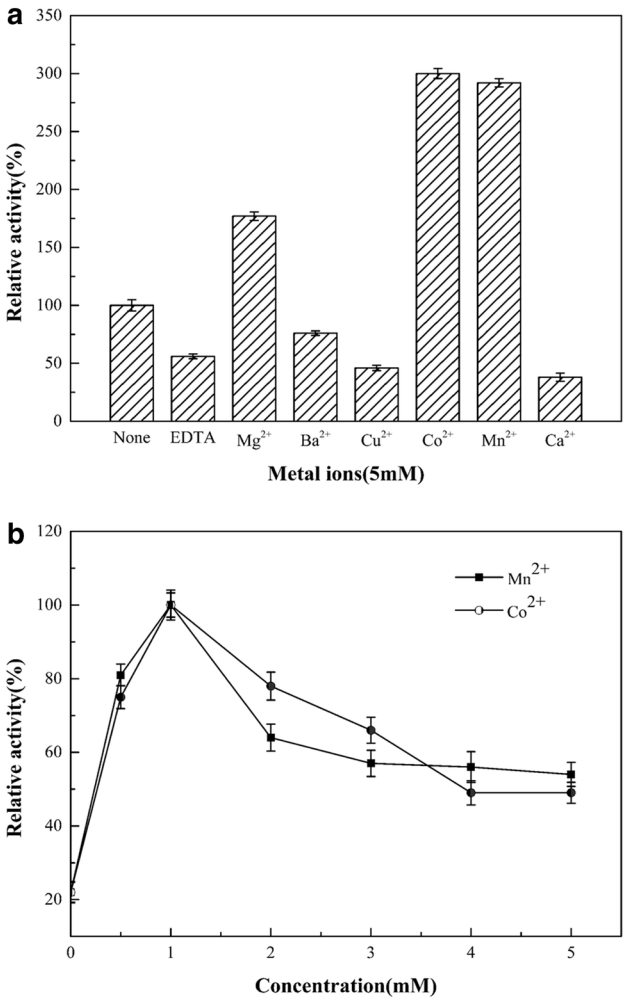

Fig. 5 **a** Effects of divalent ions on L-AI activities. Enzyme activity at the absence of metal ions was defined as 100%. Error bars represent the standard deviation from three separate experiments. **b** Effects of various concentrations of Mn^{2+} and Co^{2+} on L-AI activities. Activities at optimal metal ion concentrations were defined as 100%. Error bars represent the standard deviation from three separate experiments

The kinetic parameters of L-arabinose isomerase

The kinetic parameters of the L-arabinose isomerase were determined using D-galactose as the substrate. Activity was measured as described above. The kinetic parameters were calculated from the Michaelis–Menten model. The K_m and V_{max} values of the enzyme at pH 7.0 and 65 °C were 129 mM and 0.045 ± 0.0012 mM min^{-1}, respectively (Fig. 6).

Production of D-tagatose from D-galactose

To determine the production of the D-tagatose, the conversion rate was measured using D-galactose as the substrate containing 1 mM Co^{2+} or no metal ions. The results demonstrated that the rate of conversion of D-galactose to D-tagatose, in the absence of metal ions, was 31% at 65 °C; whereas in the presence of Co^{2+}, the rate of conversion was up to 43%.

Discussion

In this work, an L-AI from wild-type strain *L. brevis* (MF 465792) was successfully purified. The pure enzyme, although presented in low amount after Native-PAGE extraction exhibited an 83-fold increase in specific activity. The traditional enzyme purification methods using the ion-exchange chromatography and the gel filtration chromatography failed to yield pure L-AI. Hence, we used the Native-PAGE gel extraction method to purify the L-AI and obtained completely pure enzyme. The methodology presented here offers a convenient and timesaving approach to purify enzyme with high purity, which is not attainable using conventional chromatography methods.

The properties of the L-arabinose isomerases vary from various organisms. Up to now, a number of L-arabinose

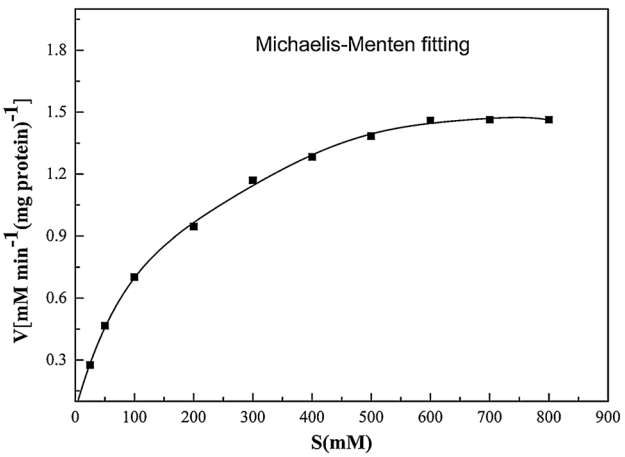

Fig. 6 Michaelis–Menten fitting plot of L-AI reaction

isomerases have been identified and obtained either from wild-type strains or from cloning and heterogeneous expressions. Table 2 reviews the biochemical characteristics of the L-AIs from various bacteria. In our study, the optimum temperature and pH of L-arabinose isomerase from *L. brevis* were determined to be 65 °C and pH 7.0. We found that the L-arabinose isomerase was perfectly stable below 65 °C, in the absence of metal ions, suggesting that this enzyme is remarkably thermostable. On the other hand, we found that slightly acidic or neutral conditions are beneficial for the production of D-tagatose, because a slightly acidic pH range could reduce browning and the formation of by-products [3]. The neutral optimum pH and the suitable optimum temperature make *L. brevis* L-AI a good candidate to produce D-tagatose under ideal industrial conditions.

Like most of the L-arabinose isomerase, the *L. brevis* L-AI can be activated in the presence of certain metal ions. We observed maximum activation of *L. brevis* L-AI in the presence of Mn^{2+} or Co^{2+} (Fig. 5a). In addition, Mg^{2+} can also activate *L. brevis* L-AI to some degree, but exhibited a lower enhancement of enzyme activity compared to Mn^{2+} or Co^{2+}. Upon the addition of 1 mM Mn^{2+} or 1 mM Co^{2+}, the enzyme activity dramatically increased at least threefold and the K_m increased to 73 mM instead of 129 mM (Figure not shown), indicating an increased affinity for D-galactose. In the presence of Cu^{2+}, however, the enzyme activity significantly decreased. These results indicate that there is an absolute requirement of metal ions for its catalytic activity [2].

The kinetic parameters of the wild-type *L. brevis* L-AI for L-arabinose isomerase were determined under optimal temperature and pH condition (pH 7.0, 65 °C). The apparent K_m, V_{max}, and catalytic efficiency kcat K_m^{-1} of *L. brevis* L-AI using D-galactose as a substrate were 129 mM, 0.045 ± 0.0012 mM min^{-1}, and 0.12 min^{-1} mM^{-1}, respectively. In addition, Table 3 summarizes the kinetic parameters of L-AIs isolated from various organisms. Compared with most L-AIs from heterologous expression, the *L. brevis* L-AI does not exhibit a very high catalytic efficiency. Although a direct comparison is difficult because of the different reaction temperatures, *L. brevis* L-AI appears to have a lower K_m and a higher efficiency than *B. coagulans* NL01 [4] L-AI and *B. longum* [29] L-AI according to the previous reports. The apparent K_m of *B. coagulans* NL01 and *B. longum* L-AI were 355.1 mM and 590 mM, respectively. Moreover, the *L. brevis* is a type of probiotic strain, which is considered as a GRAS strain. Therefore, the *L. brevis* L-AI is well-suited to produce D-tagatose, which is mainly used as a food additive, to ensure the safety of D-tagatose production.

Table 2 Biochemical characteristics of L-AIs from various organisms

Organism	Optimum temperature (°C)	Optimum pH	Metal requirement for enzyme activity	Metal inhibitors	References
L. brevis	65	7.0	Mn^{2+}, Co^{2+}, Mg^{2+}	Cu^{2+}, Ca^{2+}	This study
L. fermentum CGMCC2921[a]	65	6.5	Mn^{2+}, Co^{2+}	Cu^{2+}	[17]
L. plantarum NC8[a]	60	7.5	Mn^{2+}, Co^{2+}	Cu^{2+}	[18]
L. plantarum WU14[a]	60	7.17	Mn^{2+}	Cu^{2+}, Zn^{2+}	[19]
Thermus sp. IM6501[a]	60	8	Mn^{2+}	Zn^{2+}, Ni^{2+}	[20]
B. stearothermophilus US100[a]	80	7.5	Mn^{2+}, Co^{2+}	Cu^{2+}	[21]
B. stearothermophilus IAM 11001[a]	65	7.5	Mn^{2+}	Ca^{2+}, Ba^{2+}	[22]
A. flavithermus[a]	95	10.5	Ni^{2+}	Cu^{2+}, Fe^{3+}	[23]
T. maritima[a]	90	7.5	Mn^{2+}, Co^{2+}	Ca^{2+}, Fe^{2+}, Ni^{2+}	[24]
B. licheniformis[a]	50	7.5	Mn^{2+}, Co^{2+}	Cu^{2+}, Hg^{2+}	[25]
B. thermoglucosidasius[a]	40	7.0	Mn^{2+}	Cu^{2+}, Zn^{2+}, Fe^{2+}	[26]
T. neapolitana[a]	85	7.0	Mn^{2+}, Co^{2+}	Cu^{2+}, Zn^{2+}	[27]
B. coagulans[a]	70	7.0	Mn^{2+}	Cu^{2+}, Zn^{2+}	[28]
P. pentosaceus PC-5[a]	50	6.0	Mn^{2+}, Co^{2+}	Cu^{2}	[3]
S. flexneri[a]	40	8.0	Mn^{2+}, Co^{2+}	Hg^{2+}	[7]
B. coagulans NL01[a]	60	7.5	Mn^{2+}, Co^{2+}	Cu^{2+}	[4]
B. longum[a]	55	6.0	Mn^{2+}, Mg^{2+}	Fe^{2+}	[29]
A. cellulolytics ATCC 43068[a]	75	7.5	Mn^{2+}, Co^{2+}	–	[8]
L. sakei 23K[a]	30–40	5.0–7.0	Mn^{2+}, Mg^{2+}	–	[30]
L. gayonii[b]	35	6.0–7.0	Mn^{2+}	Cu^{2+}, Hg^{2+}	[31]
L. plantarum SK-2[b]	50	7.0	Mn^{2+}, Fe^{3+}, Fe^{2+}, Ca^{2+}	Cu^{2+}, Ag^{+}, Hg^{2+},Pb^{2+}	[13]

[a]The L-AIs were obtained from cloning and heterogeneous expression

[b]The L-AIs were obtained from wild-type strains

Table 3 Kinetic parameters of L-AIs from various organisms

Organism	Reaction temperature (℃)	D-galactose			References
		K_m (mM)	V_{max} (U mg^{-1})	kcat K_m^{-1} (min^{-1} mM^{-1})	
L. brevis	65	129	1.7	0.12	This study
L. fermentum CGMCC2921[a]	65	60	9.8	9.0	[17]
L. plantarum NC8[a]	60	69.7	7	1.6	[18]
B. stearothermophilus US100[a]	80	57	8.9	8.84	[21]
A. flavithermus[a]	95	25.19	–	5.16	[23]
T. maritima[a]	90	60	8.9	8.5	[24]
B. thermoglucosidasius[a]	40	175	–	2.8	[26]
T. neapolitana[a]	85	250	14.3	3.24	[27]
P. pentosaceus PC-5[a]	50	66	7.8	2.9	[3]
S. flexneri[a]	40	104.8	2.02	0.104	[7]
B. coagulans NL01[a]	60	355.1	6.8	1.0	[4]
B. longum[a]	55	590	7.7	0.72	[29]
A. cellulolytics ATCC 43068[a]	75	28.9	4.9	9.3	[8]
L. sakei 23K[a]	30–40	59	76	10.3	[30]
L. plantarum SK-2[b]	50	119	–	–	[13]

[a]The L-AIs were obtained from cloning and heterogeneous expression

[b]The L-AIs were obtained from wild-type strains

Up to now, there have been few reports about the purification and characterization of L-AIs from wild-type strains. Nakamatu et al. [31] reported that the *L. gayonii* L-AI has optimum activity at 35 °C, pH 6.0–7.0. Besides, the *L. gayonii* L-AI requires Mn^{2+} for its activity, and the Cu^{2+} and Hg^{2+} ions appear to be the inhibitors. But the apparent K_m and other parameters using D-galactose as a substrate were not determined in this paper. In addition, the characterization of *L. plantarum* SK-2 L-AI [13] showed that its optimum temperature and pH were 50 °C and pH 7.0. The *L. plantarum* SK-2 L-AI exhibited high activity in the presence of Mn^{2+}, Fe^{3+}, Fe^{2+}, and Ca^{2+}, whereas metal ions including Cu^{2+}, Ag^{+}, Hg^{2+}, and Pb^{2+} inhibited the enzyme activity. The apparent K_m indicated that the *L. plantarum* SK-2 L-AI catalyzes the conversion of D-galactose to D-tagatose similarly as the *L.brevis* L-AI (Table 3). However, the catalytic efficiency of kcat K_m^{-1} and the V_{max} of the *L. plantarum* SK-2 L-AI were not reported. In this report, we conducted a systematic characterization for the *L. brevis* L-AI, and determined its optimal reaction conditions and the kinetic parameters.

Generally, a major index in a biotransformation process is the conversion efficiency. Table 4 reviews the conversion rate of L-AIs from various organisms. Compared with previous studies of L-AIs, the conversion yield of *L. brevis* L-AI was higher. Under the optimal conditions, *L. brevis* converted the D-galactose (50 mM) into D-tagatose at a high

Table 4 Conversion rate of L-AIs from various organisms

Organism	Reaction temperature (°C)	Reaction time (h)	Metal ions (mM)	Conversion rate (%)	Concentration of D-galactose (mM)	References
L. brevis	65	23	1 Co^{2+}	43	50	This study
S. flexneri	40	24	1 Mn^{2+}	22.3	50	[7]
Thermus sp. IM6501	60	72	5 Mn^{2+}	54	6	[20]
L. plantarum SK-2	35	96	–	39	550	[13]
B. thermoglucosidasius	40	30	1 Mn^{2+}	45.6	200	[26]
B. stearothermophilus US100	70	7	0.2 Co^{2+}, 1 Mn^{2+}	48	28	[21]
B. stearothermophilus IAM 11001	65	12	1 Mn^{2+}	36	50	[22]
L. sakei 23K	45	7	0.8 Mn^{2+}, 0.8 Mg^{2+}	36	50	[30]

conversion rate of 43% after 23 h at 65 °C, in the presence of 1 mM Co^{2+}, and no by-product was observed. Under the same conditions, Xu et al. [6] reported that the equilibrium between D-galactose and D-tagatose was about 35.4%. With *S. flexneri* [7] and *B. stearothermophilus* IAM 11001 [22] L-AIs, the conversion rates of D-galactose were 22.3% at 40 °C and 36% at 65 °C, respectively. These results imply that the *L. brevis* L-AI catalyzes the conversion of D-galactose into D-tagatose more efficiently.

Taken together, this is, to our knowledge, the first time that the L-AI from *L. brevis* has been purified and characterized. We established an efficient and robust purification protocol for L-AI, which could be widely applied to other enzymes. Our work opened up an exciting and appealing way for D-tagatose production.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with animals performed by any of the authors.

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