Electronic Supplementary Material (ESI) for Chemical Communications

Supporting Information

Supramolecular stabilization of the acid tolerant L-arabinose isomerase from the food-grade *Lactobacillus sakei*

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Contents:

Experimental details:

Synthesis of Noria and NoriaPG

The synthesis of Noria was carried out according to the method described by Kudo et al. [1]. To synthesize NoriaPG, the resorcinol used in the method of Kudo et al. [1] was replaced by pyrogallol, as described below:

A mixture of pyrogallol (20 mmol) and 1,5-pentanedial (4.0 mmol) in ethanol was stirred at 80 °C for 48 h in the presence of concentrated HCl (3.0 mL), then poured into a large amount of diethyl ether to precipitate a white solid. The solid was washed with diethyl ether several times and then dried *in vaccuo* at 60 °C for 12h.

NoriaPG analysis

IR (film): υ =3202 (υO-H), 2932 (υC-H), 1601, 1503, and 1435 cm^{-1} (υC=C aromatic). ^{1}H NMR (500 MHz, [D6]DMSO, tetramethylsilane): δ = 0.96-2.15 (m, 36H, -CH2CH2CH2), 4.28 (m, 12H, >CH-), 7.25 (m, 6H, Ar-H), 8.8–9.56 ppm (m, 36H, OH); MALDI-TOF-MS: m/z $[M+H]^{+}$ calculated for ($C_{102}H_{96}O_{36}$+H): 1898.8, found: 1900.3.

Bacterial strains, plasmids and media

Escherichia coli BL21 was grown in Luria Bertani (LB) medium supplemented, when necessary, with ampicillin (100 µg/ml) and IPTG at 160 µg/ml. The pMR36 plasmid encoding the *Lactobacillus sakei* L-AI gene was previously reported [2].

Crude cell-lysate preparation and enzyme purification

Escherichia coli strain (MRS36) was grown as indicated above until OD_{600} of 1.0, the culture was induced overnight using IPTG (160 µg/ml) followed by harvesting of the cells by centrifugation (7500 × g, for 10 min at 4 °C). Pellets were resuspended in 100 mM sodium acetate buffer pH 5.0. The cell suspensions were incubated for 2 h on ice in presence of 5 mg/ml lysozyme and one Complete™ protease inhibitor cocktail tablet (Roche®). Cell

disruption was carried out by sonication at 4 °C for 2 min (pulsations of 3 s, amplify 90) using a Vibra-Cell™ 72405 Sonicator and cell debris were removed by centrifugation (30 000 × g, for 30 min at 4 °C). The protein was purified as previously described by Rhimi et al. [2].

Protein quantification, electrophoresis and molecular mass determination

Protein concentration was determined using Bradford's method with bovine serum albumin as the standard [3]. The protein samples were separated in 12% SDS-PAGE according to the Laemmli method [4].

Enzyme assay

L-Arabinose isomerase activity was measured by determining the amount of L-ribulose or D-tagatose formed. Under standard conditions, the reaction mixture contained 50 µl of 1 mg/ml enzyme preparation, 50 mM of L-arabinose (D-galactose) and 100 mM sodium acetate buffer (pH 5.0) in order to bring the final volume to 1 ml. The reaction mixture was incubated for 2 min at 35 °C in the presence of L-arabinose (or D-galactose) and the reaction was stopped by heating the mixture at 99 °C for 5 min. Then, the amount of L-ribulose (or D-tagatose) was determined colorimetrically using the cystein-carbazol-sulphuric-acid method and the absorbance was measured at 560 nm [5].

Temperature, pH and thermostability profiles

The effect of temperature on the activity was determined by incubating the purified enzyme at temperatures ranging from 4 to 55 °C. The *L. sakei* L-AI pH profile was obtained by measuring the activity at various pH values ranging from 3 to 8.5 (3 to 5 with sodium acetate buffer, 6 to 7 with MES buffer and 7.5-8.5 with bicine buffer). The enzyme stability as a function of temperature and pH was investigated by incubating the enzyme at different temperature and pH, withdrawing the samples at defined intervals, placing them on ice and measuring residual activity at 35 °C and pH 5.0 during 2 min.

Supporting Figures:

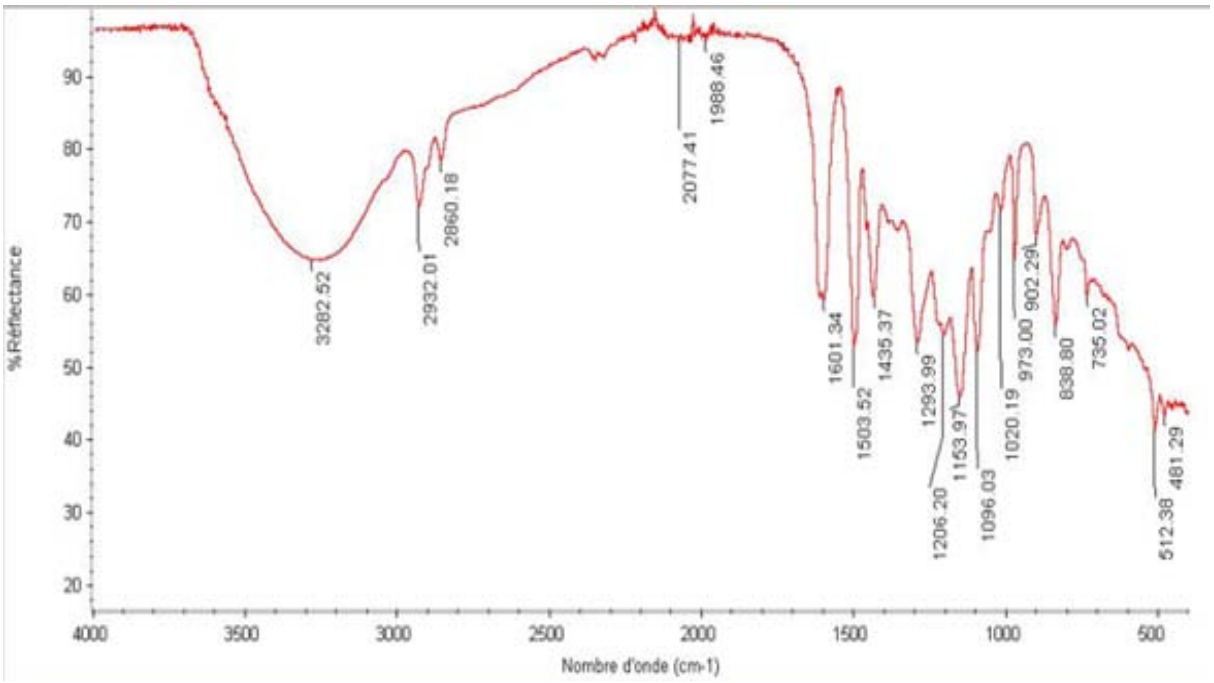

Fig. S1 IR Spectrum of NoriaPG

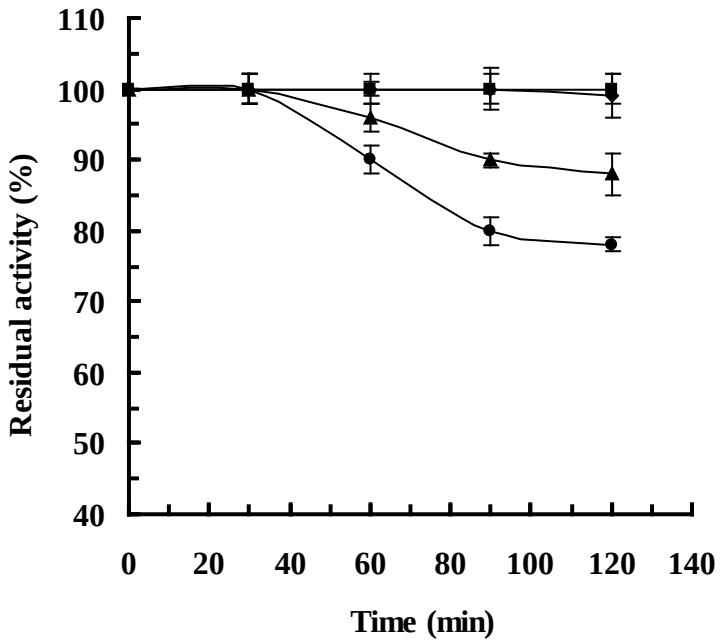

Fig. S2. Thermostability profiles of the *L. sakei* L-AI in presence of 0.8 mM Mg^{2+} and 0.8 mM Mn^{2+}. (■): 35 °C, (♦): 40 °C, (▲): 45 °C and (●): 50 °C. The initial activity was defined as 100% and corresponds to 218 U/mg. Error bars represent the standard deviation from three separate experiments.

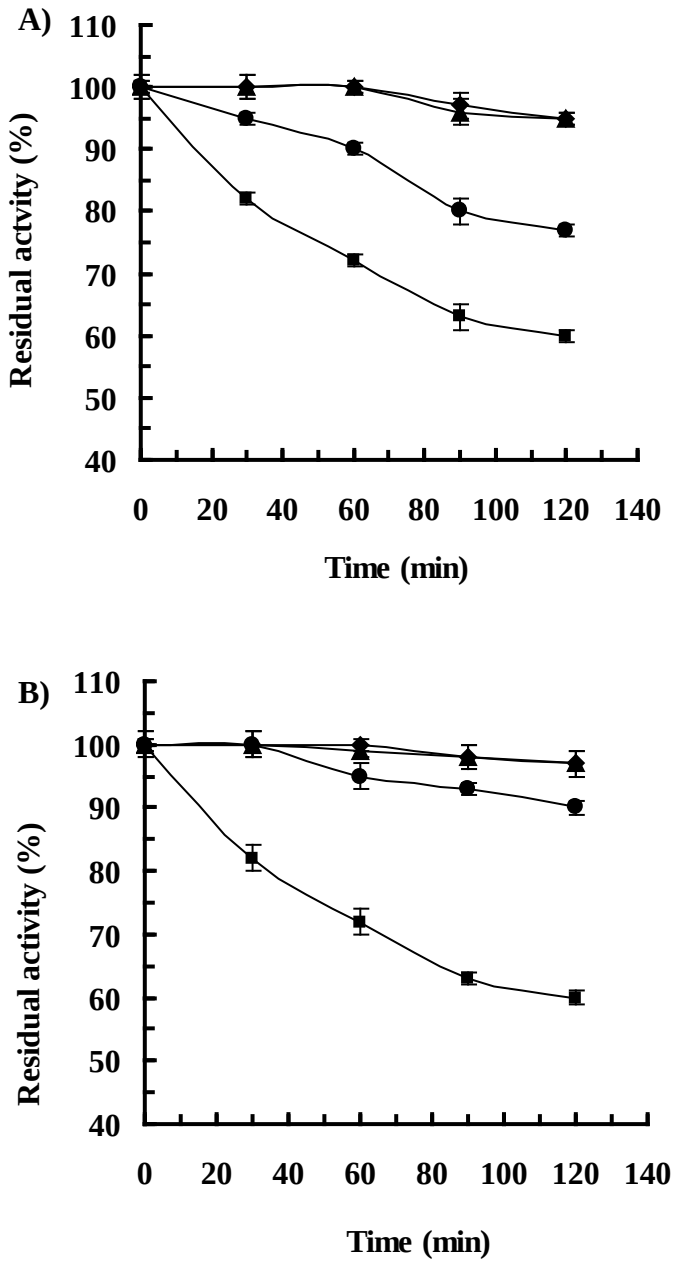

Fig. S3. Effect of Noria (A) and NoriaPG (B) concentrations on the thermostability of the *L. sakei* L-AI. (■): free enzyme, (●): 0.5 mM, (▲): 1 mM and (♦): 1.5 mM. Studies were carried out at 45 °C and pH 5.0. Error bars represent the standard deviation from three separate experiments.

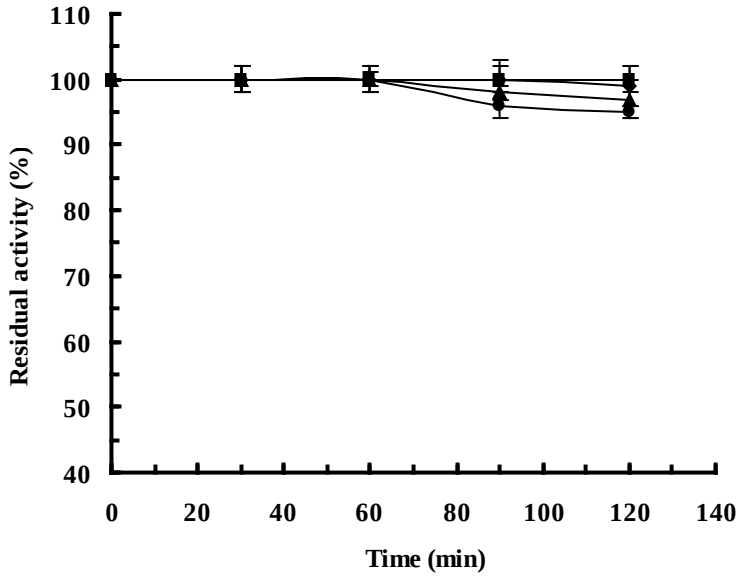

Fig. S4 . Thermostability profiles of the *Shewanella* sp ANA-3 L-AI in presence of 1mM, Noria, 1 mM NoriaPG, 0.8 mM Mg^{2+} and 0.8 mM Mn^{2+}. (■): 35 °C, (♦): 40 °C, (▲): 45 °C and (●): 50 °C. The initial activity was defined as 100% and corresponds to 218 U/mg at pH 5.0. Error bars represent the standard deviation from three separate experiments.

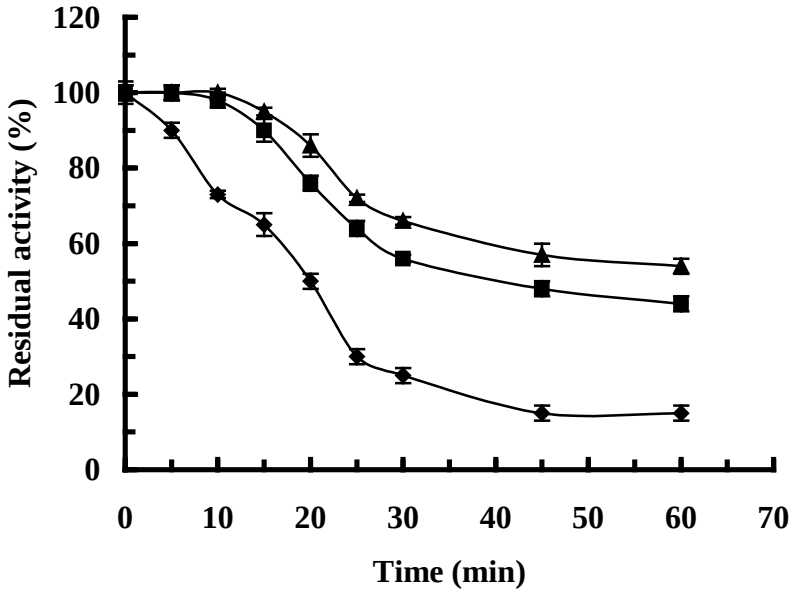

Fig. S5 . Thermostability profile of the Q268K US100 L-AI in presence of (♦): 0.2 mM Co^{2+} and 1 mM Mn^{2+}, (■): 1 mM NoriaPG, (▲): 1 mM NoriaPG, 0.2 mM Co^{2+} and 1 mM Mn^{2+}. Reaction was done at 80 °C and pH 6.0 as previously reported [6]. The initial activity was defined as 100% and corresponds to 185 U/mg. Error bars represent the standard deviation from three separate experiments.

References and Note

[1] H. Kudo, R. Hayashi, K. Mitani, T. Yokozawa, N.C. Kasuga, T. Nishikubo, *Angew. Chem. Int. Ed.*, 2006, **45**, 7948 -7952.

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