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Supporting Information

A new L-arabinose isomerase with copper ion tolerance is suitable for creating protein-inorganic hybrid nanoflowers with enhanced enzyme activity and stability

Zheng Xu,[ψ1,2] Rui Wang,[ψ1,2] Chao Liu,[1,2] Bo Chi,[1,2] Jian Gao,[3] Beining Chen,[4] Hong Xu*[1,2]

Materials and Methods

Bacteria screening

To obtain the bacteria containing L-arabinose isomerase, we screened approximately 500 candidate microorganisms. Among them, the No. 222 bacterium showed the highest enzyme activity for the conversion from L-arabinose to L-ribulose. This bacterium was identified to be gram-positive bacterium *Paenibacillus polymyxa* by 16s rDNA sequencing and the result was set for BLAST in the GenBank. This strain was renamed as strain NX-1 and preserved in our laboratory.

Gene cloning and expression

The encoding gene of *Paenibacillus polymyxa* AI (PPAI) was amplified through polymerase chain reaction (PCR) from *Paenibacillus polymyxa* NX-1. To express this gene in *E. coli*, the PCR product was double digested by NdeI and XhoI, and ligated with the same digested pET-28a vector. The ligation product was transformed into the competent cells of *E. coli* BL21 (DE3). The recombinant PPAI enzyme was purified to homogeneity by nickel affinity and gel filtration chromatography. This enzyme showed optimal reaction temperature and pH at 50 °C and 7.5, respectively.

Preparation of protein-inorganic hybrid nanoflowers

To synthesize the protein-inorganic hybrid nanoflowers, 20 μL aqueous $CuSO_4$ solution (120 mM) was added to 3 mL of PBS (pH 7.4) buffer containing proteins with different concentrations in a tube, followed by incubation at 25 °C for three days. The precipitation was shown at the bottom of the tube. Before SEM analysis, the precipitation was filtered and dried on a 0.22 μm membrane and sputter-coated with gold. The protein concentration was measured by Bradford protein assay using bovine serum albumin (BSA) as standard (1976). To create nanoflowers using different divalent ions, the equal amount of ions were respectively mixed with protein solution and stillness for three days until nanoflowers appear.

Catalytic stability of hybrid nanoflowers

To determine the thermal stability of the hybrid nanoflowers, they were incubated at high temperatures (50, 55, and 60 °C) for 20 min. Samples were withdrawn for certain time intervals and the residual activity was determined by the cysteine-carbazole-sulfuric acid method. To determine the protein leakage of nanoflowers after long time usage (0~100 h), the protein concentration in the supernatant was determined by the Bradford method (Bradford 1976).

Batch production of L-ribulose and D-tagatose by hybrid nanoflowers

To test the catalytic stability of PPAI hybrid nanoflowers, the nanoflowers were used for batch production of rare sugars. After every batch, the nanoflowers were recovered by filtration using a 0.22 μm filter. The concentrations of formed L-ribulose and D-tagatose were determined by HPLC.

Enzyme activity assay

The enzymatic activities of PPAI free enzyme and PPAI-inorganic hybrid nanoflowers were measured by determining the formation of L-ribulose and D-tagatose. The reaction was performed in 2 mL system with 0.1 mg PPAI, 1 mM Mn^{2+}, and 50 mM L-ribulose (or D-galactose) for 30 min at 40 °C and pH 7.5. The L-ribulose (or D-tagatose) concentration was determined by the cysteine-carbazole-sulfuric acid method or by HPLC using a Rezex RCM-Monosaccharide column (operating temperature: 70 °C) and a refractive index detector (SHODEX RI-101) using water as the mobile phase at a flow rate of 1 mL min^{-1}. The concentration of lactose and D-galactose were also determined by HPLC. The standards used were 10 g L^{-1} solutions of each sugar (Sigma, USA).

Table S1. Effect of different metal ions on the activity of PPAI.

Metal ion and EDTA	Relative activity (%)	
	1mM	10 mM
None	100 ± 2	100 ± 1
EDTA	117 ± 1	128 ± 2
$ZnCl_2$	95 ± 1	62 ± 1
$MgCl_2$	110 ± 3	124 ± 2
$MnCl_2$	108 ± 1	102 ± 1
$CoCl_2$	135 ± 2	116 ± 1
$CaCl_2$	113 ± 2	133 ± 2
$CuCl_2$	120 ± 1	62 ± 1
$NiCl_2$	120 ± 1	110 ± 1
$BaCl_2$	128 ± 2	156 ± 2

Table S2-A. The k_{cat} values of immobilized enzyme over the pH range (reaction temperature was 50 °C, with the addition of 1 mM Mn^{2+})

pH	Enzyme activity (%)	k_{cat} (min^{-1})
5.0	62	5092
6.0	71	5703
7.0	81	6300
7.5	100	9184
8.0	92	8680
9.0	90	8407
10.0	89	8334

Table S2-B. The k_{cat} values of immobilized enzyme over the temperature range (reaction pH was 7.5, with the addition of 1 mM Mn^{2+})

Temperature (°C)	Enzyme activity (%)	k_{cat} (min^{-1})
20	31	1930
30	70	5235
40	87	8447
50	100	9184
60	50	4438
70	40	3061
80	20	1056

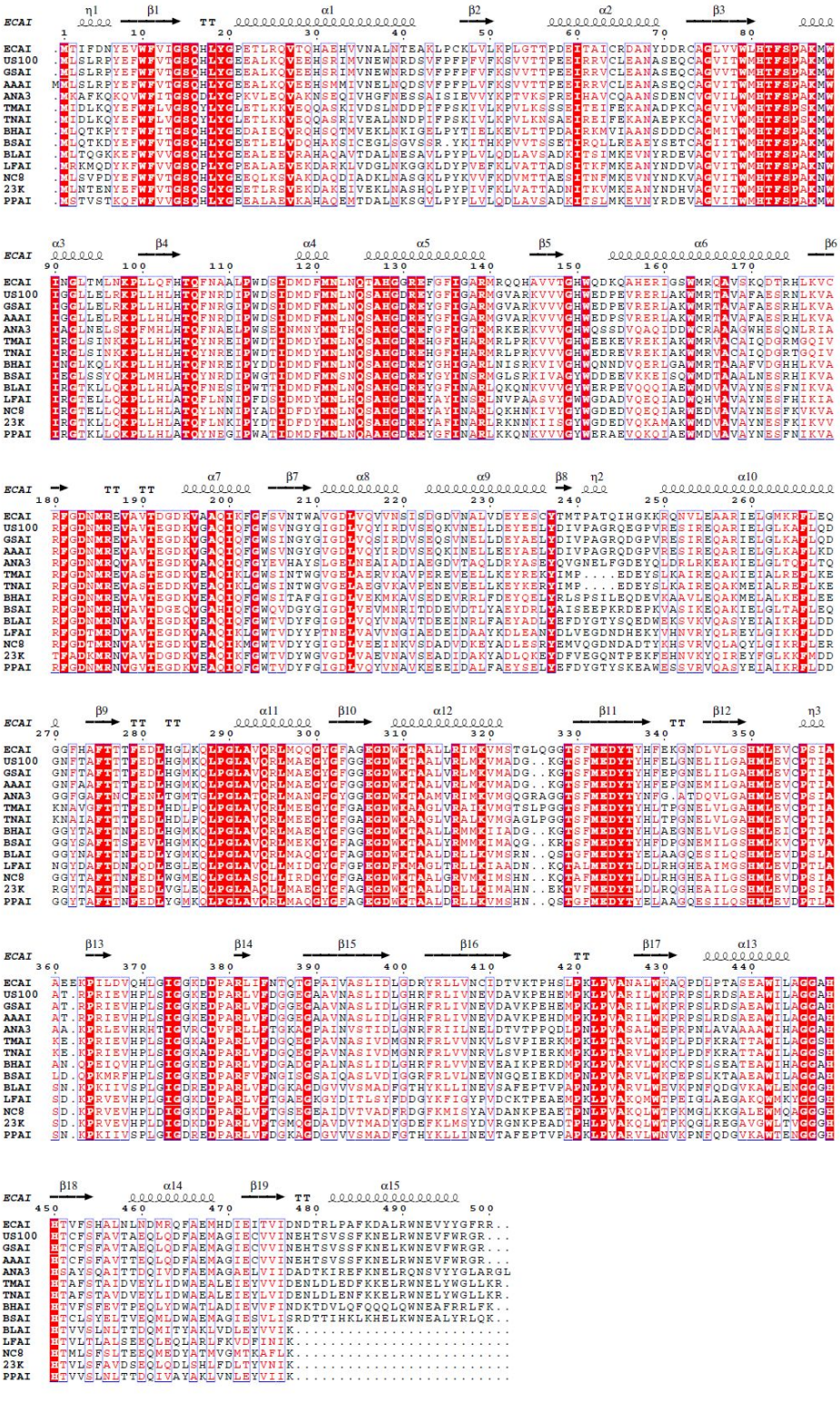

Fig. S1 Multiple sequence alignment of AIs from *E. coli* (ECAI, YP_488368.1), *B. stearothermophilus* US100 (BSAI US100, CAI29261.1), *G. stearothermophilus* (GSAI, Genbank accession number: ABY84698.1), *A. acidocaldarius* (AAAI, AAY68209.1), *Shewanella* sp. ANA-3 (SSAI ANA-3, ABK48296.1), *T. maritima* (TMAI, NP_228089.1), *T. neapolitana* (TNAI, AAK18729.1), *B. halodurans* (BHAI, WP_010898034.1), *B. subtilis* (BSAI, ACT82395.1), *B. licheniformis* (BLAI, KFM93600.1), *L. fermentum* CGMCC2921 (LFAI, ADJ94948.1), *L. plantarum* NC8 (NC8, CAM91953.1), *L. sakei* 23K (23K, YP_396468.1), and *P. polymyxa* (PPAI, this study). The alignment was carried out using Clustal X.

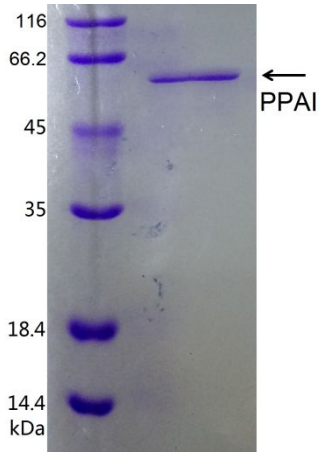

Fig. S2 The SDS-PAGE of purified PPAI enzyme. The molecular weight (kDa) of the protein was showed.

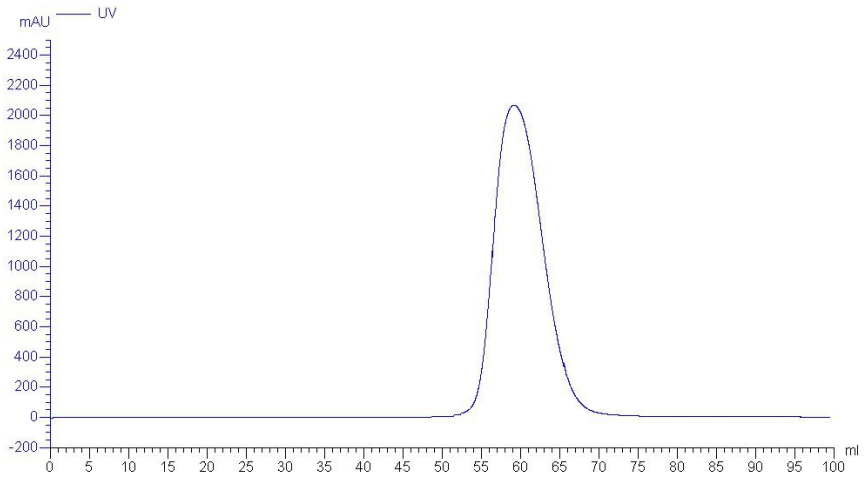

Fig. S3-A The gel filtration profile of purified recombinant PPAI enzyme

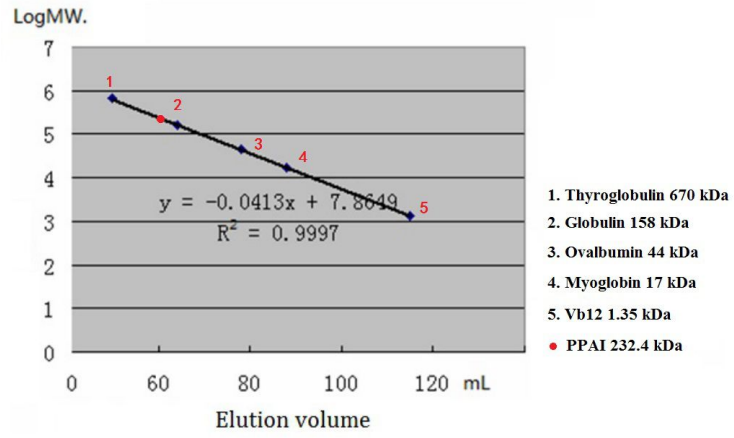

Fig. S3-B Molecular weight calculation of purified recombinant PPAI enzyme

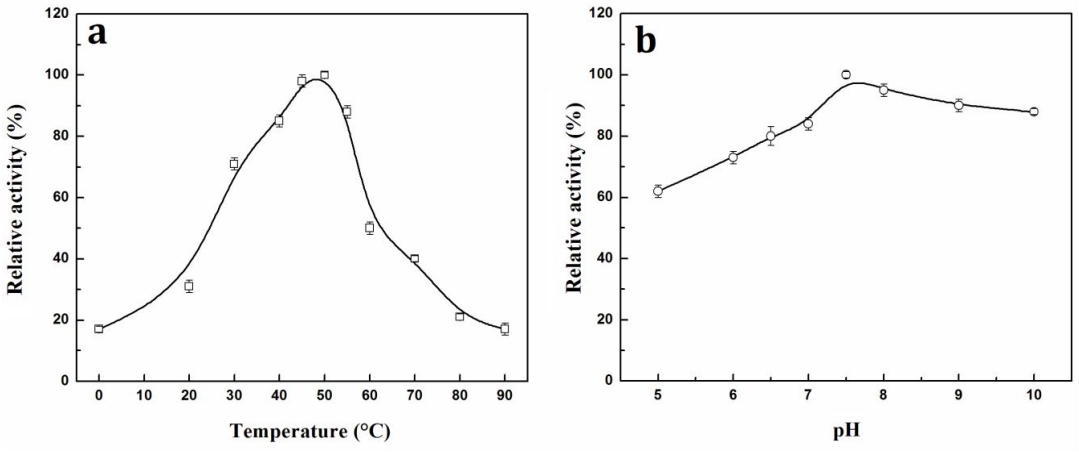

Fig. S4 Effect of temperature (a) and pH (b) on the activity of PPAI. Activities at the optimal temperature and pH were defined as 100%. Error bars represent the standard deviation from three separate experiments.

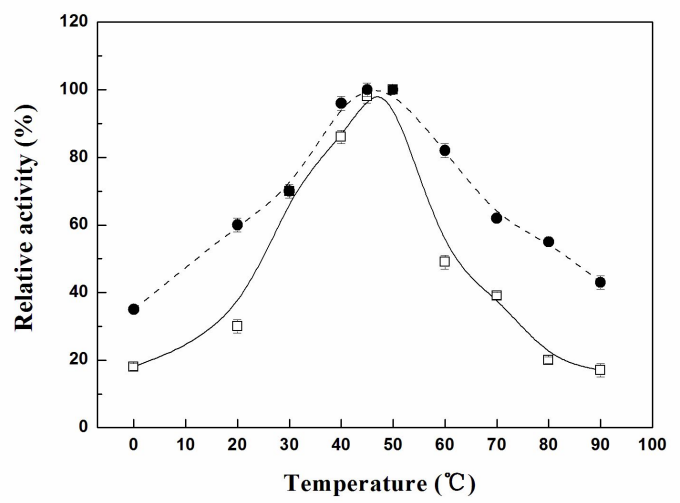

Fig. S5-A Effect of temperature on the activity of free (□) and immobilized (●) PPAI. Activities at the optimal temperature were defined as 100%. Error bars represent the standard deviation from three separate experiments.

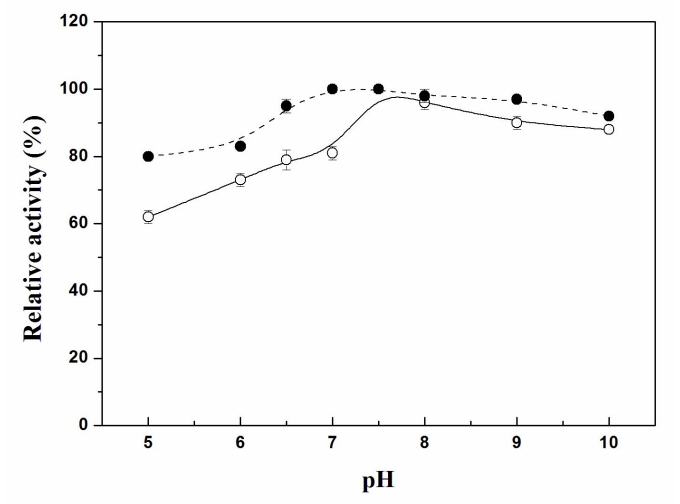

Fig. S5-B Effect of pH on the activity of free (○) and immobilized (●) PPAI. Activities at the optimal pH were defined as 100%. Error bars represent the standard deviation from three separate experiments.

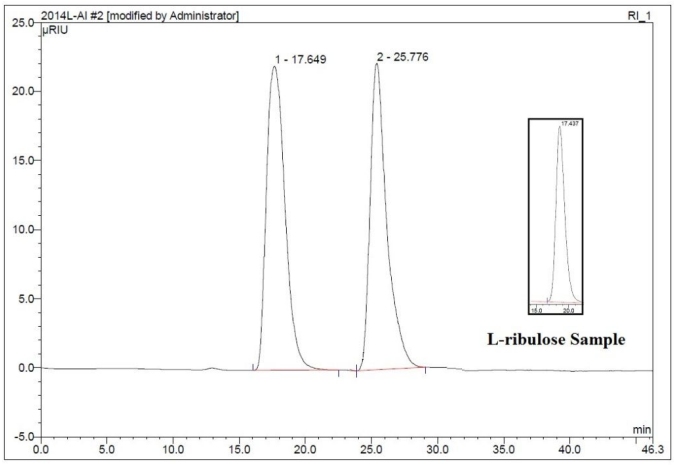

Fig. S6 HPLC analysis of produced L-ribulose (or D-tagatose) and comparison with standards. Peak 1: L-ribulose (retention time: 17.649 min), peak 2: L-arabinose (25.776 min). The retention time of L-ribulose standard from Sigma is 17.437 min.

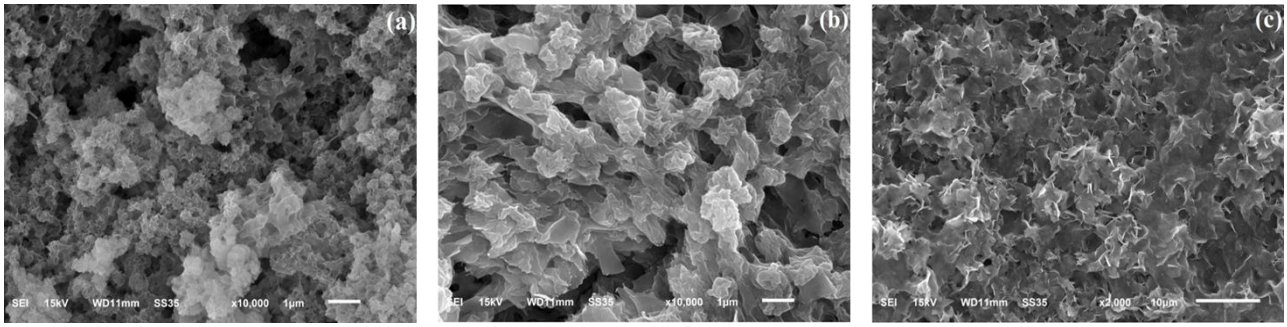

Fig. S7 The SEM images of (a) $CaCl_2$, (b) $CoCl_2$, and (c) $MnCl_2$ made precipitates containing PPAI enzyme (0.1 mg mL^{-1}).