Supporting information

Molecular Engineering of *Bacillus paralicheniformis* Acid Urease to Degrade Urea and Ethyl Carbamate in Model Chinese Rice Wine

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Single SSM and double mutagenesis library construction:

Plasmid pRSFDuet-Bpure-2 was used as the template, which was constructed in our previously work[1]. For single SSM mutagenesis library construction, such as Ala166 mutation library: PCR reaction system was composed of 25 μL of 2× PrimerSTAR HS Premix (TAKARA, JAPAN), 1.0 μL of the forward primer A166-F (10 μM), 1.0 μL of the reverse primer A166-R (10 μM), 1.0 μL of the template (1 μg/mL), and 22 μL of double-distilled water. PCR conditions were as follows: 98 ^{o}C for 30 s; 98 ^{o}C for 10 s, 60 ^{o}C for 5 s, 72 ^{o}C for 1 kb/min; 30 cycles, final extension at 72 ^{o}C for 10 min. After PCR reaction, 1 μL of DpnI (TAKARA, JAPAN) was added to the mixture, and then incubated at 37 ^{o}C for 30 min to digest the template. After digesting, PCR products were directly transformed into BL21(DE3) to construct recombinant strains that harboring the mutated plasmids. For double mutagenesis construction, L287I-F, L287I-R and L287N-F, L287N-R were used as primer and the plasmid that harboring the Leu253Pro mutation was used as template for construction of the double mutants L253P/L287I and L253P/L287N, respectively. The following procedures were the same as the procedures of the single SSM mutagenesis library construction.

Determination of the molecular masses and k_{cat} value of BpUrease and its variants

The BpUrease and its variants were purified using a StrepTrapTM HP affinity column, as described in our previously work[1]. Then, 500 μL of the purified BpUrease or

the standard molecular weight protein myoglobulin (17 kDa), ovalbumin (44 kDa), albumin (66 kDa), lgG (158 kDa) and ferritin (440 kDa) was applied to the HiLoad 16/600 Superdex 200 pg (GE Healthcare Life Sciences) column. Subsequently, they were eluted by 10 mM phosphate buffer, 0.15 M NaCl, pH 7.4, at the speed of 0.5 mL/min. The standard curve was made by taking the logarithm of the molecular weight (lgMr) of standard protein as the ordinate and the elution volume as the abscissa, which was y = -4.2253x + 4.5078, where y was lgMr, x was $V_e/120$, V_e was the elution volume (mL) and the column volume was 120 mL. The V_e of BpUrease and its variants were 61.7 mL (Fig. S1), which means the molecular weight of BpUrease and its variants were 216 kDa. This result was consistent with the results of the native-PAGE in our previous work[1].

Determination of the k_{cat} values of BpUrease and its variants

The k_{cat} values was calculated by equation $k_{cat} = V_{max}/[E]$, where [E] was the amount of enzyme in mole. For example, for the wild-type BpUrease with EC as substrate, V_{max} according to Table 3 is 125 μmol/min/mg protein, which is 2.08 μmol/s/mg. For a urease mol mass of 216 kDa, 1 μmol of enzyme is 216 mg. So k_{cat} is 2.08*216 = 449 s^{-1}.

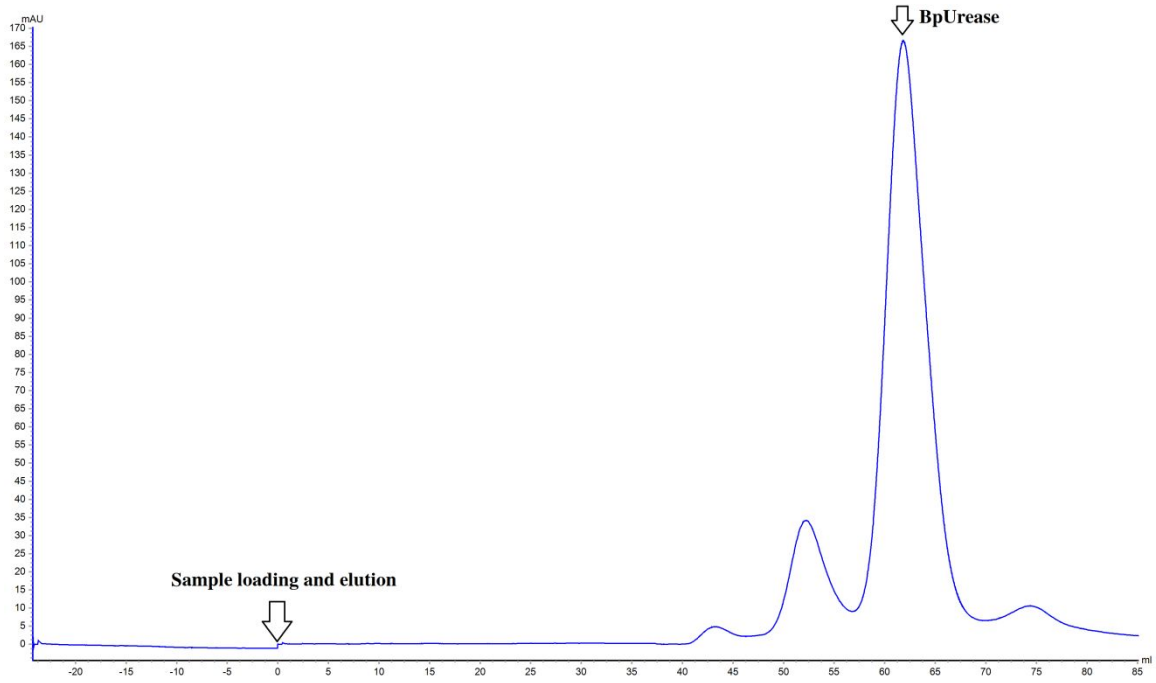

Figure S1 Purification of the recombinant BpUrease and its variants using the HiLoad 16/600 Superdex 200 pg (GE Healthcare Life Sciences) column

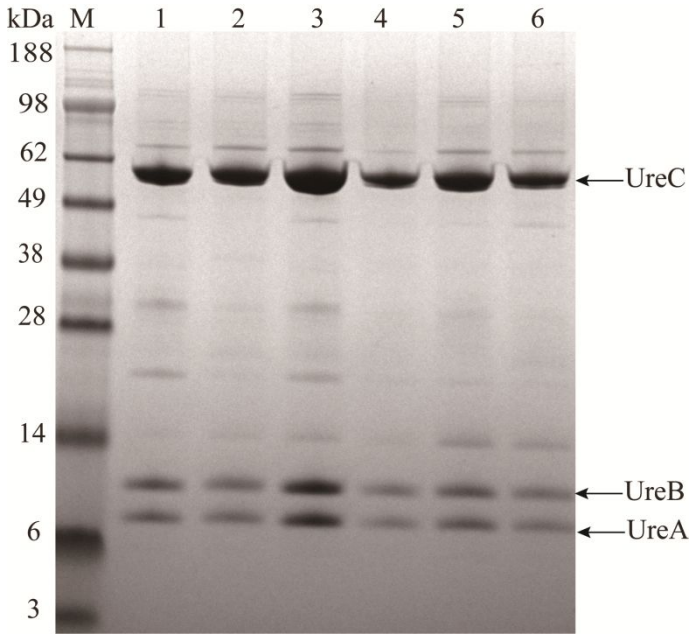

Figure S2 SDS-PAGE analysis of the purified BpUrease and its variants. Line 1 to line 6 was represent as BpUrease, L253P, L287I, L287N, L253P/L287I, L253P/L287N, respectively.

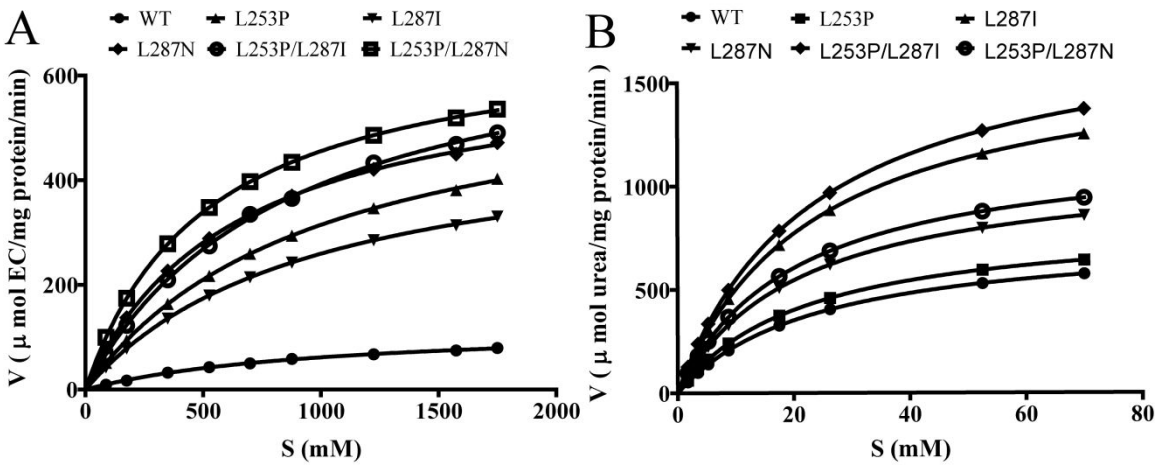

Figure S3 Michaelis–Menten curves of the recombinant BpUrease and its variants. (A): EC as the substrate, with a concentration of 100-1800 mM, (B):Urea as the substrate, with a concentration of 2-80 mM. V was defined as μmol urea (or EC) hydrolyzed per mg protein per minute at 37^{o}C and pH 4.5.

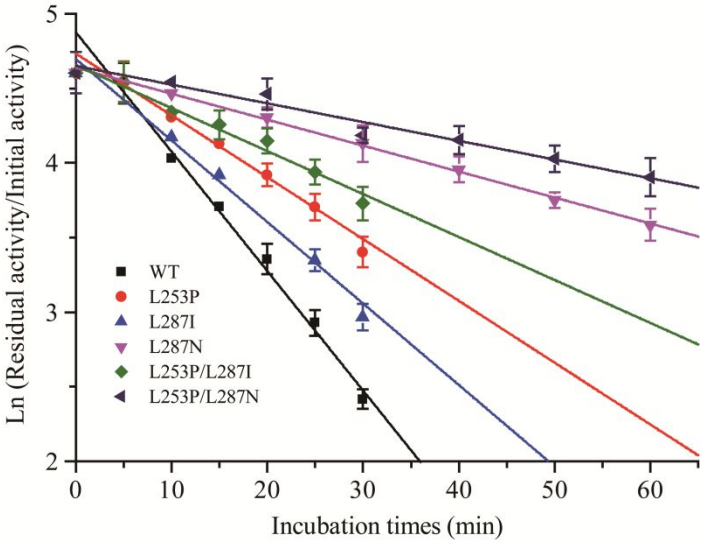

Figure S4 Thermo-stability of BpUrease and mutants at 70°C.

Reference

1. Liu, Q.; Chen, Y.; Yuan, M.; Du, G.; Chen, J.; Kang, Z. A *Bacillus paralicheniformis* iron-containing urease reduces urea concentrations in rice wine. *Appl Environ Microbiol* **2017,** 83 (17), 11.