Supporting information for：

A *Bacillus paralicheniformis* iron-containing urease reduces urea concentrations in rice wine

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PCR reaction condition

PCR reaction system was composed of 25 μL of 2× PrimerSTAR HS Premix (TAKARA, JAPAN), 1.0 μL of the forward primer (10 μM), 1.0 μL of the reverse primer (10 μM), 1.0 μL of the circulated DNA (1 μg/mL) or 1.0 μL of the genomic DNA (100 μg/mL), and 22 μL of double-distilled water. PCR conditions were as follows: 98 ºC for 30 s; 98 ºC for 10 s, 55 ºC for 5 s, 72 ºC for 1 kb/min; 30 cycles, final extension at 72 ºC for 10 min.

Gibson assembly

For single gene assemble with plasmid: the Gibson assembly reaction system was composed of 4 μL of 5× CE II buffer, Exnase®2 μL (Vazyme, NanJing, China), linearized vector 0.03 pmol, gene fragment 0.06 pmol, double-distilled water up to 20 μL. Then 37 ºC for 30 min and put it on the ice for transformation.

Phosphorylation and ligation

The fragment were phosphorylated and ligated by Blunting Kination Ligation (BKL) Kit (TAKARA, JAPAN). The phosphorylation reaction system were composed of 0.5 μL of 10× Blunting Kination Buffer, 0.25μL of Blunting Kination Enzyme Mix, 4.25 μL of PCR fragment, then 37 ºC for 30 min and 70 ºC for 5 min to inactivation of the enzyme. After cooling down the mixture to 4 ºC, 5μL of Ligation Solution I were added and mixed,

and ligated at 16 °C for 1 h, then the mixture were used for transformation.

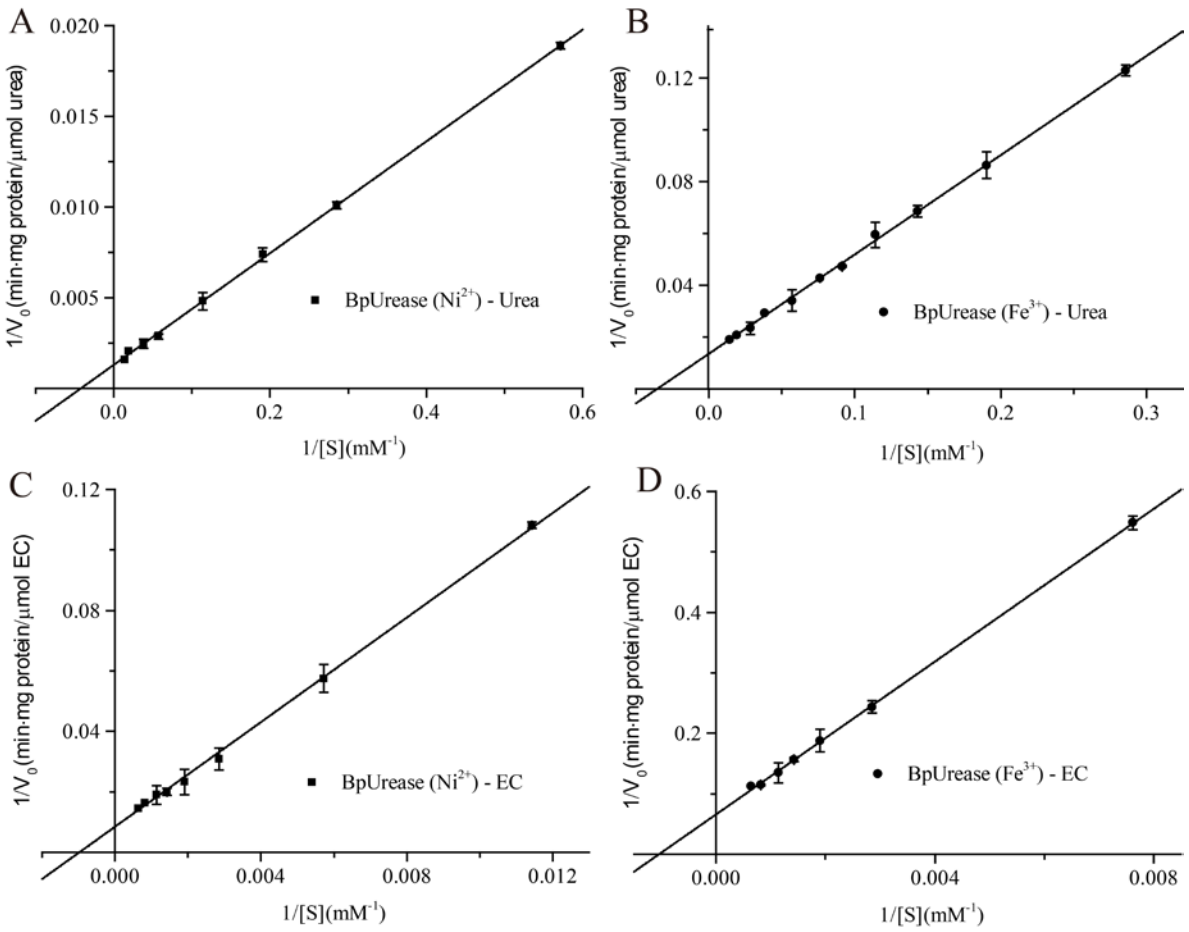

Figure S1. Lineweaver-Burk plot for K_m and V_{max} values of the recombinant urease. (A), (B):Urea as the substrate, with a concentration of 2-80 mM. (C), (D): EC as the substrate, with a concentration of 100-1800 mM. V_0 was defined as μmol urea (or EC) hydrolyzed per mg protein per minute at 37°C and pH 4.5.