# Functional characterization of PLP fold type IV transaminase from *Psendonocardia ammonioxydans*

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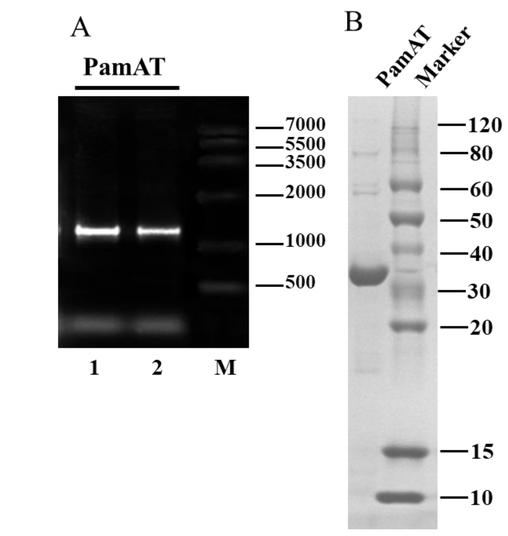
## Abstract

The asymmetric synthesis of chiral amines and unnatural amino acids using aminotransferase as catalyst is one of the hot topics in bioprocess engineering. Pyridoxal-5′-phosphate (PLP)-dependent transaminases are industrially important enzymes catalyzing the stereoselective amination of ketones and keto acids. Here, we report biochemical characterization of a aminotransferase from *Psendonocardia ammonioxydans*, which is active towards keto analogs of D-amino acids and (R)-2-aminopentane. The enzyme named PamAT is characterized by an alkaline pH optimum (pH 7-9.5), a tolerance to high dimethyl sulfoxide concentrations (up to 50%) and a tolerance to high ligand concentrations (up to 200 mM). These results complement our knowledge of the catalytic diversity of transaminases and indicate the need for further research to synthesis chiral amines in these enzymes.

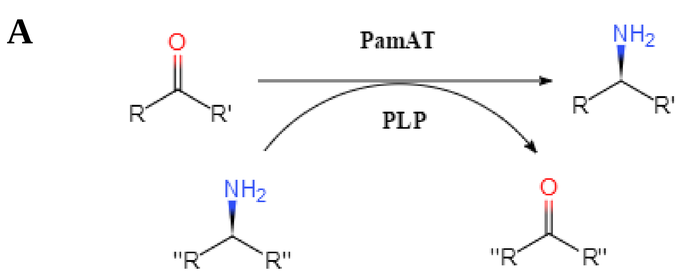
## Results

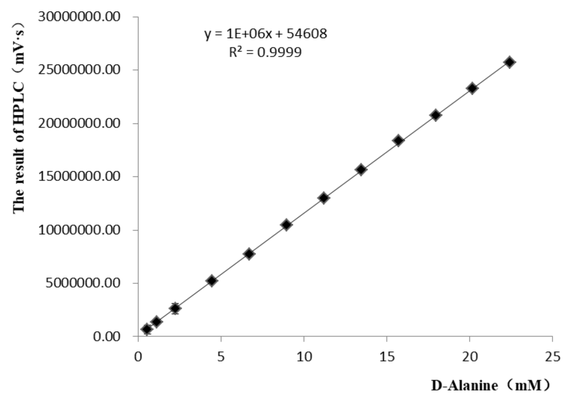
### 1. Purification of *P. ammonioxydans* aminotransferase (PamAT) from recombinant E. coli

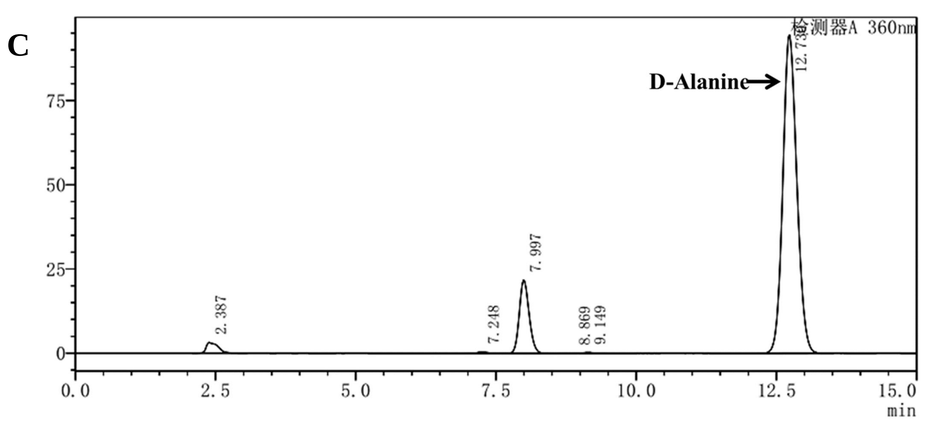



**Purification of PamAT from recombinant E. coli**.
(A) Target fargment of PseAT gene. (B) SDS-PAGE of purified PamAT. The left and right lanes show molecular mass markers and the PamAT elution pattern, respectively.

### 2. Analysis and determination of enzyme activity

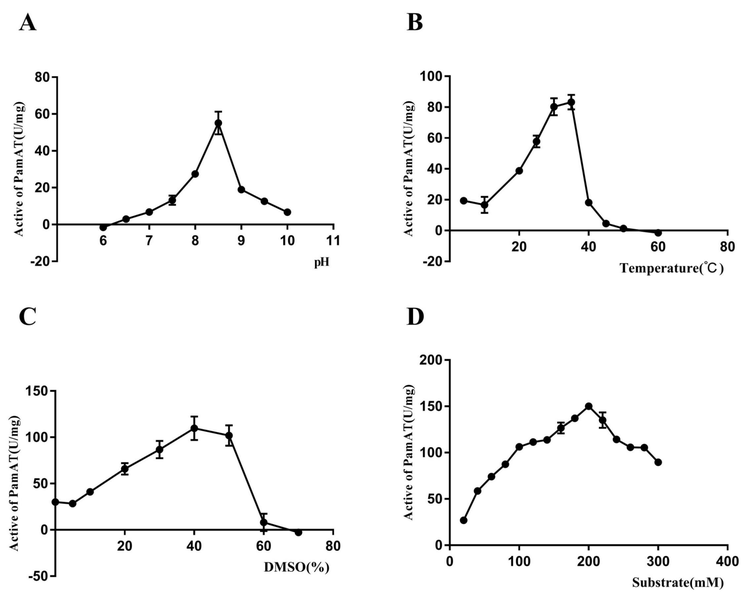


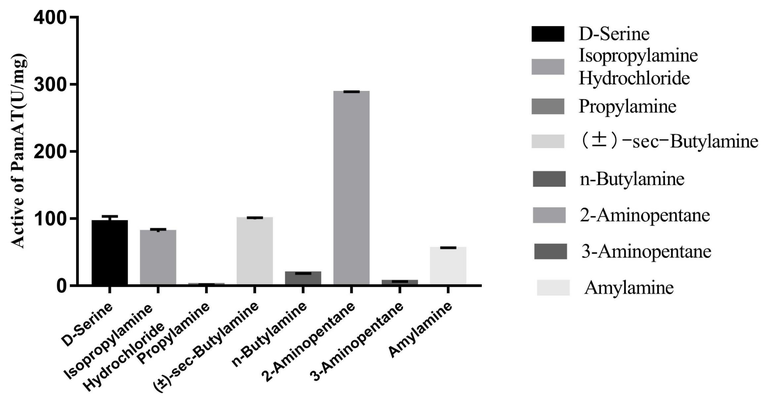

The activity with isopropylamine hydrochloride and pyruvate was detected using HPLC assay the product D-alanine . (A) Principle of transamination reaction. (B) Standard curve of D-Alanine concentration in C18 column. (C) Chromatogram of the enzyme. The spectra of the native enzyme (solid line) and the reduced enzyme in the presence of 50 mM sodium borohydride (broken line) were measured at 30 °C and pH 8.5. The enzyme concentration was 0.76 mg/ml.

### 3 Effect of pH, temperature, DMSO and substrate on PamAT enzyme activity



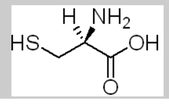
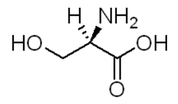
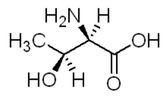
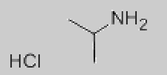
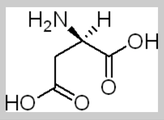
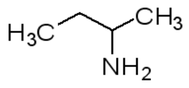
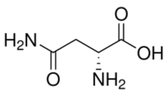
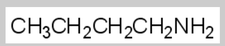
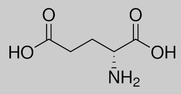
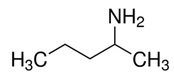
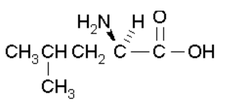
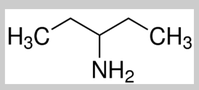
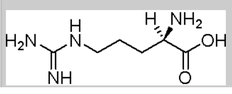

**Effect of pH, temperature, DMSO and substrate on enzyme activity.**
U=50 mM amino donor with amino acceptor react for 1 h at 30 ℃ to produce 1 μM D-alanine. (A) The enzyme was incubated at 50mM substrate concentrations , 30 °C and at the indicated pHs (n =3). (B) The enzyme was assayed at 50mM substrate concentrations, pH 8.5 and at the indicated temperatures (n =3). (C) The enzyme was assayed at 50mM substrate concentrations, pH 8.5, 30 °C and at the indicated DMSO (n =3). (D) The enzyme was assayed at pH 8.5, 30 °C and at the indicated substrate concentrations (n =3).

### 4 Identification of potent substrate specificity of PamAT




**The pyruvate amino donor on enzyme activity**
The enzyme was assayed at 50mMsubstrate concentrations, pH 8.5, 30 °C (n =3).

**Table. Amino donor spectrum of PamAT with pyruvate as the amino acceptor**

| No. | Substrate | Structure | Specific activity U/mg | No. | Substrate | Structure | Specific activity U/mg |
|---|---|---|---|---|---|---|---|
| | D-Serine |  | 94.92±8.63 | | D-cysteine |  | n.d. |
| | Isopropylamine Hydrochloride |  | 80.16±3.89 | | D-Threonine |  | n.d. |
| | Propylamine |  | 1.25±0.46 | | D-Aspartic acid |  | n.d. |
| | (±)-sec-Butylamine |  | 99.24±2.79 | | D-Asparagine monohydrate |  | n.d. |
| | n-Butylamine |  | 18.40±0.14 | | D-Glutamic acid |  | n.d. |
| | 2-Aminopentane |  | 287.08±2.02 | | D-2-Amino-4-methylpentanoic acid |  | n.d. |
| | 3-Aminopentane |  | 5.96±0.80 | | D-Arginine |  | n.d. |
| | Amylamine | $CH_3(CH_2)_3CH_2NH_2$ | 54.86±2.12 | | | | |