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Biosynthesis of 4-hydroxyphenylpyruvic acid from L-tyrosine using recombinant *Escherichia coli* cells expressing membrane bound L-amino acid deaminase☆

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Abstract: 4-Hydroxyphenylpyruvic acid (4-HPPA), a kind of α-keto acid, is an intermediate in the metabolism of tyrosine and has a wide range of application in food, pharmaceutical and chemical industry. Using amino acids as raw material to produce the corresponding α-keto acid is thought to be both economic and efficient. Among the enzymes that convert amino acid to α-keto acid, membrane bound L-amino acid deaminase (mL-AAD), which is anchored to the outer side of the cytomembrane, become an ideal enzyme to prepare α-keto acid since there is no cofactors needed and H_2O_2 production during the reaction. In this study, the mL-AAD from *Proteus vulgaris* was used to prepare whole-cell catalysts to produce 4-HPPA from L-tyrosine. The secretory efficiency of mL-AAD conducted by its own twin-arginine signal peptide (twin-arginine translocation pathway, Tat) and integrated pelB (the general secretory pathway, Sec)-Tat signal peptide were determined and compared firstly, using two pET systems (pET28a and pET20b). It was found that the Tat pathway (pET28a-*mlaad*) resulted in higher cell-associated mL-AAD activity and cell biomass, and was more beneficial to prepare biocatalyst. In addition, expression host Bl21(DE3) and 0.05 mmol·L^{-1} IPTG were found to be suitable for mL-AAD expression. The reaction