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Biosynthesis of Salvianic Acid from L-dopa via a Two-Step Process

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Extended Abstract

Salvianic acid A (SAA, 3-(3',4'-dihydroxyphenyl)-2-hydroxypropanoic acid), as the main bioactive component of traditional Chinese herb *Salvia miltiorrhiza*, has important application value in the treatment of cardiovascular diseases [1]. Traditionally, SAA was mainly isolated from dried root of *S. miltiorrhiza* by a water-extraction process. However, the amount of SAA in crude root is very low (0.045%), which restricted its large-scale applications. Although several chemical methods for SAA synthesis have been developed, these methods also suffered from intractable enantioselectivities, complicated procedure, and environmental pollution [2]. Therefore, developing efficient and eco-friendly methods for SAA production was highly desirable. In this study, a two-step biocatalytic cascade reaction to produce SAA from inexpensive L-dopa with high efficiency by using whole-cell biocatalysts was developed. In the first step, the recombinant *Escherichia coli* cells expressing mL-AAD from *Proteus vulgaris* (BL21(DE3)-pET-28a-*mlaad*) were employed to deaminize L-dopa to form 3,4-dihydroxyphenylalanine (DHPPA). Subsequently, the permeabilized recombinant *E. coli* cells co-expression of d-lactate dehydrogenase (d-LDH) from *Pediococcus acidilactici* and FDH from *Mycobacterium vaccae* N10 (NADH regeneration system) (BL21(DE3)-pETDuet-*pddh-fdh*) were used to convert DHPPA in the raw reaction solution to SAA.

Results: (1) The effect of bioconversion conditions for BL21(DE3)-pET-28a-*mlaad* to convert L-dopa to DHPPA were evaluated firstly. The optimal conditions for the deamination reaction were as follows: 0.42 g/L cell biomass, 50 mM L-dopa, 37 °C, pH 7.5 and 160 min (operation time). (2) To overcome the permeability barrier of cell envelope to substrates and products, the hexane-permeabilized BL21(DE3)-pETDuet-*pddh-fdh* was used to convert DHPPA to SAA. The optimal pH and temperature for the reaction were 6.0 and 30 °C, respectively. (3) Two-step catalytic synthesis of SAA from L-dopa: DHPPA was prepared with BL21(DE3)-pET-28a-*mlaad* under the optimum deamination conditions described above, and 48.6 mM DHPPA was obtained from 50 mM L-dopa. Next, the recombinant BL21(DE3)-pET-28a-*mlaad* cells were removed from the reaction solution by centrifugation, 100 mM sodium formate were added and the reaction pH value was adjusted to 5.5. Then 0.31-0.93 mg/mL permeabilized BL21(DE3)-pETDuet-*pddh-fdh* were added to the reaction solution to start the conversion of DHPPA to SAA. The yields of SAA from DHPPA in our experimental ranges could all reach more than 97.7%, and the SAA production rate was accelerated with increasing cell concentration. When cell concentration above 0.62 mg/mL, DHPPA could almost be converted to SAA in 4.5 h. In case of 0.31 mg/mL cell concentration, SAA production rate from DHPPA could over 97.7% after 5.5 h. Overall, in our developed a two-step biotransformation process, L-dopa was efficiently deaminized to DHPPA with a high yield of 97.7 % in mL-AAD bioconversion, then DHPPA was effectively converted to SAA by the permeabilized recombinant *E. coli* cells co-expression of d-LDH and FDH. The total yield of SAA from L-dopa could reach approximately 96.5% by the two-step biocatalytic reaction in the optimum reaction conditions.

Therefore, the methods developed herein provided excellent production efficiency and reflected good industrial application prospects.

References

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