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

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Extended Abstract

Salvinic 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 deaminate 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