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Regioselective synthesis of piceatannol from resveratrol: catalysis by two-component flavin-dependent monooxygenase HpaBC in whole cells

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ABSTRACT

Piceatannol, a valuable biologically active stilbene derivative, was efficiently synthesized from resveratrol. Whole-cell catalysis with HpaBC monooxygenase enabled the regioselective hydroxylation of resveratrol to produce 23 mM (5.2 g L^{-1}) of piceatannol.

Piceatannol (3,4,3′,5′-tetrahydroxy-*trans*-stilbene) is a biologically active molecule that naturally occurs in several plants, including grapes, Japanese knotweed, and passion fruit.[1–3] This molecule is a powerful antioxidant. It exhibits much higher antioxidant activity in radical scavenging experiments than resveratrol (3,5,4′-trihydroxy-*trans*-stilbene), an extensively studied analog of piceatannol.[4,5] Furthermore, piceatannol can suppress proliferation of a variety of tumor cells, including leukemia, lymphoma, and melanoma.[6,7] In addition to having antioxidant and anticancer activities, recent reports indicate that piceatannol not only exerts positive effects on human dermal cells through inhibition of melanogenesis and synthesis of collagen, but also exhibits vasorelaxant effects in rat thoracic aorta.[8,9] Notably, these effects of piceatannol are more pronounced than those of resveratrol. These beneficial properties encourage the use of piceatannol in health and functional foods, as well as in pharmaceuticals and cosmetics. Indeed, an extract of passion fruit seed that contains concentrated piceatannol is marketed under the trade name Passienol (Morinaga & Co., Ltd., Japan).

Piceatannol has been found in several plants, but generally at very low levels,[1–3] making naturally extracted piceatannol extremely expensive. In contrast, resveratrol is a relatively inexpensive compound common in many plants and at higher concentrations.[1–3] Thus, oxidation catalysts that are able to regioselectively hydroxylate resveratrol might provide a new, efficient, and cost-effective synthetic approach to piceatannol (Scheme 1). This oxidation reaction cannot be performed by chemical methods, and there have been only a few reports of biocatalysts (i.e., enzymes and microorganisms) that can oxidize resveratrol to piceatannol. Human and bacterial cytochrome P450s were shown on an analytical scale to be capable of this catalytic conversion, but with very low activity.[10–12] It was also reported that *Streptomyces avermitilis* converted 78% of a 100 μM resveratrol solution to piceatannol.[13]

In this study, we found that the two-component flavin-dependent monooxygenase, HpaBC, from *Pseudomonas aeruginosa* has catalytic activity for the regioselective hydroxylation of resveratrol to piceatannol. Enzymes in the HpaBC family consist of a flavin-dependent monooxygenase (HpaB) and a NAD(P)H:flavin oxidoreductase (HpaC), and catalyze hydroxylation using molecular oxygen as an innocuous oxidant under ambient conditions.[14–16] HpaBC monooxygenases are involved in the 4-hydroxyphenylacetate degradation pathway. These enzymes reportedly catalyze the hydroxylation of monocyclic aromatic compounds, including 4-hydroxyphenylacetate, L-tyrosine, and halophenols.[16–20] Recently, we reported that HpaBC from *P. aeruginosa* can synthesize hydroxycinnamic acids.[21] While the present Letter was in preparation, a review article was published that contained a short sentence regarding unpublished data; it indicated that HpaBC from *Escherichia coli* exhibits oxidation activity for resveratrol.[22,23] However, no research papers describing

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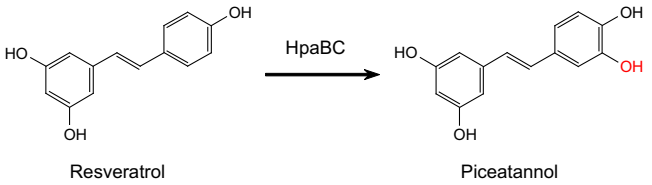

Scheme 1. Regioselective hydroxylation of resveratrol to piceatannol by HpaBC.

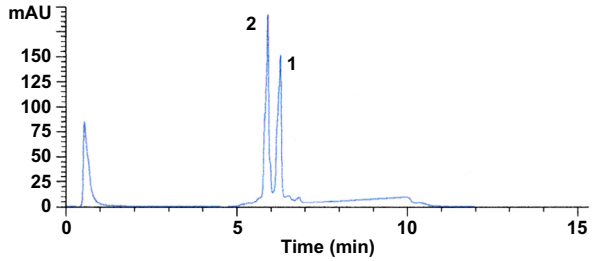

Figure 1. HPLC chromatogram of the reaction of HpaBC whole-cell catalyst with resveratrol. Peaks 1 and 2 were found to correspond to resveratrol and piceatannol, respectively.

piceatannol synthesis by any enzyme in the HpaBC family have been published. Here, we examined in detail the catalytic activity of HpaBC from *P. aeruginosa* toward resveratrol, and investigated the application of the HpaBC whole-cell catalyst to the flask-scale production of piceatannol.

We explored the catalytic potential of HpaBC for resveratrol hydroxylation by whole-cell assays using *E. coli* cells expressing HpaBC (experimental details are shown in Supplementary data). Reaction conditions previously optimized for *p*-coumaric acid[21] were first employed (Table 1, entry 1). When resveratrol (10 mM) was reacted with the transformed *E. coli* cells (8 mg of dry cell weight per milliliter) in a microtube containing 100 μl of the reaction mixture, HPLC analysis showed a major peak (retention time, 5.9 min) in addition to the substrate peak (6.3 min) (Fig. 1). This new peak was not detected in the reaction mixture containing *E. coli* cells carrying the empty vector without the *hpaBC* genes (data not shown). The compound corresponding to this peak was confirmed to be a monooxygenation product of resveratrol based on the determination of its mass value ($[M-H]^-$, $m/z = 243.2$). Furthermore, the ^1H NMR and ^{13}C NMR spectra of this product were in agreement with those of piceatannol that had been previously determined (see Supplementary data).[24,25] Based on these observations, this new product was identified as piceatannol (Scheme 1). The whole cells regioselectively hydroxylated the C-3′ position of resveratrol without any detectable by-products. Using these reaction conditions (Table 1, entry 1), 4.3 mM piceatannol was produced from 10 mM resveratrol in 24 h.

We next examined reaction conditions to further enhance the productivity of piceatannol (Table 1). As the concentrations of resveratrol substrate and the catalyst cells increased, the amount of piceatannol product increased (Table 1, entries 1–5). When 30 mM resveratrol was reacted with 32 mg mL^{-1} of the transformed *E. coli* cells, the production of piceatannol reached 8.8 mM (Table 1, entry 5); however, the conversion yield decreased. We reasoned that the relatively large molecular size of resveratrol might hamper the entrance of this substrate into *E. coli* cells. Non-ionic detergents such as Tween 80 and Triton X-100 reportedly improve the permeability of bacterial cell membranes for substrate compounds without significantly damaging the cells.[26,27] We found that the addition of Tween 80 or Triton X-100 to the reaction mixture significantly enhanced the productivity of piceatannol (Table 1, entries 6–9). In particular, the whole cells produced 25 mM piceatannol in the presence of 1% (v/v) Tween 80, with a conversion yield of 83% (Table 1, entry 7). This amount of product was 2.8 times higher than that in the absence of any detergents.

Finally, we attempted to produce piceatannol on a flask scale using the optimized reaction conditions. Resveratrol (30 mM) was reacted with the HpaBC whole-cell catalyst (32 mg mL^{-1}) in a 500-ml flask that contained 20 ml of the reaction mixture. The time course of the conversion is shown in Figure 2. The initial rate of resveratrol hydroxylation by the whole cells in the presence of 1% (v/v) Tween 80 was estimated as 3.2 μmol per g of cells per min. This rate was approximately 1.5 times higher than that in

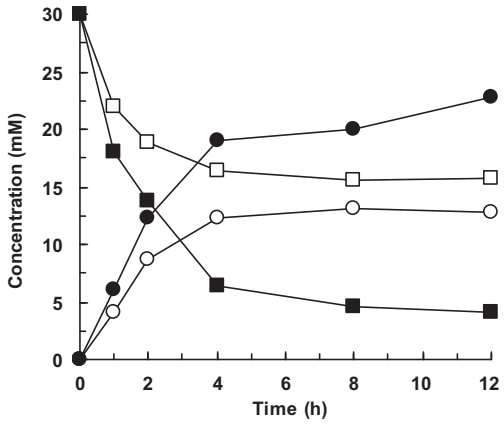

Figure 2. Flask-scale production of piceatannol by HpaBC whole-cell catalyst. The whole-cell catalyst was reacted with resveratrol in the absence (white symbols) or in the presence (black symbols) of Tween 80 (1%). The time courses of resveratrol consumption (squares) and of piceatannol production (circles) are shown.

Table 1
Production of piceatannol from resveratrol by a HpaBC whole-cell catalyst

Entry	Substrate (mM)	Catalyst[a] (mg mL^{-1})	Detergent (% v/v)	Product (mM)	Conversion[b] (%)
1	10	8	0	4.3	43
2	10	16	0	6.2	62
3	20	16	0	7.2	36
4	20	32	0	8.1	41
5	30	32	0	8.8	29
6	30	32	0.1 (Tween 80)	13	43
7	30	32	1 (Tween 80)	25	83
8	30	32	0.1 (Triton X-100)	9.9	33
9	30	32	1 (Triton X-100)	22	73

[a] Milligram of dry cell weight per milliliter.
[b] Conversion (%) = product (mM)/substrate (mM) × 100.

the absence of the detergent. In the presence of Tween 80, the production of piceatannol continued to increase and reached 23 mM (5.2 g L^{-1}) in 12 h (Fig. 2). This production is the highest level so far attained by any biocatalytic synthesis of piceatannol, and is 300 times higher than that achieved by cells of *S. avermitilis*.[13]

In conclusion, we demonstrated that HpaBC from *P. aeruginosa* is useful for the high-yield and regioselective synthesis of piceatannol from resveratrol. Piceatannol can be also prepared by chemical synthesis through other routes,[24,25] as well as by extraction from plant sources; however, our strategy provides an efficient and green alternative for the production of piceatannol. Moreover, it is noteworthy that HpaBC is able to hydroxylate resveratrol, a relatively large molecule that includes two benzene rings, since enzymes in the HpaBC family had been believed to exhibit catalytic activity only for monocyclic aromatic compounds. Thus, the findings of this study open up possibilities for further exploitation of the catalytic potential of HpaBC. For example, the approach presented here might be also applicable to the oxyfunctionalization of other valuable polycyclic compounds, for example stilbenes and flavonoids.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2014.03.076.

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