Note

Formation of 2,6-Dihydroxyphenylacetic Acid from Phenylacetic Acid by *Trichosporon cutaneum*

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In investigations on the metabolism of phenylacetic acid (PA) by microorganisms, the formation of homogentisic acid (2,5-DHPA)^{1~11)} or homoprotocatechuic acid (3,4-DHPA),^{12~14)} or both,^{11,15)} has been demonstrated. We previously reported that the ability to convert PA into 2,6-dihydroxyphenylacetic acid (2,6-DHPA) is widely present in fungi, especially in *Aspergillus* and *Penicillium* species,¹¹⁾ and that 2,6-DHPA was formed from PA via *o*-hydroxyphenylacetic acid (2-HPA) by *Aspergillus fumigatus*.⁸⁾ This paper presents the results of a screening test for yeasts which are able to produce 2,6-DHPA from PA, and reports that 3 strains of *Trichosporon cutaneum* selected as 2,6-DHPA producers, unlike the strain of *T. cutaneum* reported by Dagley's group,^{10,17)} converted PA into 2,5- and 2,6-DHPA via 2-HPA, and into 3,4-DHPA via *m*-hydroxyphenylacetic acid (3-HPA), respectively.

The following 90 strains of yeasts (18 genera, 76 species) were screened for the production of 2,6-DHPA from PA: *Saccharomyces*, 22 strains; *Candida*, 18; *Rhodotorula*, 10;

Hansenula, 8; *Pichia*, 6; *Torulopsis*, 6; *Trichosporon*, 4; *Kloeckera*, 2; *Sporobolomyces*, 3; *Cryptococcus*, 2; *Endomyces*, 2; and one each of strains of *Endomycopsis capsularis*, *Schizosaccharomyces pombe*, *Debaryomyces hansenii*, *Saccharomycodes ludwigii*, *Hanseniaspora valbyensis*, *Wickerhamia fluorescens* and *Torula rubra* var. *alpha*. These strains were grown with shaking (rotary, 200 rpm) at 30°C for 3~5 days in 100 ml of medium (pH 6.0) containing malt extract (2.0%), glucose (1.0%), peptone (0.1%) and PA (0.05%). The cells were harvested in the late logarithmic phase of growth by centrifugation and then washed three times with sterile saline. The washed cells were suspended in 20 ml of 0.2M Na₂HPO₄-0.1M citric acid buffer (pH 6.0), and then 5 ml of each of the cell suspensions was added to 50 ml of the same Na₂HPO₄-citric acid buffer in a 300 ml Erlenmeyer flask containing one of the substrates (0.05%): PA for the screening test; and PA, 2-HPA, 3-HPA and *p*-hydroxyphenylacetic acid (4-HPA) for the metabolic study. The cultures were incubated at 30°C on a rotary shaker (200 rpm) for 3~170 hr.

After incubation for a given period, the culture filtrate was acidified to pH 2.0 with H₂SO₄ and then extracted with ethylacetate. The ethylacetate extract was concentrated and subjected to thin-layer chromatography (TLC). The concentrated extract was also silylated with *N,O*-bis-(trimethylsilyl)-acetamide in acetonitrile and then submitted to gas-chromatography (GC). GC was performed with a Shimadzu GC-4B chromatograph on a glass column (1.5 m × 0.3 cm i.d.) packed with SE-30. The gas flow rate for N₂ was 30 ml/min. The temperature was increased from the initial 100°C to a final 250°C at a rate of 10°C/min. The silylated derivatives of PA and its metabolites were identified by comparing their retention times with those of authentic samples and by cochromatography. Since the derivatives of 2,5- and 3,4-DHPA showed the same retention time values, the formation of these compounds was confirmed by TLC and GC-mass spectrometry (GC-MS). Detection and identification of the metabolites by TLC, UV and GC-MS were carried out as described previously.^{4,8,19)} PA was determined by Yoshii's colorimetric method.¹⁸⁾

Among the 90 strains investigated in the screening test, 3

TABLE I. FORMATION OF VARIOUS METABOLITES FROM PHENYLACETIC ACID AND ITS HYDROXY DERIVATIVES BY *T. cutaneum* YTr-1, YTr-2 AND KUY-6A

Substrate	Metabolites					
	2-HPA	3-HPA	4-HPA	2,5-DHPA	2,6-DHPA	3,4-DHPA
PA	+	+	±	+	+	—
2-HPA	nd	—	—	+	+	—
3-HPA	—	nd	—	+	—	+
4-HPA	—	—	nd	—	—	+

nd, not determined.

FIG. 1. Proposed Metabolic Sequence for PA Catabolism in *T. cutaneum* YTr-1, YTr-2 and KUY-6A.

(1) PA; (2) 2-HPA; (3) 3-HPA; (4) 4-HPA; (5) 2,6-DHPA; (6) 2,5-DHPA; (7) 3,4-DHPA.

strains of *Trichosporon cutaneum* (*T. cutaneum* YTr-1, YTr-2 and KUY-6A) alone were able to produce 2,6-DHPA from PA. These 3 strains of *T. cutaneum* consumed about 50% of the PA on incubation for 48 hr and 90% in 168 hr. In addition to 2,6-DHPA, these strains produced 2-HPA, 3-HPA, 4-HPA (trace amount) and 2,5-DHPA from PA, however, 3,4-DHPA was not detected in the culture filtrates. When monohydroxyphenylacetic acid was used as a substrate, the 3 strains converted 2-HPA into 2,5- and 2,6-DHPA, 3-HPA into 2,5- and 3,4-DHPA, and 4-HPA into 3,4-DHPA, respectively (Table I).

The conversion of 2-HPA to 2,5-DHPA^{1,5-9)} and 2,6-DHPA^{8,16)} that of 3-HPA to 2,5-DHPA^{8-10,15,21)} and 3,4-DHPA,²⁰⁾ and also that of 4-HPA to 3,4-DHPA^{10,12-15,17)} have been verified in microorganisms, however, no report on the conversions described above, except for the conversion of 3-HPA to 2,5-DHPA¹⁰⁾ and that of 4-HPA to 3,4-DHPA^{10,17)} have so far appeared for yeasts. This is the first report of the formation of 2,5- and 2,6-DHPA from PA via 2-HPA and that of 3,4-DHPA from 3-HPA by a yeast, *T. cutaneum*. 3,4-DHPA was not detected in the culture filtrate on incubation with PA, however, the formation of 3-HPA and 4-HPA from PA and that of 3,4-DHPA from 3-HPA and 4-HPA were revealed (Table I), and hence, the 3 strains of *T. cutaneum* may have a metabolic pathway from PA through 3-HPA and 4-HPA to 3,4-DHPA. From the results obtained in this work, a metabolic sequence for the PA catabolism in *T. cutaneum* YTr-1, YTr-2 and KUY-6A is proposed (Fig. 1).

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