Pathochemical Studies on Rhizoctonia Disease. I. Meta-hydroxylation of Phenylacetic Acid by Rhizoctonia solani

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Low molecular wt metabolites as well as enzymes are thought to be involved in pathogenesis by *Rhizoctonia solani* (3, 13, 15, 16). Recently, phenylacetic (1, 11), *m*-hydroxyphenylacetic (1, 11), *p*-hydroxyphenylacetic (1, 4, 11), 3-nitro-4-hydroxyphenylacetic (10, 11), and β-furoic (1) acids were isolated as possible phytotoxins from culture filtrates of *R. solani*. We have reported on the biogenesis of these phenolic acids by the pathogen (10, 12). Phenylacetic acid, which is produced via the phenylalanine-phenylpyruvic acid pathway, is the immediate precursor of *m*-hydroxyphenylacetic acid. We could find no evidence that phenylacetic acid was converted to the *p*-hydroxy isomer, which was formed biosynthetically via the tyrosine-*p*-hydroxyphenylpyruvic acid pathway.

The hydroxylation of phenylacetic acid has been investigated in several biological systems, and two alternative pathways have been suggested to predominate in microbial hydroxylation. One is a route via *p*-hydroxy- and 3,4-dihydroxyphenylacetic acids and the other is via the *o*-hydroxy isomer. Bocks et al. (2) and Faulkner & Woodcock (6) reported that *Aspergillus niger* (Mulder strain) can transform phenylacetic acid to ortho-, meta-, and para-hydroxylated derivatives of the acid. The meta and para isomers were minor products. In contrast to these results, we have found that *m*-hydroxyphenylacetic acid is the major product obtained from the metabolism of phenylacetic acid by a number of isolates of *Rhizoctonia solani* Kuehn (10, 12).

We report here the results of a survey to determine if other species of fungi can hydroxylate phenylacetic acid predominately by meta-hydroxylation. An abstract describing some of this work has been published (9).

All fungi used were from our own culture collection. The isolates were grown in still culture at room temp in a modified Richards' medium containing 0.5% polypeptone (Wako Chemicals, Japan) and 0.1% yeast extract. The mycelial mats from 2- to 3-week-old cultures were washed three times with sterile distilled water and suspended in 100 ml phosphate buffer (0.067 M, pH 6.5) containing phenylacetic acid (4 mg/ml). To determine the amount of hydroxylated phenylacetic acid derivatives in the replacement culture, the acidic products were extracted with ethyl acetate and were chromatographed with benzene:acetic acid (5:1, v/v) on Toyo Roshi No. 50 paper.

After being dried at room temp, chromatograms were sprayed with 1% *p*-nitrobenzene diazonium fluoroborate in 20% sodium acetate aqueous solution, or with 0.05% bromophenol blue in 95% ethyl alcohol (w/v). Mono-hydroxylated phenylacetic acid content of each sample was determined by comparing color intensity and size of spots with those of known amounts of authentic compounds. The position of hydroxylation, i.e., para- or meta-phenylacetic acid in the product, was determined from infrared (IR) spectra of extracts from paper chromatograms. For colorimetric determination of *m*-hydroxyphenylacetic acid, the R_F 0.2 to 0.5 zone, corresponding to that of the authentic acid on paper, was removed and eluted with 5 ml distilled water. Color was developed with Folin-Ciocalteu reagent (7), and the optical density was measured at 660 mμ within 40 min.

Results showed that all the isolates of *R. solani* had the ability to hydroxylate phenylacetic acid in the meta position (Table 1). In a typical replacement culture in which 400 mg of phenylacetic acid was incubated with isolate No. 450 of *R. solani*, 159.9 mg of recrystallized *m*-hydroxyphenylacetic acid was recovered. This was identified by IR spectra and mixture melting points. No para- or ortho-hydroxy derivatives were detected. In contrast, isolates of *Alternaria, Aspergillus, Botrytis, Cladosporium, Cochliobolus, Fusarium, Gloeosporium, Glomerella, Penicillium, Pestalotia,* and *Sclerotinia* hydroxylated phenylacetic acid in the ortho position (Table 2). Of these isolates, *Penicillium citrinum, P. frequentans,* and *Gloeosporium laeticolor* appear to produce some meta-isomer as a minor metabolite. However, the yield was so small that quantitative tests by methods other than paper chromatography were not possible. Only *o*-hydroxyphenylacetic acid was detected as a major metabolite of phenylacetic acid, which agrees with the data of Isono (8) and Faulkner & Woodcock (6). This was analogous to metabolism of phenoxyacetic acid by *Aspergillus niger* (5). Fourteen additional species of fungi used in the experiment did not metabolize phenylacetic acid to hydroxylated derivatives under these cultural condi-

TABLE 1. The production of meta-hydroxyphenylacetic acid from phenylacetic acid by isolates of *Rhizoctonia solani* in replacement cultures[a]

Isolate no.	Source	Yield of *m*-hydroxyphenylacetic acid
		mg/100 ml
210	Paddy rice	150
208	Paddy rice	70
204	Paddy rice	60
202	Paddy rice	20
205	Corn	5
6	Barley	5
86	Sugarbeet	50
450	Sugarbeet	250
302	Sugarbeet	220
317	Sugarbeet	190
94	Sugarbeet	50

[a] Phenylacetic acid (400 mg) was added to 100 ml phosphate buffer (0.067 M, pH 6.5) as a replacement medium.

TABLE 2. Hydroxylation of phenylacetic acid by various fungi in replacement cultures

Fungi	Mono-hydroxy phenylacetic acid produced[a]		
	ortho	meta	para
Alternaria japonica Yoshii, 0-34	+	—	—
Alternaria kikuchiana Tanaka, 0-9	+	—	—
Alternaria solani (Ell. & Mart.) Sor., IFO 5924	++	—	—
Alternaria mali Roberts	++	—	—
Aspergillus niger v. Tiegh., IAM 3001	++	—	—
Botrytis cinerea Pers.	++	—	—
Cephalosporium diospyri Crandall	—	—	—
Cercospora beticola Sacc.	—	—	—
Cladosporium carpophilum Theum.	—	—	—
Cladosporium cucumerinum Ell. & Arth.	++	—	—
Cladosporium fulvum Cke.	++	—	—
Cochliobolus miyabeanus (Ito & Kurib.) Drechs., No. 13	++	—	—
Colletotrichum lagenarium (Pass.) Ell. & Halst.	—	—	—
Endothia parasitica (Murr.) And. & And.	—	—	—
Fusarium oxysporum f. *cucumerinum* Owen	++	—	—
f. *lycopersici* (Sacc.) Snyd. & Hans., R 5-6	—	—	—
f. *vasinfectum* (Atk.) Snyd. & Hans.	+	—	—
Fusarium moniliforme Schlecht	—	—	—
Fusarium roseum f. *cerealis* (Cke.) Snyd. & Hans.	—	—	—
Gloeosporium laeticolor Berk.	+	±	—
Gloeosporium kaki Hori	—	—	—
Glomerella cingulata (Ston.) Spauld. & Schrenk, IAM 8050	+	—	—
Helminthosporium sigmoideum var. *irregulare* Cralley & Tullis	—	—	—
Irpex lacteus Fr., Ps-8a	—	—	—
Penicillium citrinum Thom, ATCC 9849	±	±	—
Penicillium digitatum Sacc., IAM 7234	+	—	—
Penicillium frequentans Westling, IAM 7082	+	±	—
Pestalotia funerea Desm.	+	—	—
Pyricularia oryzae Cav., A-1	—	—	—
Rhizopus nigricans Ehrenb.	—	—	—
Sclerotinia sclerotiorum (Lib.) de Bary, S-7	++	—	—
Verticillium dahliae Kleb., F-94	—	—	—

[a] Phenylacetic acid (400 mg) was added to 100 ml phosphate buffer (0.067 M, pH 6.5) as a replacement medium, and the yield of conversion products was recorded as follows: ++ = 20-100 mg/100 ml; + = 1-19 mg/100 ml; ± = less than 1 mg/100 ml; — = not detected.

tions (Table 2), although they made vigorous growth.

These experiments indicate that relatively few plant-pathogenic fungi have the ability to hydroxylate phenylacetic acid in the meta position. Of the 33 species and forms of fungi tested, only *R. solani* had this ability. The possible significance of this in disease development is not known. We can expect such hydroxylation to occur in the host plant, since the proper precursors should be present. A recent report by Tomaszewski & Thimann (14) indicates that monophenols such as *p*-coumaric and *p*-hydroxybenzoic acids stimulate the decarboxylation of IAA, while caffeic, ferulic, and chlorogenic acids enhanced IAA-induced growth by inhibiting IAA decarboxylation. It is possible that some hydroxylated metabolites act as phytotoxins, induce phytoalexins, and inhibit or activate host metabolic systems. Further study will be required to understand this interesting microbial hydroxylation and its possible pathological significance.

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