APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Feb. 1981, p. 442-448
0099-2240/81/020442-07$02.00/0

Vol. 41, No. 2

Catabolism of Substituted Benzoic Acids by *Streptomyces* Species†

JOHN B. SUTHERLAND, DON L. CRAWFORD,* AND ANTHONY L. POMETTO III

Department of Bacteriology and Biochemistry, Idaho Agricultural Experiment Station, University of Idaho, Moscow, Idaho 83843

Four thermotolerant actinomycetes from soil, identified as *Streptomyces albulus* 321, *Streptomyces sioyaensis* P5, *Streptomyces viridosporus* T7A, and *Streptomyces* sp. V7, were grown at 45°C in media containing either benzoic acid or hydroxyl- and methoxyl-substituted benzoic acids as the principal carbon sources. Benzoic acid was converted to catechol; *p*-hydroxybenzoic, vanillic, and veratric acids were converted to protocatechuic acid; and *m*-hydroxybenzoic acid was converted to gentisic acid. Catechol, protocatechuic acid, and gentisic acid were cleaved by catechol 1,2-dioxygenase, protocatechuate 3,4-dioxygenase, and gentisate 1,2-dioxygenase, respectively. Dioxygenases appeared only in induced cultures. *m*-Hydroxybenzoic, *m*-anisic, and *p*-anisic acids were gratuitous inducers of dioxygenases in some strains. One strain converted vanillic acid to guaiacol.

Microorganisms that catabolize aromatic compounds convert them to hydroxylated intermediates and then cleave the rings with ring-fission dioxygenases (1, 7). Among the organisms included in the *Actinomycetales*, several catabolic pathways are known for the *Nocardiaceae*, but little is known about aromatic metabolism in the *Streptomycetaceae*. Various *Nocardia* spp. strains that are now classified in *Rhodococcus* spp. (16) metabolize benzoate through catechol (25); metabolize *p*-anisate, *p*-hydroxybenzoate, vanillate, and veratrate through protocatechuate (3, 5, 12); and metabolize *m*-anisate and *m*-hydroxybenzoate through gentisate (14). Vanillate and veratrate are converted to guaiacol by one strain of *Streptomyces* sp. (13).

Six ring-cleaving dioxygenases have been reported in the *Actinomycetales*. Catechol is metabolized in *Rhodococcus* spp. by either a 1,2-dioxygenase or a 2,3-dioxygenase (2, 15, 29). Protocatechuate 3,4-dioxygenase and gentisate 1,2-dioxygenase occur in *Rhodococcus* spp. (3, 15) and *Streptomyces* spp. (9), and protocatechuate 4,5-dioxygenase is found in a strain of *Nocardia* sp. (18). Homogentisate 1,2-dioxygenase has been reported in *Rhodococcus* spp. (15).

The objective of this investigation was to identify the main catabolic pathways for aromatic compounds in *Streptomyces* spp. Using four thermotolerant strains known to metabolize aromatic carbon sources, we investigated the pathways in which hydroxyl- and methoxyl-substituted benzoic acids were converted into substrates for ring-cleaving dioxygenases. We also studied the induction of dioxygenases by substituted benzoic acids.

† Paper no. 79513 of the Idaho Agricultural Experiment Station.

MATERIALS AND METHODS

Organisms and growth conditions. The strains of *Streptomyces* spp. studied were isolated from soil by enrichment at 45°C by D. L. Sinden (M.S. thesis, University of Idaho, Moscow, 1979). Strain 321 conformed to the emended description of *Streptomyces albulus* (28); strain P5 conformed to the description of *S. sioyaensis* (27), except that it did not use sucrose; and strain T7A conformed to the description of *S. viridosporus* (28). Strain V7 was not identified to species.

m-Anisic acid (98%), *p*-anisic acid (98%), and veratric acid (99+%) were obtained from Aldrich Chemical Co., Milwaukee, Wis.; benzoic acid, guaiacol, protocatechuic acid, sodium gentisate, and vanillic acid (reagent grade) were obtained from Sigma Chemical Co., St. Louis, Mo.; catechol (98%) and *m*-hydroxybenzoic acid (98+%) were from Eastman Kodak Co., Rochester, N.Y.; and *p*-hydroxybenzoic acid (reagent grade) was from Nutritional Biochemicals Corp., Cleveland, Ohio.

The streptomycetes were inoculated into flasks of a liquid medium containing 1 g of an aromatic substrate, 4 g of Na_2HPO_4, 3 g of $(NH_4)_2SO_4$, 1 g of KH_2PO_4, 0.50 g of yeast extract, 0.2 g of NaCl, 0.2 g of $MgSO_4 \cdot 7H_2O$, and 0.05 g of $CaCl_2 \cdot 2H_2O$ per liter of distilled water. Cultures were grown for 2 to 3 days in a shaker incubator (New Brunswick Scientific Co., New Brunswick, N.J.) at 45°C.

Cell-free extracts. Mycelia were centrifuged at 4,200 × *g* for 10 min and washed with cold 0.85% (wt/vol) KCl solution. The washed pellets were resuspended in 5 ml of cold 500 mM tris(hydroxymethyl)aminomethane-acetate buffer (pH 7.4) and treated with a Biosonik III sonic disintegrator (Bronwill Scientific Co., Rochester, N.Y.) for a total of 2 to 3 min to produce a cell-free extract. The extract was

centrifuged at 25,000 × *g* for 20 min, and the pellet was discarded. Protein content of cell-free extracts was determined by the method of Lowry et al. (21), using bovine serum albumin as the standard.

Oxygenase assays. Oxygenases in cell-free extracts were assayed at 45°C in the same buffer with an oxygen electrode (Gilson Medical Electronics, Middleton, Wis.). The solubility of O_2 in water at 45°C is approximately 22.0 μl g^{-1} (23). Reaction mixtures contained 1.4 ml of buffer, 0.1 ml of crude extract, and 10 μl of a 10 mM solution of a known oxygenase substrate. For determination of monooxygenase activity, 0.1 μmol of reduced nicotinamide adenine dinucleotide (NADH) or reduced nicotinamide adenine dinucleotide phosphate (NADPH) (Sigma) was also added to the reaction mixture. Specific activities of oxygenases were calculated as micromoles of O_2 consumed per minute per milligram of protein.

UV absorption spectra. Utilization of aromatic compounds by whole cells was demonstrated by following the disappearance of ultraviolet (UV) absorption peaks with a Coleman 124 double-beam recording spectrophotometer (Perkin-Elmer Corp., Norwalk, Conn.). A strain was considered to degrade a compound if the UV absorption peaks characteristic of that compound disappeared from the culture medium during growth of the organism, but were still present in sterile control media incubated for the same period.

Products of ring cleavage in cell-extract reaction mixtures were identified spectrophotometrically. Catechol 1,2-dioxygenase (catechol:oxygen 1,2-oxidoreductase, EC 1.13.11.1) was demonstrated by a temporary increase in absorbance at 260 nm (17), corresponding to the formation of *cis,cis*-muconate (λ_{max} = 258 nm); protocatechuate 3,4-dioxygenase (protocatechuate:oxygen 3,4-oxidoreductase, EC 1.13.11.3) was demonstrated by a temporary increase in absorbance at 260 nm (22), corresponding to the formation of β-carboxy-*cis,cis*-muconate (λ_{max} = 255 nm); and gentisate 1,2-dioxygenase (gentisate:oxygen 1,2-oxidoreductase, EC 1.13.11.4) was demonstrated by a temporary increase in absorbance at 334 nm (11), corresponding to the formation of maleylpyruvate (λ_{max} = 334 nm). Catechol 2,3-dioxygenase and protocatechuate 4,5-dioxygenase were assayed at 375 and 410 nm, respectively (18, 19).

Thin-layer chromatography. Spent growth media from 3- to 7-day cultures were decanted, acidified with 12 M HCl to pH 1 to 2, and extracted with diethyl ether. After the ether had evaporated, the residue was redissolved in 95% (vol/vol) ethanol at a concentration of 10 mg ml^{-1} and applied as streaks to 500- or 1000-μm-thick silica gel plates (Absorbosil-5; Applied Science Laboratories, State College, Pa.). Standards (1 μmol) of suspected ring-cleavage intermediates were spotted at one side of each plate.

Chromatographic solvents used were benzene-ethyl acetate-formic acid (85:15:1) (13) and benzene-methanol-acetic acid (45:10:1). Plates were developed, air dried, and examined immediately under short- and long-wave UV lamps. To detect catechol and guaiacol, which did not fluoresce under UV lamps, the standard spots on the plates were sprayed with an indicator [1% (wt/vol) $K_3Fe(CN)_6$ and 1% (wt/vol) $FeCl \cdot 6H_2O$, prepared separately and mixed in equal volumes] (6). All of the compounds except benzoic acid could be detected by the indicator spray. R_f values and colors of spots were compared with those of the standards (Table 1).

Unsprayed bands corresponding to suspected intermediates were scraped from the chromatograms, suspended in 10 ml of 95% ethanol, and centrifuged for 30 min at 5,000 × *g*. The clarified ethanol solutions were then decanted and evaporated to dryness at 100°C. If further purification was desired, the compounds were chromatographed again, using the other solvent. To confirm the identity of purified intermediates from thin-layer chromatograms, UV absorption spectra of recovered compounds were compared with spectra of standards chromatographed in the same way.

Pyruvate determination. A procedure based on the spectrophotometric method of Collinsworth et al. (8) as modified by Crawford (10) was used to demonstrate the quantitative conversion of *m*-hydroxybenzoate through gentisate to pyruvate. Cuvettes containing 2 ml of 500 mM tris(hydroxymethyl)aminomethane–acetate buffer and 1 ml of a cell extract from *m*-hydroxybenzoate-grown cells were incubated at 45°C. Next, 0.1 μmol of *m*-hydroxybenzoate and 0.1 μmol of NADH were added to sample cuvettes, which then were aerated with O_2 for 2.5 h until maleylpyruvate had formed and disappeared, as determined from the absorbance at 340 nm. Then 0.1 μmol of NADH was added, followed 2.5 min later by 5 μl (7.5 U) of D-(−)-lactic dehydrogenase from *Lactobacillus leichmannii* (Sigma Chemical Co.). The decrease in absorbance at 340 nm over 51 min, indicating the oxidation of NADH, and the extinction coefficient of NADH at 340 nm (ε = 6,200) were used to calculate the amount of pyruvate produced from 0.1 μmol of *m*-hydroxybenzoate (10).

RESULTS

All four *Streptomyces* spp. strains had the ability to metabolize one or more hydroxyl- or methoxyl-substituted benzoic acids (Table 2). The enzymes which cleaved the aromatic rings, as shown by the substrate specificity, O_2 uptake assays, and UV absorption spectra of products

TABLE 1. *R_f values for aromatic compounds on silica gel thin-layer plates*

Compound	R_f values	
	Solvent 1[a]	Solvent 2[b]
Benzoic acid	0.54	0.88
Catechol	0.38	0.72
Gentisic acid	0.26	0.51
Guaiacol	0.72	0.84
m-Hydroxybenzoic acid	0.22	0.65
p-Hydroxybenzoic acid	0.22	0.64
Protocatechuic acid	0.08	0.43
Vanillic acid	0.26	0.72
Veratric acid	0.29	0.79

[a] Solvent 1: Benzene-ethyl acetate-formic acid (85:15:1).

[b] Solvent 2: Benzene-methanol-acetic acid (45:10:1).

TABLE 2. *Specific activities of dioxygenases in cell extracts from four Streptomyces spp. grown on substituted benzoic acids*

Strain	Growth substrate[a]	Utilization of aromatic growth substrate[b]	Sp act[c] of:		
			Catechol	Protocatechuate	Gentisate
S. albulus 321	Benzoic acid	+	1.10[d]	<0.01	<0.01
	m-Hydroxybenzoic acid	+	<0.01	1.00	0.93[d]
	p-Hydroxybenzoic acid	+	<0.01	1.78[d]	<0.01
	m-Anisic acid	−	<0.01	0.04	0.46
	p-Anisic acid	−	<0.01	0.01	<0.01
	Vanillic acid	+	<0.01	0.66[d]	<0.01
	Veratric acid	−	<0.01	0.02	<0.01
	Succinic acid[e]	−	<0.01	0.01	<0.01
S. sioyaensis P5	Benzoic acid	−	<0.01	<0.01	<0.01
	m-Hydroxybenzoic acid	−	<0.01	1.32	<0.01
	p-Hydroxybenzoic acid	+	<0.01	1.06[d]	<0.01
	m-Anisic acid	−	<0.01	0.01	<0.01
	p-Anisic acid	−	<0.01	0.05	<0.01
	Vanillic acid	+[f]	<0.01	0.06	<0.01
	Veratric acid	−	<0.01	0.01	<0.01
	Succinic acid	−	<0.01	0.02	<0.01
S. viridosporus T7A	Benzoic acid	+	0.40[d]	<0.01	<0.01
	m-Hydroxybenzoic acid	+	<0.01	<0.01	1.33[d]
	p-Hydroxybenzoic acid	−	<0.01	0.05	0.01
	m-Anisic acid	−	<0.01	<0.01	0.01
	p-Anisic acid	−	<0.01	0.03	<0.01
	Vanillic acid	−	0.01	0.01	<0.01
	Veratric acid	−	0.01	<0.01	0.01
	Succinic acid	−	<0.01	<0.01	<0.01
Streptomyces sp. V7	Benzoic acid	+	2.49[d]	0.23	<0.01
	m-Hydroxybenzoic acid	+	0.05	0.04	0.88[d]
	p-Hydroxybenzoic acid	+	<0.01	0.71[d]	0.01
	m-Anisic acid	−	<0.01	0.05	0.01
	p-Anisic acid	−	<0.01	0.15	<0.01
	Vanillic acid	+	<0.01	1.26[d]	<0.01
	Veratric acid	+	<0.01	0.89[d]	<0.01
	Succinic acid	−	<0.01	0.05	<0.01

[a] Cultures were grown at 45°C for 2 to 3 days in a liquid medium containing 0.1% (wt/vol) of an aromatic compound and 0.05% (wt/vol) yeast extract.

[b] As shown by changes in UV absorption spectra of culture media over 7 days.

[c] Specific activity is expressed as micromoles of O_2 consumed per minute per milligram of protein.

[d] Substrates for ring cleavage detected in ether extracts of culture media by thin-layer chromatography and UV spectrophotometry.

[e] Succinic acid was used as a control.

[f] Vanillate was converted to guaiacol and not metabolized further.

of ring cleavage, were catechol 1,2-dioxygenase, protocatechuate 3,4-dioxygenase, and gentisate 1,2-dioxygenase. All of the dioxygenases were inducible and were detected at high specific activities only in cultures containing appropriate aromatic compounds.

S. albulus 321 produced high levels of catechol 1,2-dioxygenase when grown with benzoate; produced high levels of protocatechuate 3,4-dioxygenase when grown with *p*-hydroxybenzoate, *m*-hydroxybenzoate, or vanillate; and produced high levels of gentisate 1,2-dioxygenase when grown with *m*-hydroxybenzoate or *m*-anisate (Table 2). *S. sioyaensis* P5 produced high levels of protocatechuate 3,4-dioxygenase when grown with *m*-hydroxybenzoate or *p*-hydroxybenzoate (Table 2); no other dioxygenase was detected in this strain. *S. viridosporus* T7A produced high levels of catechol 1,2-dioxygenase and gentisate 1,2-dioxygenase when grown with benzoate and *m*-hydroxybenzoate, respectively (Table 2). *Streptomyces* sp. V7 produced high levels of catechol 1,2-dioxygenase when grown with benzoate; produced high levels of protocatechuate 3,4-dioxygenase when grown with vanillate, veratrate, *p*-hydroxybenzoate, benzoate,

TABLE 3. *Dioxygenases found in cell extracts from Streptomyces spp. strains grown on substituted benzoic acids*[a]

Strain	Inducer	Detection of ring-cleavage enzyme:[b]		
		Catechol 1,2-dioxygenase	Protocatechuate 3,4-dioxygenase	Gentisate 1,2-dioxygenase
S. albulus 321	*m*-Anisate	−	−	+[c]
	Benzoate	+	−	−
	m-Hydroxybenzoate	−	+[c]	+
	p-Hydroxybenzoate	−	+	−
	Vanillate	−	+	−
S. sioyaensis P5	*m*-Hydroxybenzoate	−	+[c]	−
	p-Hydroxybenzoate	−	+	−
S. viridosporus T7A	Benzoate	+	−	−
	p-Hydroxybenzoate	−	−	+
Streptomyces sp. V7	*p*-Anisate	−	+[c]	−
	Benzoate	+	−	−
	m-Hydroxybenzoate	−	−	+
	p-Hydroxybenzoate	−	+	−
	Vanillate	−	+	−
	Veratrate	−	+	−

[a] Cultures were grown at 45°C for 2 to 3 days in a liquid medium containing 0.1% (wt/vol) of an aromatic compound and 0.05% (wt/vol) yeast extract.

[b] Dioxygenases were identified by observing the increases in UV absorbance at 260 nm for catechol 1,2-dioxygenase and protocatechuate 3,4-dioxygenase or at 334 nm for gentisate 1,2-dioxygenase (11, 17, 22). Assays were also run for catechol 2,3-dioxygenase and protocatechuate 4,5-dioxygenase at 375 and 410 nm, respectively (18, 19), but these enzymes were not detected.

[c] Gratuitous induction.

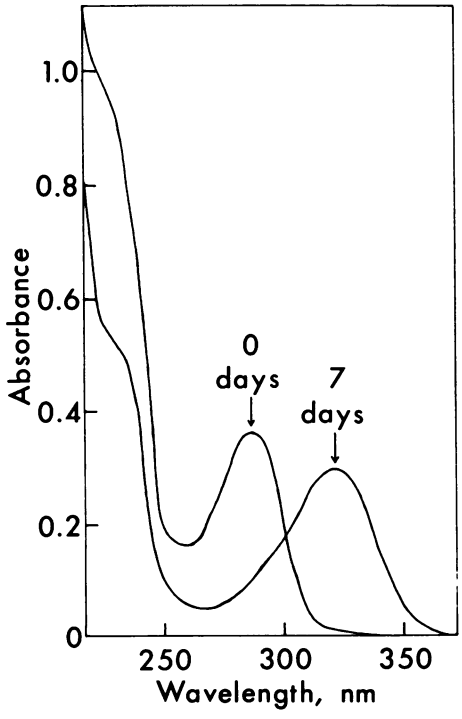

FIG. 1. *Changes in the UV absorption spectrum of the medium during growth of S. albulus 321 on m-hydroxybenzoate; 0 Days, spectrum of m-hydroxybenzoate; 7 days, spectrum identical to that of gentisate, with a* λ_{max} *of 324 nm at pH 7.4.*

or *p*-anisate; and produced high levels of gentisate 1,2-dioxygenase when grown with *m*-hydroxybenzoate (Table 2).

UV absorption spectra of culture media sampled periodically during incubation showed that many of the substituted benzoic acids that induced dioxygenase activity completely disappeared from the media, but others did not disappear (Table 2). No changes in characteristic UV spectra were observed in sterile control flasks. Gratuitous induction was assumed when the addition of a substrate for ring cleavage to a cell-free extract resulted in O_2 uptake, even though the spent culture medium showed no change in UV absorption that would indicate catabolism of the aromatic compound. Among the gratuitous inducers were *m*-anisate in *S. albulus* 321, *m*-hydroxybenzoate in *S. albulus* 321 and *S. sioyaensis* P5, and *p*-anisate in *Streptomyces* sp. V7 (Table 2). For instance, although UV absorption spectra of spent culture media showed that *S. sioyaensis* P5 did not metabolize *m*-hydroxybenzoate, cell-free extracts demonstrated a high specific activity of protocatechuate 3,4-dioxygenase (Table 2). However, the ex-

tracts did not take up O_2 when incubated with *m*-hydroxybenzoate and either NADH or NADPH as a cofactor, indicating the absence or instability of an *m*-hydroxybenzoate hydroxylase. Furthermore, none of the strains catabolized the methoxybenzoates *m*- and *p*-anisate, even though the *m*- and *p*-hydroxylated benzoates and the methoxylated compounds vanillate and veratrate were catabolized by some strains.

Since O_2 uptake data did not distinguish between different ring-cleavage mechanisms for a single substrate, products were identified by increases in UV absorbance at selected wavelengths (Table 3). The dioxygenases found in these strains of *Streptomyces* spp. were catechol 1,2-dioxygenase, protocatechuate 3,4-dioxygenase, and gentisate 1,2-dioxygenase. Catechol 2,3-dioxygenase and protocatechuate 4,5-dioxygenase were not detected.

Thin-layer chromatography and UV spectrophotometry were used to isolate and identify intermediate compounds in the catabolic pathways. Catechol was detected in cell-free extracts from benzoate-grown cultures of *S. albulus* 321, *S. viridosporus* T7A, and *Streptomyces* sp. V7. Protocatechuate was detected in extracts from *p*-hydroxybenzoate-grown cultures of *S. albulus* 321, *S. sioyaensis* P5, and *Streptomyces* sp. V7; from vanillate-grown cultures of *S. albulus* 321 and *Streptomyces* sp. V7; and from veratrate-grown cultures of *Streptomyces* sp. V7. Vanillate was detected in extracts from veratrate-grown cultures of *Streptomyces* sp. V7, and guaiacol in extracts from vanillate-grown cultures of *S. sioyaensis* P5. Gentisate, but not protocatechuate, was detected in extracts from *m*-hydroxybenzoate-grown cultures of *S. albulus* 321, *S. viridosporus* T7A, and *Streptomyces* sp. V7.

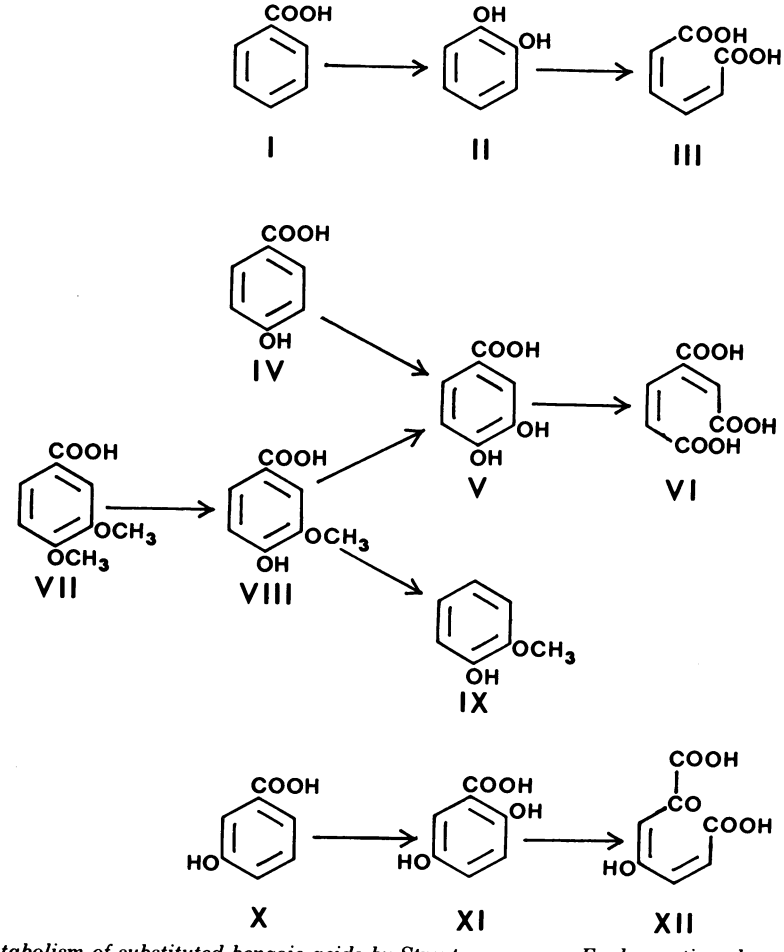

FIG. 2. *Catabolism of substituted benzoic acids by Streptomyces spp. Each reaction shown was found in one or more strains. I, Benzoic acid; II, catechol; III, cis,cis-muconic acid; IV, p-hydroxybenzoic acid; V, protocatechuic acid; VI, β-carboxy-cis,cis-muconic acid; VII, veratric acid; VIII, vanillic acid; IX, guaiacol; X, m-hydroxybenzoic acid; XI, gentisic acid; XII, maleylpyruvic acid.*

Since *S. albulus* 321 produced high levels of both protocatechuate 3,4-dioxygenase and gentisate 1,2-dioxygenase when grown on *m*-hydroxybenzoate (Table 2), the pathway of *m*-hydroxybenzoate catabolism was investigated further in this strain. Cultures grown on *m*-hydroxybenzoate produced a compound that had a UV absorption spectrum (Fig. 1) and an R_f value (Table 1) identical to those of gentisate (λ_{max} = 324 nm). In the pyruvate determination, cell-free preparations converted 0.1 μmol of *m*-hydroxybenzoate to 0.1 μmol of pyruvate, showing that this compound was catabolized by the gentisate pathway.

DISCUSSION

The principal catabolic pathways for substituted benzoic acids in *Streptomyces* spp. are summarized in Fig. 2. They were demonstrated by patterns of oxygenase induction, by UV spectra demonstrating the disappearance of aromatic substrates and the appearance of products, and by pathway intermediates isolated and identified by thin-layer chromatography and UV spectrophotometry. Dilution of cell-free extracts may inhibit oxygenase activity (4), so that a low specific activity does not necessarily indicate complete absence of the enzyme. Subsequent metabolism of the products of ring cleavage was presumably through the gentisate pathway (20) and the catechol and protocatechuate branches of the β-ketoadipate pathway (24).

m-Hydroxybenzoate is catabolized through gentisate in some strains of *Streptomyces* spp. (9) and *Rhodococcus* spp. (14, 26). However, it induces a protocatechuate dioxygenase in some strains of *Nocardia* spp. (3). As shown by the accumulation of pyruvate in reaction mixtures, the strains reported here used the gentisate pathway for catabolism of *m*-hydroxybenzoate.

Production of guaiacol from vanillate, observed in *S. sioyaensis* P5, is also known in *Streptomyces* sp. strain 179 (13). Although Crawford and Olson (13) suggest that decarboxylation of vanillate might be a step in a reaction sequence leading to the production of catechol, strain 179 does not metabolize guaiacol further. Guaiacol also accumulated, without further metabolism, in *S. sioyaensis* P5.

Our results show that *Streptomyces* spp. strains catabolized substituted benzoic acids through catechol, protocatechuate, and gentisate; all three of the ring-cleavage dioxygenases were inducible. Other *Streptomyces* spp. strains are known to attack polymeric aromatic compounds such as lignin (9); it is now clear that streptomycetes are also able to catabolize a variety of single-ring aromatic compounds. Thus, these organisms are likely to play an important role in recycling aromatic residues in nature.

ACKNOWLEDGMENTS

This work was supported by grant PFR-78-09453 from the National Science Foundation and by the Idaho Agricultural Experiment Station.

We thank M. P. Lechevalier of Rutgers University for cell-wall analysis of strain T7A and R. L. Crawford of the University of Minnesota for helpful comments.

LITERATURE CITED

1. **Cain, R. B., R. F. Bilton, and J. A. Darrah.** 1968. The metabolism of aromatic acids by micro-organisms: metabolic pathways in the fungi. Biochem. J. **108**:797-828.
2. **Cain, R. B., and N. J. Cartwright.** 1960. On the properties of some aromatic ring-opening enzymes of species of the genus *Nocardia*. Biochim. Biophys. Acta **37**:197-213.
3. **Cartwright, N. J., and R. B. Cain.** 1959. Bacterial degradation of the nitrobenzoic acids. Biochem. J. **71**: 248-261.
4. **Cartwright, N. J., and A. R. W. Smith.** 1967. Bacterial attack on phenolic ethers: an enzyme system demethylating vanillic acid. Biochem. J. **102**:826-841.
5. **Cartwright, N. J., A. R. W. Smith, and J. A. Buswell.** 1970. Bacterial attack on phenolic ethers: inducible oxidative metabolism of phenylmethyl ethers by bacteria. Microbios **2**:113-143.
6. **Catroux, G., J. C. Fournier, and J. Rivière.** 1969. Mise en évidence rapide de l'utilisation des acides-phénols par des souches bactériennes isolées du sol. Ann. Inst. Pasteur (Paris) **116**:99-102.
7. **Chapman, P. J.** 1972. An outline of reaction sequences used for the bacterial degradation of phenolic compounds, p. 17-55. *In* Division of Biology and Agriculture (ed.), Degradation of synthetic organic molecules in the biosphere. National Academy of Sciences, Washington, D.C.
8. **Collinsworth, W. L., P. J. Chapman, and S. Dagley.** 1973. Stereospecific enzymes in the degradation of aromatic compounds by *Pseudomonas putida*. J. Bacteriol. **113**:922-931.
9. **Crawford, D. L., and J. B. Sutherland.** 1979. The role of actinomycetes in the decomposition of lignocellulose. Dev. Ind. Microbiol. **20**:143-151.
10. **Crawford, R. L.** 1975. Novel pathway for degradation of protocatechuic acid in *Bacillus* species. J. Bacteriol. **121**:531-536.
11. **Crawford, R. L., S. W. Hutton, and P. J. Chapman.** 1975. Purification and properties of gentisate 1,2-dioxygenase from *Moraxella osloensis*. J. Bacteriol. **121**: 794-799.
12. **Crawford, R. L., E. McCoy, J. M. Harkin, T. K. Kirk, and J. R. Obst.** 1973. Degradation of methoxylated benzoic acids by a *Nocardia* from a lignin-rich environment: significance to lignin degradation and effect of chloro substituents. Appl. Microbiol. **26**:176-184.
13. **Crawford, R. L., and P. P. Olson.** 1978. Microbial catabolism of vanillate: decarboxylation to guaiacol. Appl. Environ. Microbiol. **36**:539-543.
14. **Eggeling, L., and H. Sahm.** 1980. Degradation of coniferyl alcohol and other lignin-related aromatic compounds by *Nocardia* sp. DSM 1069. Arch. Microbiol. **126**:141-148.
15. **Engelhardt, G., H. G. Rast, and P. R. Wallnöfer.** 1979. Degradation of aromatic carboxylic acids by *Nocardia* spec. DSM 43251. FEMS Microbiol. Lett. **5**:245-251.
16. **Goodfellow, M., and G. Alderson.** 1977. The actinomycete-genus *Rhodococcus*: a home for the 'rhodochrous' complex. J. Gen. Microbiol. **100**:99-122.
17. **Hayaishi, O., M. Katagiri, and S. Rothberg.** 1957.

Studies on oxygenases: pyrocatechase. J. Biol. Chem. **229**:905-920.

18. **Klages, U., and F. Lingens.** 1979. Degradation of 4-chlorobenzoic acid by a *Nocardia* species. FEMS Microbiol. Lett. **6**:201-203.
19. **Kojima, Y., N. Itada, and O. Hayaishi.** 1961. Metapyrocatechase: a new catechol-cleaving enzyme. J. Biol. Chem. **236**:2223-2228.
20. **Lack, L.** 1959. The enzymic oxidation of gentisic acid. Biochim. Biophys. Acta **34**:117-123.
21. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265-275.
22. **MacDonald, D. L., R. Y. Stanier, and J. L. Ingraham.** 1954. The enzymatic formation of β-carboxymuconic acid. J. Biol. Chem. **210**:809-820.
23. **Morrison, T. J., and F. Billett.** 1952. The salting-out of non-electrolytes. Part II. The effect of variation in non-electrolyte. J. Chem. Soc. **1952**:3819-3822.
24. **Ornston, L. N., and R. Y. Stanier.** 1966. The conversion of catechol and protocatechuate to β-ketoadipate by *Pseudomonas putida*. I. Biochemistry. J. Biol. Chem. **241**:3776-3786.
25. **Rann, D. L., and R. B. Cain.** 1973. Regulation of the enzymes of aromatic ring fission in the genus *Nocardia*. Biochem. Soc. Trans. **1**:658-661.
26. **Rast, H. G., G. Engelhardt, and P. R. Wallnöfer.** 1980. Degradation of aromatic compounds in the actinomycete-genus *Rhodococcus*. FEMS Microbiol. Lett. **7**:1-6.
27. **Shirling, E. B., and D. Gottlieb.** 1968. Cooperative description of type cultures of *Streptomyces*. II. Species descriptions from first study. Int. J. Syst. Bacteriol. **18**: 69-189.
28. **Shirling, E. B., and D. Gottlieb.** 1972. Cooperative description of type strains of *Streptomyces*. V. Additional descriptions. Int. J. Syst. Bacteriol. **22**:265-394.
29. **Treccani, V., E. Galli, D. Catelani, and C. Sorlini.** 1969. Induction of 1,2- and 2,3-diphenol oxygenases in *Pseudomonas desmolyticum*. Z. Allg. Mikrobiol. **8**:65-69.