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Catabolism of Benzoate and Monohydroxylated Benzoates by *Amycolatopsis* and *Streptomyces* spp.

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Eight actinomycetes of the genera *Amycolatopsis* and *Streptomyces* were tested for the degradation of aromatic compounds by growth in a liquid medium containing benzoate, monohydroxylated benzoates, or quinate as the principal carbon source. Benzoate was converted to catechol. The key intermediate in the degradation of salicylate was either catechol or gentisate, while *m*-hydroxybenzoate was metabolized via gentisate or protocatechuate. *p*-Hydroxybenzoate and quinate were converted to protocatechuate. Catechol, gentisate, and protocatechuate were cleaved by catechol 1,2-dioxygenase, gentisate 1,2-dioxygenase, and protocatechuate 3,4-dioxygenase, respectively. The requirement for glutathione in the gentisate pathway was dependent on the substrate and the particular strain. The conversion of *p*-hydroxybenzoate to protocatechuate by *p*-hydroxybenzoate hydroxylase was gratuitously induced by all substrates that were metabolized via protocatechuate as an intermediate, while protocatechuate 3,4-dioxygenase was gratuitously induced by benzoate and salicylate in two *Amycolatopsis* strains.

Several catabolic pathways for the breakdown of aromatic compounds are known, and most of this knowledge comes from studies of members of the genus *Pseudomonas* (15, 20, 30). This genus is regarded as ubiquitous and successful because of its well-known capacity to utilize an extraordinarily wide range of compounds, including many aromatics, as nutrients. In fact, the elective culture techniques for the isolation of microorganisms with unusual catabolic activities favor the selection of these organisms, which have faster growth rates and rapidly dominate the culture. Also, gram-negative bacteria are favored since the methods for in vitro recombination are well developed. The nutritional versatility of the soil actinomycetes is quite comparable with that of pseudomonads (6). However, there is only a little information available on the degradation routes for aromatic compounds by this interesting group of organisms. It has been known for many years that nocardioform actinomycetes, especially members of the genus *Rhodococcus*, are able to degrade a wide variety of aromatic substrates, including nitroaromatic compounds (4, 5), pyridine (29), 4-chlorobenzoate (13), toluate (18), and pentachlorophenol (3). We have extended these studies to another related genus, *Amycolatopsis*, because all members of this genus were able to grow on benzoate or monohydroxylated benzoic acids as the sole sources of carbon and energy. This genus has been described in 1985 (16) and, like the *Rhodococcus* spp., belongs to the taxonomical group of nocardioform bacteria. Most of the members of the genus *Amycolatopsis* are known to produce antibiotics (16), but they never have been examined with regard to their abilities to grow on aromatic substrates.

For comparison, we also examined a few *Streptomyces* strains because some members of this genus are known to degrade lignin and many other aromatic compounds (2, 7, 21, 22, 26, 27). We selected some *Streptomyces* species that have not been looked at before by other authors because we wanted to demonstrate that the ability to grow on aromatic substrates is widespread within this genus. We intended to detect possible differences between these two groups of organisms in the routes and induction patterns for the degradation of benzoate and monohydroxylated benzoic acids. Quinic acid and protocatechuic acid were included in our studies in order to demonstrate enzyme induction due to the formation of protocatechuate.

MATERIALS AND METHODS

Organisms and growth conditions. *Amycolatopsis* and *Streptomyces* spp. were obtained from the German culture collection (DSM-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Federal Republic of Germany), except for *Streptomyces ghanaensis* FH 1290, which was obtained from W. Wohlleben (Universität Bielefeld).

Sodium benzoate, *p*-hydroxybenzoic acid, *m*-hydroxybenzoic acid, salicylic acid, and gentisic acid were obtained from E. Merck AG, Darmstadt, Federal Republic of Germany; protocatechuic acid, *N*-ethylmaleimide, NAD, NADH, and NADPH were obtained from Sigma Chemie GmbH, Deisenhofen, Federal Republic of Germany.

The culture medium was made according to the method of Seiler (24) and contained 1 to 3 g of the aromatic substrate per liter. After inoculation, cultures were grown for 3 to 5 days on a rotary shaker at 30°C.

Preparation of cell extracts. Mycelia were harvested by centrifugation at 4,400 × *g* for 10 min and washed with cold 50 mM Tris hydrochloride buffer, pH 8.0. The washed pellets were suspended in 5 ml of the same buffer, and cells were disrupted by two passes through an AMINCO French pressure cell (SLM Instruments Inc., Urbana, Ill.) at 18,000 lb/in^2. The extract was cleared by centrifugation at 25,000 × *g* for 30 min, and the pellet was discarded. The protein concentration of the cell extracts was determined by the biuret method (1) and was usually 7 to 25 mg/ml.

Oxygenase assays. Oxygenase activity in the cell extracts was assayed at 25°C with an oxygen electrode (Rank Brothers, Bottisham, Cambridge, England). The solubility of O_2 in water at 25°C is approximately 250 μmol/liter (28). Reaction mixtures contained 0.1 to 0.5 ml of crude extract, 1.0 μmol

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of a known oxygenase substrate, and the appropriate buffers in a final volume of 3 ml. For determination of monooxygenase activity, 0.3 μmol of NADH or NADPH was added to the reaction mixtures. Specific activities were calculated as nanomoles of O_2 consumed per minute per milligram of protein. The following enzyme activities were measured: benzoate 1,2-dioxygenase (EC 1.13.99.2; 31), salicylate hydroxylase (EC 1.14.13.1; 32), *m*-hydroxybenzoate hydroxylases (EC 1.14.13.23 and 1.14.13.24; 11, 17), *p*-hydroxybenzoate hydroxylase (EC 1.14.13.2; 12), catechol 1,2-dioxygenase (EC 1.13.11.1; 5), protocatechuate 3,4-dioxygenase (1.13.11.3; 25), and gentisate 1,2-dioxygenase (EC 1.13.11.4; 9).

Products of ring cleavage generated in cell extracts were identified spectrophotometrically. Catechol 1,2-dioxygenase activity was demonstrated by a temporary increase in the A_{260} (5), corresponding to the formation of *cis,cis*-muconate, as was the activity of protocatechuate 3,4-dioxygenase, which corresponded to the formation of β-carboxy-*cis,cis*-muconate (25). Gentisate 1,2-dioxygenase was detected by a temporary increase in the A_{334} (9) which was due to the formation of maleylpyruvate. *N*-Ethylmaleimide was used for estimating the glutathione dependency of the gentisate pathway (8).

The monooxygenases like salicylate hydroxylase, *m*-hydroxybenzoate hydroxylase, and *p*-hydroxybenzoate hydroxylase were detected spectrophotometrically by a decrease in the A_{340}, corresponding to the disappearance of NADH or NADPH, respectively. Unlike the *m*-hydroxybenzoate 4-hydroxylase of *Pseudomonas testosteroni*, which requires NADPH as a reductant (17), the *m*-hydroxybenzoate hydroxylase of *Amycolatopsis* spp. requires NADH. For the glutathione-dependent isomerization of maleylpyruvate, 0.3 μmol of reduced glutathione was added to the reaction mixture since in the spectrophotometric test for *m*-hydroxybenzoate hydroxylase, the formation of maleylpyruvate raises the A_{340}, interfering with the detection of NADH.

Detection of aromatic compounds. The disappearance of the aromatic substrates and, in some cases, the transient formation of gentisate or protocatechuate was followed by high-pressure liquid chromatography analysis. Samples were diluted and injected directly onto an Rp-18 column (125 by 4.6 mm, packed with Spherisorb ODS II [5 μm] [Bischoff Chromatography, Leonberg, Federal Republic of Germany]). Two different solvent systems were used, one with 17% methanol in 0.1 M potassium phosphate buffer (pH 3.0) for the separation of polar compounds like gentisate (retention volume, 3.28 ml), protocatechuate (retention volume, 3.54 ml), catechol (retention volume, 4.19 ml), *p*-hydroxybenzoate (retention volume, 5.98 ml), and *m*-hydroxybenzoate (retention volume, 7.46 ml) and a second one with 45% methanol in the same buffer for the separation of less polar compounds like salicylate (retention volume, 2.95 ml) and benzoate (retention volume, 4.18 ml). The high-pressure liquid chromatography system consisted of a high-precision pump model 300 C and a C-R3A integrator (Gynkotek GmbH, Germering, Federal Republic of Germany) and a Spectroflow 757 absorbance detector (Kratos Analytical Instruments, Ramsey, N.J.). The analysis was done at a flow rate of 1 ml/min, and the compounds were detected by their UV A_{220}.

RESULTS

All four *Streptomyces* and *Amycolatopsis* strains were able to metabolize benzoate or one or more of the hydroxylated derivatives. We were able to demonstrate by high-pressure liquid chromatography the transient accumulation of gentisate or protocatechuate in some cultures during growth on the appropriate substrate. The data are summarized in Table 1. Enzymes cleaving the aromatic ring, as shown by the substrate specificity, O_2 uptake assays, and UV absorption, were catechol 1,2-dioxygenase, protocatechuate 3,4-dioxygenase, and gentisate 1,2-dioxygenase (Table 2). All dioxygenases are inducible, and high specific activities were observed only when the cultures contained the appropriate aromatic compounds.

TABLE 1. Aromatic substrates tested for degradation by four strains of *Amycolatopsis* spp. and *Streptomyces* spp. and accumulation of characteristic intermediates (when detected)

Species	Use[a] of:				
	Benzoate	Salicylate	*m*-OHB	*p*-OHB	Quinate
Amycolatopsis spp.					
A. mediterranei (DSM 40501)	−	−	−	+	+
A. rugosa (DSM 43194)	+	−	−	+	−
DSM 43387	+	+	Prot.	Prot.	Prot.
DSM 43388	+	+	Gent.	+	Prot.
Streptomyces spp.					
S. ghanaensis (FH 1290)	+	−	Gent.	−	Prot.
S. niger (DSM 40302)	+	+	−	+	+
S. olivaceiscloticus (DSM 40595)	+	+	−	+	+
S. umbrinus (DSM 40278)	+	Gent.	−	+	+

[a] −, No growth; +, utilization of the substrate; Gent., accumulation of gentisate; Prot., accumulation of protocatechuate; *m*-OHB, *m*-hydroxybenzoate; *p*-OHB, *p*-hydroxybenzoate.

Benzoate. Benzoate was converted to catechol by all strains able to grow on this substrate. Further metabolism proceeded via the catechol branch of the β-ketoadipate pathway. This is corroborated by the occurrence of high specific activities of catechol 1,2-dioxygenase (Table 2). In two *Amycolatopsis* strains, a gratuitous induction of protocatechuate 3,4-dioxygenase (Table 2, E2) which was not observed in *Amycolatopsis rugosa* and the four *Streptomyces* strains examined occurred. Another interesting observation was the gratuitous induction of salicylate hydroxylase in these two strains when they were cultivated in the presence of benzoate (Table 2, E5).

Salicylate. For the degradation of salicylate, we were able to demonstrate the existence of two different routes within the genus *Streptomyces* (Table 2 and Fig. 1). *Streptomyces niger* DSM 40302, *Streptomyces olivaceiscleroticus* DSM 40595, and *Amycolatopsis* spp. DSM 43387 and 43388 converted salicylate to catechol, and further metabolism was via the catechol branch of the β-ketoadipate pathway. The pattern of enzymes induced in these strains very much resembled that obtained with benzoate (Table 2). However, there was one exception: *Streptomyces umbrinus* (DSM 40278) converted salicylate to gentisate (Table 1), and we could demonstrate gentisate 1,2-dioxygenase activity, while no other ring-cleaving dioxygenase was detectable for this strain. Furthermore, no reduced glutathione was required for the gentisate pathway induced by salicylate (Fig. 2).

***m*-Hydroxybenzoate.** *m*-Hydroxybenzoate was degraded by only three of the eight strains investigated. Interestingly, we found two different degradation routes within the genus *Amycolatopsis*. Strain DSM 43387 metabolized this sub-

TABLE 2. Specific activities of monooxygenases and dioxygenases in cell extracts of *Amycolatopsis* and *Streptomyces* spp. grown on different substrates

Species	Substrate[a]	Sp act[b] in:						
		E1	E2	E3	E4	E5	E6	E7
A. mediterranei (DSM 40501)	*p*-OHB	–	99	–	–	–	–	228
	Quinate	–	42	–	–	–	–	12
	Protocat.	–	65	–	–	–	–	38
A. rugosa (DSM 43194)	Benzoate	330	–	–	12	–	–	–
	p-OHB	–	139	–	–	–	–	87
	Protocat.	–	10	–	–	–	–	–
Amycolatopsis sp. (DSM 43387)	Benzoate	168	28	–	17	13	–	–
	Salicylate	569	72	–	–	83	–	–
	m-OHB	9	22	–	–	–	18	12
	p-OHB	13	13	–	–	–	–	43
	Quinate	5	38	–	–	–	–	10
	Protocat.	10	40	–	–	–	–	7
Amycolatopsis sp. (DSM 43388)	Benzoate	222	25	–	53	26	–	–
	Salicylate	103	84	–	15	134	–	–
	m-OHB	–	–	250	–	11	199	–
	p-OHB	35	19	25	–	–	–	–
	Quinate	6	65	16	–	–	–	–
	Protocat.	5	17	35	–	–	–	–
S. ghanaensis (FH 1290)	Benzoate	27	–	–	–[c]	–	–	–
	m-OHB	–	–	128	–	–	44	–
	Quinate	–	11	–	–	–	–	–
S. niger (DSM 40302)	Benzoate	127	–	–	–[c]	–	–	–
	Salicylate	136	–	–	–	–[c]	–	–
	p-OHB	–	114	–	–	–	–	75
	Quinate	–	22	–	–	–	–	20
S. olivaceiscloticus (DSM 40595)	Benzoate	206	–	–	–[c]	–	–	–
	Salicylate	102	–	–	–	–[c]	–	–
	p-OHB	–	105	–	–	–	–	99
	Quinate	–	113	–	–	–	–	24
S. umbrinus (DSM 40278)	Benzoate	194	–	–	–[c]	–	–	–
	Salicylate	–	–	94	–	–[c]	–	–
	p-OHB	–	58	–	–	–	–	110
	Quinate	–	15	–	–	–	–	35

[a] *p*-OHB, *p*-Hydroxybenzoate; Protocat., protocatechuate; *m*-OHB, *m*-hydroxybenzoate.

[b] Expressed as nanomoles of substrate oxidized per minute per milligram of protein. E1, Catechol 1,2-dioxygenase; E2, protocatechuate 3,4-dioxygenase; E3, gentisate 1,2-dioxygenase; E4, benzoate 1,2-dioxygenase; E5, salicylate hydroxylase; E6, *m*-hydroxybenzoate hydroxylase; E7, *p*-hydroxybenzoate hydroxylase; –, no enzyme activity detected.

[c] The benzoate 1,2-dioxygenase and salicylate hydroxylase activities of *Streptomyces* spp. were not detectable by our methods, probably because of the instability of these enzymes.

strate via protocatechuate; accordingly, we detected *m*-hydroxybenzoate hydroxylase (Table 2, E6) and protocatechuate 3,4-dioxygenase activities. In addition to these expected enzyme activities, we observed induction of *p*-hydroxybenzoate hydroxylase and, to some extent, catechol 1,2-dioxygenase. This pattern of induced enzymes somehow resembles that obtained after growth on *p*-hydroxybenzoate, quinate, and protocatechuate (Table 2). Another *Amycolatopsis* strain (DSM 43388) able to grow on *m*-hydroxybenzoate as the sole carbon source and *S. ghanaensis* (FH 1290) converted this substrate to gentisate (Table 1). In both strains, induction of the gentisate 1,2-dioxygenase occurred while no other ring-cleaving dioxygenase was detected in cell extracts. However, these two strains differed with regard to the glutathione requirements of their gentisate pathways. In *Amycolatopsis* sp. strain DSM 43388, the maleylpyruvate disappeared even without the addition of reduced glutathione and in the presence of *N*-ethylmaleimide (Fig. 3). In contrast, the gentisate pathway in *S. ghanaensis* is glutathione dependent (Fig. 4).

***p*-Hydroxybenzoate.** *p*-Hydroxybenzoate was converted to protocatechuate by all strains that were able to grow on this substrate and was further degraded via the β-ketoadipate pathway as demonstrated by the presence of protocatechuate 3,4-dioxygenase activities (Table 2, E2). In addition to this enzyme, all strains with the exception of *Amycolatopsis* sp. strain DSM 43388 exhibited an induction of the *p*-hydroxybenzoate hydroxylase. *Amycolatopsis* sp. strain DSM 43388 had a lag period of 7 to 10 days until growth on *p*-hydroxybenzoate was initiated, and concentrations higher than 1 g/liter inhibited growth. Thus, it seems that the *p*-hydroxybenzoate hydroxylase of this strain is quite unstable and has a low specific activity. In the two *Amycolatopsis* spp. strains DSM 43387 and DSM 43388, we could demonstrate a weak induction of the catechol 1,2-dioxygenase by *p*-hydroxybenzoate, and interestingly, in strain DSM 43388, the gentisate 1,2-dioxygenase was induced by this substrate as well (Table 2). The induction was about 10% of that observed with *m*-hydroxybenzoate as the substrate. This is the first example of such an unusual induction pattern within the actinomycetes.

Nearly identical patterns of enzyme induction were observed after growth on *p*-hydroxybenzoate, quinate, or protocatechuate (Table 2). In addition to protocatechuate 3,4-dioxygenase, there was always an induction of the *p*-hydroxybenzoate hydroxylase. There were only two exceptions: in *A. rugosa*, we never observed any gratuitous induction, and in *Amycolatopsis* sp. strain DSM 43388, *p*-hydroxybenzoate hydroxylase was not detected at all. We therefore propose that protocatechuate may serve as an inducer for *p*-hydroxybenzoate hydroxylase within the genus *Streptomyces* and within at least two of the four *Amycolatopsis* strains. The activity of this enzyme was highest with *p*-hydroxybenzoate at the level of the fully induced enzyme, while other substrates always resulted in lower enzyme activities. In *Amycolatopsis* sp. strain DSM 43388, we observed the unexpected induction of the gentisate 1,2-dioxygenase by all substrates that were degraded via protocatechuate, which seemed to act here as a weak inducer for this enzyme. However, we cannot rule out the possibility that the observed enzymatic activity resulted from an unspecific cleavage of gentisate by protocatechuate 3,4-dioxygenase.

DISCUSSION

The most likely catabolic pathways for benzoate and the monohydroxylated benzoates in *Amycolatopsis* and *Streptomyces* spp. are outlined in Fig. 1. Our conclusions are derived from the pattern of oxygenase induction, by high-pressure liquid chromatography analysis of the culture fluid, and by spectrophotometry of reaction mixtures containing cell extracts.

With respect to the degradation routes, the two genera *Streptomyces* and *Amycolatopsis* resemble each other. Differences appear only in the degradation of salicylate and *m*-hydroxybenzoate. In the genus *Streptomyces*, salicylate could be catabolized by two different pathways. All *Amycolatopsis* strains and two of the three streptomycetes able to

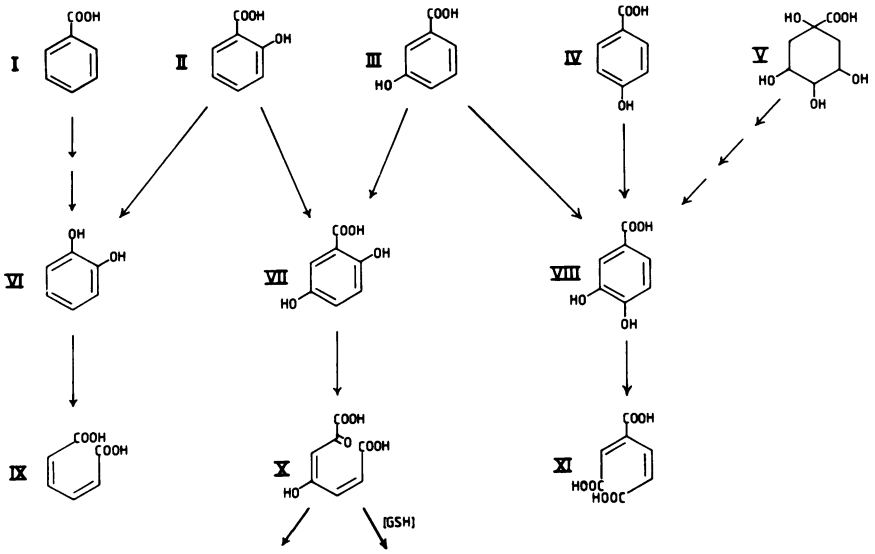

FIG. 1. Catabolism of benzoate and monohydroxylated benzoates by *Amycolatopsis* and *Streptomyces* spp. Each reaction shown was found in one or more strains. I, Benzoic acid; II, salicylic acid; III, *m*-hydroxybenzoic acid; IV, *p*-hydroxybenzoic acid; V, quinic acid; VI, catechol; VII, gentisic acid; VIII, protocatechuic acid; IX, *cis,cis*-muconic acid; X, maleylpyruvic acid; XI, β-carboxy-*cis,cis*-muconic acid; GSH, reduced glutathione.

grow on this substrate degraded the compound via catechol, while in *S. umbrinus*, the gentisate pathway was induced (Tables 1 and 2). This indicates that there may exist two different salicylate hydroxylases in streptomycetes: the salicylate 1-hydroxylase, leading to the formation of catechol, and the salicylate 5-hydroxylase, which forms gentisate. It should be mentioned that this reflects a well-known situation in the genus *Pseudomonas*, where two different degradation routes for salicylate exist, one via catechol and a second via gentisate (33).

The common catabolism of *m*-hydroxybenzoate in strep-

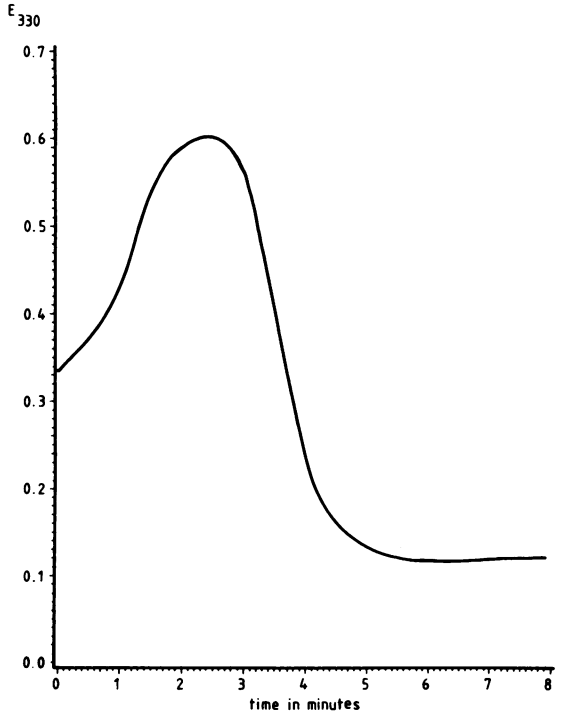

FIG. 2. Glutathione independency of the gentisate pathway in *S. umbrinus*. The strain was grown on salicylate. The transient increase in the A_{330} (E_{330}) in the presence of 1 mM *N*-ethylmaleimide is due to the rapid isomerization of maleylpyruvate. The reaction did not require the addition of reduced glutathione.

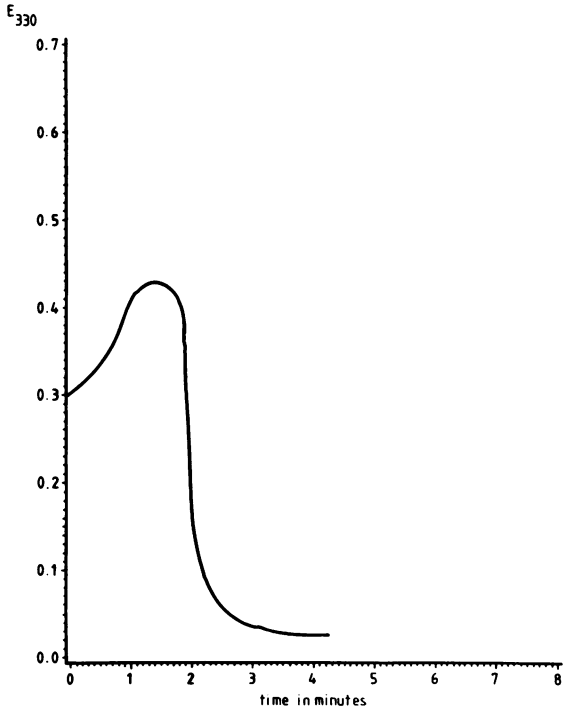

FIG. 3. Glutathione independency of the gentisate pathway in *Amycolatopsis* sp. strain DSM 43388. The strain was grown on *m*-hydroxybenzoate. The transient increase in the A_{330} (E_{330}) in the presence of 1 mM *N*-ethylmaleimide was due to the rapid isomerization of maleylpyruvate. The reaction did not require addition of reduced glutathione.

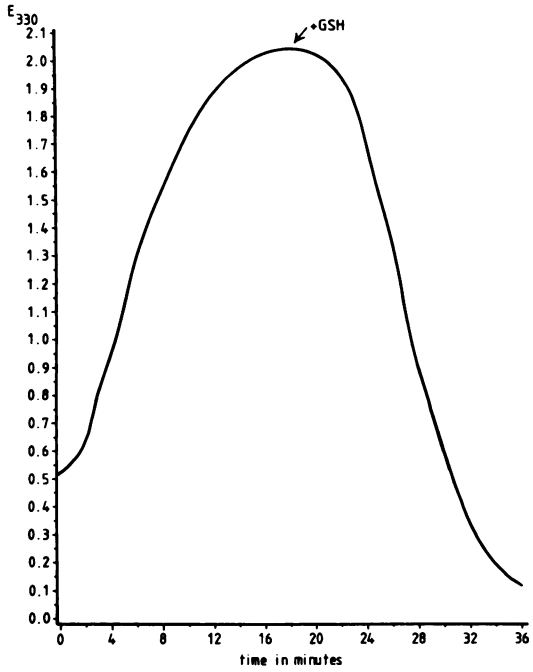

FIG. 4. Glutathione dependency of the gentisate pathway in *S. ghanaensis*. The strain was grown on *m*-hydroxybenzoate. The A_{330} (E_{330}) in the presence of 1 mM *N*-ethylmaleimide (accumulation of maleylpyruvate) was followed. Arrow indicates addition of 1 mM reduced glutathione (GSH).

tomycetes is via gentisate (25). In our study, *S. ghanaensis* was the only strain of the genus *Streptomyces* able to metabolize *m*-hydroxybenzoate, and indeed, we found an induction of gentisate 1,2-dioxygenase. The genus *Amycolatopsis* had two degradation routes for *m*-hydroxybenzoate, either via gentisate or via protocatechuate (Table 2). Again, this reflects a well-known situation in the genus *Pseudomonas*, where two different *m*-hydroxybenzoate hydroxylases have been observed (12, 17, 33). Therefore, we propose that there exists a *m*-hydroxybenzoate 4-hydroxylase as well as a *m*-hydroxybenzoate 6-hydroxylase within the genus *Amycolatopsis*. However, this position assignment is based only on the appearance of intermediates of *m*-hydroxybenzoate metabolism and on induction of the corresponding dioxygenases. To prove this proposal, it is necessary to characterize the enzyme reaction products, which was not possible in crude cell extracts that were used in our investigations. Therefore, no position assignments for the salicylate hydroxylases or for the *m*-hydroxybenzoate hydroxylases are made in Table 2.

Interestingly, there was a difference in the glutathione requirement of the gentisate pathway when induced either by salicylate or by *m*-hydroxybenzoate. The enzyme induced by *m*-hydroxybenzoate in *S. ghanaensis* required reduced glutathione for the isomerization of maleylpyruvate (Fig. 4), while pathways induced by salicylate in *S. umbrinus* and by *m*-hydroxybenzoate in *Amycolatopsis* sp. strain DSM 43388 are glutathione independent (Fig. 2 and 3). As has been pointed out by Crawford and Frick (8), there are two possible explanations for the reduced glutathione-independent gentisate pathway. The maleylpyruvate may be directly cleaved to maleate and pyruvate, or the isomerization of maleylpyruvate to fumarylpyruvate which normally is catalyzed by a reduced glutathione-requiring isomerase may be reduced glutathione independent. Unfortunately, we were not able to distinguish between these two possibilities.

Regarding the regulation of enzyme induction, there were some differences observed between the two genera *Amycolatopsis* and *Streptomyces*. The genus *Amycolatopsis* belongs to the taxonomical group of nocardioform actinomycetes (16), and for these organisms, Cain (6) has reported that the enzymes of the protocatechuate branch of the β-ketoadipate pathway are induced by β-ketoadipate. Indeed, these enzymes were gratuitously induced in the two *Amycolatopsis* spp. strains DSM 43387 and DSM 43388 when the cells were grown on substrates that were degraded via catechol (Table 2). In *A. rugosa*, however, we never observed a gratuitous induction of enzymes involved in the breakdown of aromatic compounds, and all streptomycetes tested showed no activity of the enzymes of the protocatechuate branch on substrates that are not metabolized via protocatechuate. This shows that differences in the regulation of enzyme induction appear even within one genus and that the streptomycetes exhibit induction patterns different from those of *Amycolatopsis* spp.

For both genera, we observed a gratuitous induction of the *p*-hydroxybenzoate hydroxylase on all substrates that were catabolized via protocatechuate (Table 2). Therefore, we propose that protocatechuate is an inducer of this enzyme in *Streptomyces* and *Amycolatopsis* spp. This contradicts the observations of Hosokawa and Stanier (12) and Cain (6), who found an induction of *p*-hydroxybenzoate 3-hydroxylase by *p*-hydroxybenzoate itself. However, only a weak induction of *p*-hydroxybenzoate hydroxylase was observed in strains grown on substrates which were degraded via protocatechuate (Table 2). This may indicate that induction of this enzyme is accomplished by more than one inducer; however, *p*-hydroxybenzoate is necessary for full induction of the enzyme. Another unexpected result was the induction of gentisate 1,2-dioxygenase in *Amycolatopsis* sp. strain DSM 43388 by all substrates that were degraded via protocatechuate (Table 2).

Our data show that the catabolic diversity for the degradation of monocyclic aromatic compounds within the genera *Streptomyces* and *Amycolatopsis* is quite similar to that observed within the pseudomonads. However, we never detected any *meta*-cleaving dioxygenases by the photometric tests described by Kojima et al. (14) for metapyrocatechase and by Ono et al. (19) for protocatechuate 4,5-dioxygenase. This is in agreement with reports by other workers on nocardioform actinomycetes (6, 10, 18) and streptomycetes (26) that *meta* cleavage seems to be a rare event in actinomycetes and, if it occurs, depends very strongly on the substrates that serve as inducers (23). To summarize, our data support the notion, which deserves further investigation, that the soil actinomycetes play an important role in the recycling of aromatic residues in nature.

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