APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Feb. 1993, p. 567–572
0099-2240/93/020567-06$02.00/0

Vol. 59, No. 2

Metabolism of Tetralin (1,2,3,4-Tetrahydronaphthalene) in *Corynebacterium* sp. Strain C125

JAN SIKKEMA* AND JAN A. M. DE BONT

Division of Industrial Microbiology, Department of Food Science, Wageningen Agricultural University, P.O. Box 8129, 6700 EV Wageningen, The Netherlands

Received 5 August 1992/Accepted 18 November 1992

***Corynebacterium* sp. strain C125, originally isolated on *o*-xylene, was selected for its ability to grow on tetralin (1,2,3,4-tetrahydronaphthalene) as the sole source of carbon and energy. The catabolism of tetralin in *Corynebacterium* sp. strain C125 was shown to proceed via initial hydroxylation of the benzene nucleus at positions C-5 and C-6, resulting in the formation of the corresponding *cis*-dihydro diol. Subsequently, the dihydro diol was dehydrogenated by a NAD-dependent dehydrogenase to 5,6,7,8-tetrahydro-1,2-naphthalene diol. The aromatic ring was cleaved in the extradiol position by a catechol-2,3-dioxygenase. The ring fission product was subject to a hydrolytic attack, resulting in the formation of a carboxylic acid-substituted cyclohexanone. This is the first report of the catabolism of tetralin via degradation of the aromatic moiety.**

Tetralin (1,2,3,4-tetrahydronaphthalene) consists of an aromatic and an alicyclic moiety. The compound occurs in coal tar and petroleum and is produced for industrial purposes either from naphthalene by catalytic hydrogenation or from anthracene by cracking. Tetralin is widely applied as a solvent in the petrochemical industry, in which it is particularly used in connection with coal liquefaction. It is also used in paints and waxes as a substitute for turpentine (11).

Tetralin was slowly degraded by mixed cultures of microorganisms (41) or in the presence of cosubstrates (19, 40), but it was persistent as a single substrate in experiments with pure cultures (33). Until recently, its metabolism had only been studied in strains that transformed tetralin under cooxidative conditions (10, 17, 19, 33).

In a previous paper, we reported eight bacteria that were able to utilize tetralin as the sole source of carbon and energy (35). It was shown subsequently that tetralin is extremely toxic to microbial cells as a result of its selective partitioning into cell membranes (36). Four of the eight tetralin-utilizing bacteria were isolated by selective enrichment on tetralin, while the other organisms had been isolated previously by others on other substrates (*o*-xylene, styrene, and mesitylene). In this paper, we report on the metabolism of tetralin in the *o*-xylene-isolated *Corynebacterium* sp. strain C125, which grew relatively well on tetralin (32).

MATERIALS AND METHODS

Microorganism and cultivation conditions. *Corynebacterium* sp. strain C125 was isolated previously from an enrichment culture with *o*-xylene as the sole source of carbon and energy (32). The strain was kept on slants of a mineral salts medium to which 15 g of Oxoid no. 3 agar liter^{-1} was added. The mineral salts medium contained the following (per liter of demineralized water): 1.55 g of K_2HPO_4, 0.85 g of $NaH_2PO_4 \cdot 2H_2O$, 2.0 g of $(NH_4)_2SO_4$, 0.1 g of $MgCl_2 \cdot 6H_2O$, 10 mg of EDTA, 2 mg of $ZnSO_4 \cdot 7H_2O$, 1 mg of $CaCl_2 \cdot 2H_2O$, 5 mg of $FeSO_4 \cdot 7H_2O$, 0.2 mg of $Na_2MoO_4 \cdot 2H_2O$, 0.2 mg of $CuSO_4 \cdot 5H_2O$, 0.4 mg of $CoCl_2 \cdot 6H_2O$, and 1 mg of $MnCl_2 \cdot 2H_2O$ (14). The organism was routinely grown in a chemostat on mineral medium with *o*-xylene added by the vapor phase (43), tetralin added with a micropump (Braun, Melsingen, Germany), or succinate (0.5% [wt/vol]) added directly to the medium.

Growth studies were performed in 100-ml serum bottles containing 10 ml of mineral medium. The hydrocarbon substrates were added in the vapor phase via small tubes placed in the bottle. Growth was assessed by monitoring the culture fluid turbidity together with the production of carbon dioxide from the supplied substrates (35).

Suspensions of washed cells and cell extracts. Cells were harvested by centrifugation in a Sorvall 5-B centrifuge at 4°C and 16,000 × *g*, washed twice with potassium phosphate buffer (pH 7.0; 50 mM), and suspended in the same buffer. Cell extracts were prepared by ultrasonication of a washed cell suspension (probe type sonicator; Branson, Danbury, Conn.) 10 times for 30 s each time at 4°C. Debris was removed by centrifugation at 27,000 × *g* for 30 min (4°C); the supernatant, containing 10 to 15 mg of protein ml^{-1}, was designated the crude cell extract. Protein was determined by the method of Bradford (3), using bovine serum albumin as a standard.

Oxygen consumption experiments. Oxygen consumption by washed suspensions of intact cells in 50 mM potassium phosphate buffer (total volume, 3 ml) was measured polarographically with a Clark type oxygen electrode (Yellow Springs Instrument Co., Yellow Springs, Ohio) at 30°C. Endogenous oxygen uptake was measured for 5 min at 30°C; subsequently, 0.05 ml of a mixture containing 10 mM substrate in *N*-dimethylformamide was added, and oxygen uptake was monitored for at least another 5 min. *N*-Dimethylformamide neither induced oxygen uptake nor inhibited respiratory activity of the cells at the concentration applied.

Enzyme assays. All enzyme assays were performed at 30°C. The aryl dioxygenase was assayed polarographically with a Clark type oxygen electrode (12) in 50 mM potassium phosphate buffer (pH 7.0) in the presence of NAD(P)H (0.1 mM), and the substrate was dissolved in *N*-dimethylformamide (final assay concentration, 0.1 mM). Results were corrected for endogenous oxygen consumption in the absence of the aromatic substrate.

The activity of *cis*-1,2-dihydro diol dehydrogenase was determined by monitoring the rate of reduction of NAD^+ at 340 nm in potassium phosphate buffer (pH 7.0). The reaction

* Corresponding author.

was started by adding the *cis*-1,2-dihydro diol to a final concentration of 1 mM (7).

The activities of the *ortho* ring fission dioxygenase with the various catechols were measured polarographically with an oxygen electrode by the method of Hayaishi et al. (15). The *meta* cleavage dioxygenase was assayed with various catechols by measuring the formation of ring fission products spectrophotometrically (22). The molar extinction coefficients, if not known, were determined by the method of Duggleby and Williams (8).

The ring fission products hydrolase and dehydrogenase were assayed by monitoring the disappearance of the substrates prepared by the method of Sala-Trepat et al. (31), except that heat-treated (55°C for 15 min) cell extracts of *Corynebacterium* sp. strain C125 (prepared from *o*-xylene-grown cells) were used. The assays were performed with dialyzed cell extracts; in the dehydrogenase assay, NAD^+ (final concentration, 1 mM) was included (30).

Incubation experiments. Incubations with whole cells were performed at 30°C in 100-ml serum bottles containing 50 mM potassium phosphate buffer, 75 μmol of tetralin, and freshly harvested cells of *Corynebacterium* sp. strain C125 (10 mg of protein) in a total volume of 10 ml.

Inhibition of the *cis*-dihydro diol dehydrogenase was achieved by adding 100 μmol of *cis*-3-methyl-3,5-cyclohexadiene-1,2-diol (*cis*-toluene glycol) as a competitive inhibitor (34). After 30 min, the cells were removed by centrifugation and the supernatant was extracted two times with 0.5 volume of ethyl acetate. The catechol-cleaving dioxygenase was inhibited by 0.05 mg of pyrogallol (1,2,3-trihydroxybenzene) ml^{-1} (16, 38). After various times of incubation, cells were removed by centrifugation, and supernatants were acidified to pH 2.5 with 5.0 N HCl and extracted three times with 0.5 volume of ethyl acetate. The solvent was removed in a rotary evaporator after drying over anhydrous Na_2SO_4, and the residue was dissolved in hexane. The hexane phase was washed twice with an equal volume of water to remove excess pyrogallol and oxidation products.

Chemical analyses. Dihydro diols were determined by gas chromatography (GC) of acidified extracts, as described previously (34). Catechols were detected by the method of Nair and Vaidyanathan (26). The presence of a free aldehyde group was assessed by the Tollens test (9). Enols were assayed by the $FeCl_3$ test, as outlined in Mann and Saunders (24). Picolinate derivatives were prepared by the method of Canonica et al. (4). Pyruvate was determined as described by Chakrabarty (5) by measuring the oxidation of NADH at 340 nm in the presence of an excess of lactic acid dehydrogenase.

Analytical techniques. Carbon dioxide production was determined by injecting 0.1-ml headspace samples on a Packard 427 GC (Packard/Becker, Delft, The Netherlands) fitted with a Porapack Q column (Chrompack B. V., Middelburg, The Netherlands).

GC of the incubation extracts was performed on a Chrompack CP 9000 GC with on-column injector (Chrompack) fitted with a fused silica WCOT CP-Sil 8 CB column (Chrompack) (25 m by 0.32 mm). Gas flow rates were as follows: $He/H_2/air$ = 30/20/300 ml/min. The temperature of the flame ionization detector was 300°C. The column oven was programmed from 80°C initial temperature to 280°C at a rate of 10°C/min.

Mass spectra of the accumulated intermediates were recorded on a MAT 6H7A mass spectrometer (MS; Finnigan-MAT, San Jose, Calif.), with an inlet temperature of 100°C and an electron impact of 70 eV. Accumulated incubation products were identified by GC/MS, using an HP 5890 GC (Hewlett-Packard, Palo Alto, Calif.) coupled to an HP 5970 mass selective detector.

Spectral analyses were performed with a Perkin-Elmer λ2 spectrophotometer (Perkin-Elmer, Ueberlingen, Germany).

Chemicals. 1,2,3,4-Tetrahydronaphthalene, catechol, *cis*-3,5-cyclohexadiene-1,2-diol, and *cis*-3-methyl-3,5-cyclohexadiene-1,2-diol were obtained from Janssen Chimica (Beerse, Belgium). 3-Methylcatechol was purchased from Lancaster (Morecamb, United Kingdom), 4-methylcatechol was obtained from Merck (Darmstadt, Germany), and 1,2-dihydroxynaphthalene and 2,3-dihydroxynaphthalene were obtained from Aldrich (Brussels, Belgium). Lactic acid dehydrogenase and all other biochemicals were purchased from Boehringer (Mannheim, Germany).

TABLE 1. Rates of oxygen consumption by washed cell suspensions of *Corynebacterium* sp. strain C125 grown on *o*-xylene, tetralin, or succinate

Assay substrate	Oxygen consumption[a] (nmol of $O_2 \cdot min^{-1} \cdot$ mg of cell protein^{-1})		
	o-Xylene	Tetralin	Succinate
o-Xylene	280	155	<5
Benzene	100	35	<5
Toluene	300	165	<5
Ethylbenzene	230	110	<5
o-Diethylbenzene	215	85	<5
Naphthalene	145	65	<5
Tetralin	180	80	<5
Biphenyl	135	ND[b]	<5
Catechol	305	180	<5
3-Methylcatechol	760	525	5
4-Methylcatechol	265	150	5
1,2-Dihydroxynaphthalene	2,800	785	5
2,3-Dihydroxynaphthalene	10	5	<5

[a] Corrected for endogenous oxygen uptake.
[b] ND, not determined.

RESULTS

Growth characteristics. *Corynebacterium* sp. strain C125 is able to use tetralin and several other aromatic hydrocarbons as the sole source of carbon and energy. Toluene, ethylbenzene, and *o*-xylene were good substrates (specific growth rate [μ] = approximately 0.17 h^{-1}), while moderate growth was observed with benzene, tetralin, naphthalene, *o*-diethylbenzene, biphenyl, and indane (μ = approximately 0.05 h^{-1}). No growth occurred with cyclohexane, cyclohexene, and decalin (decahydronaphthalene).

Oxygen consumption experiments. Oxygen consumption rates of washed cell suspensions of *Corynebacterium* sp. strain C125 grown on *o*-xylene, tetralin, or succinate were monitored in the presence of various substrates (Table 1). Cells grown on either *o*-xylene or tetralin readily oxidized related aromatic hydrocarbons and catechols. Succinate-grown cells were not adapted to the aromatic compounds.

Accumulation and identification of intermediates. Under specific conditions, the activities of enzymes involved in the metabolism of tetralin could be inhibited. Accumulation of the first intermediate in the degradative pathway of tetralin was achieved by the addition of 0.5 mM *cis*-3-methyl-3,5-cyclohexadiene-1,2-diol (*cis*-toluene glycol) as a competitive inhibitor for the *cis*-dihydro diol dehydrogenase. Whole-cell incubations in the presence of *cis*-toluene glycol resulted in

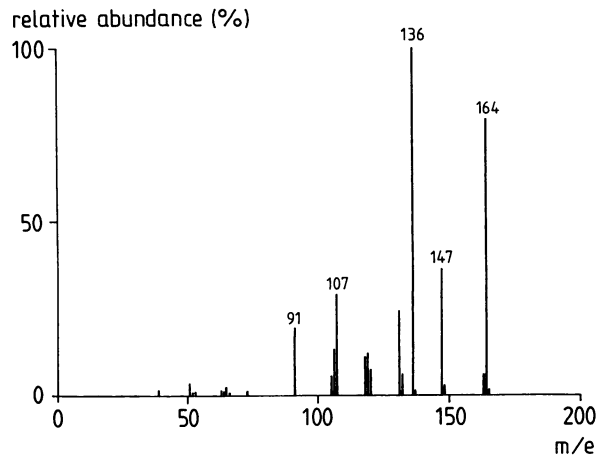


FIG. 1. Relative abundances of mass fragments in the spectrum of 5,6,7,8-tetrahydro-1,2-naphthalene diol.

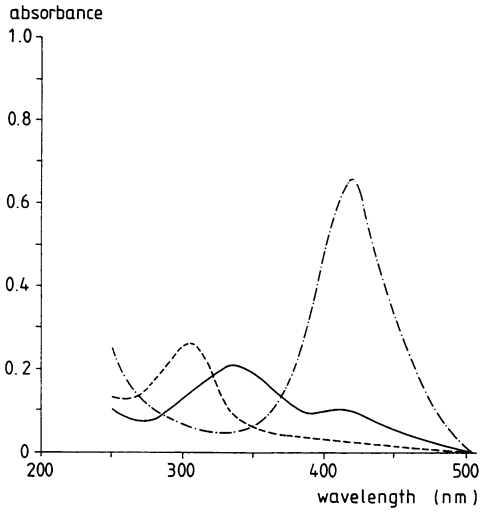


FIG. 2. Absorption spectra of the ring fission product of tetralin at different pHs. The molar extinction coefficients at the respective absorption maxima are as follows: pH 2.5, ε_{306} = 19,520 $M^{-1} \cdot cm^{-1}$; pH 7.0, ε_{336} = 16,400 $M^{-1} \cdot cm^{-1}$; pH 12.0, ε_{418} = 51,200 $M^{-1} \cdot cm^{-1}$.

the accumulation of an intermediate which disappeared upon the addition of 5 N HCl. The addition of the acid also resulted in the disappearance of *cis*-toluene glycol. GC of the acidified mixture revealed four new peaks that were identified by MS as 5,6,7,8-tetrahydro-1-naphthol, 5,6,7,8-tetrahydro-2-naphthol, *o*-cresol, and *m*-cresol. These data are consistent with 1,2,5,6,7,8-hexahydro-*cis*-1,2-naphthalene diol and *cis*-toluene glycol being the original compounds which had been acid dehydrated to the respective phenols. The ratio of 5,6,7,8-tetrahydro-1-naphthol to 2-naphthol formed by acid-catalyzed dehydration was 1:6.

Addition of pyrogallol to washed cells of *Corynebacterium* sp. strain C125 incubated with tetralin resulted in the accumulation of a compound which gave a positive reaction in the catechol assay. GC/MS analysis of ethyl acetate extracts revealed a compound with a molecular ion peak at *m/e* 164 $[M^+]$ and with other prominant peaks at *m/e* 136 $[M - 28]^+$, *m/e* 119 $[M - 28 - 17]^+$, and *m/e* 105 $[M - 28 - 17 - 14]^+$ (Fig. 1). Comparison with mass spectra of related catechols showed that this structure is consistent with a dihydroxylated tetralin.

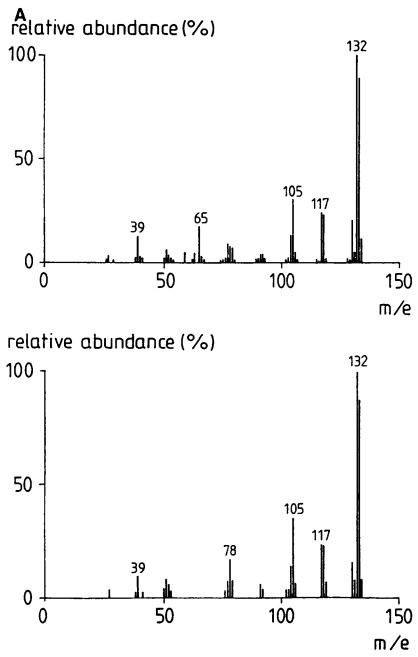
During growth of *Corynebacterium* sp. strain C125 on aromatic compounds, coloring of the medium was observed. To obtain more insight into the nature of these yellow compounds, spectra of the incubation mixtures were recorded at pH 2.5, 7.0, and 12.0. The absorption maxima observed for the ring fission products of benzene, toluene, ethylbenzene, *o*-xylene, and naphthalene were identical to values reported in the literature (2, 6, 8). In Fig. 2 the absorption spectra of the incubation mixture of *Corynebacterium* sp. strain C125 with tetralin at different pH values are presented. The shifts in absorption maxima upon changing the pH suggest that the compounds accumulated in the incubation broth were capable of keto-enol tautomerism. Extraction of incubation mixtures yielded only small quantities of a brown oil, which gave a positive $FeCl_3$ test, indicating the presence of an enol function. The isolated product showed a negative reaction in the Tollens test (9), indicating the absence of a free aldehyde group. Lyophilization of the cultivation broth of *Corynebacterium* sp. strain C125 grown on tetralin yielded a yellow powder. Dissolution of the powder in diethyl ether and subsequent GC/MS analysis revealed the presence of a number of structures consistent with carboxylic acid-substituted cyclohexanone derivatives. Only the presence of 2-oxocyclohexane propionic acid could be confirmed by comparison with the authentic compound.

Reaction of the powder with ammonia, after standing at room temperature for 5 days, yielded a new compound which showed an absorption maximum at 284 nm. The position of this maximum was not affected by changes in pH. Further analysis by GC/MS of an ether extract of the reaction solution revealed a quinoline derivative with a molecular weight of 133, which was identified as 5,6,7,8-tetrahydroquinolin (Fig. 3A).

The absence of an aldehyde and the presence of an enol, the observed formation of a quinoline derivative, and the identification of a cyclohexanone moiety suggest that fission of the catechol occurred between positions C-5 and C-9 (Fig. 4). A major peak in the GC of the lyophilized cultivation broth of *Corynebacterium* sp. strain C125 showed a mass spectrum with a small molecular ion peak at 196 $[M^+]$ (Fig. 3B). Other peaks in the mass spectrum were at *m/e* 96, *m/e* 81, *m/e* 68, and *m/e* 41. These data indicate that 4-(2'-oxocyclohexane)-2-hydroxy-buta-2,4-dienoic acid is the ring fission product, analogous to the product observed in the metabolism of naphthalene (6).

Enzyme activities in cell extracts. Extracts from cells of *Corynebacterium* sp. strain C125 grown on *o*-xylene, tetralin, or succinate were assayed for activities of enzymes involved in the initial steps in the degradation of aromatic compounds in general and tetralin in particular (Table 2). The intermediates of the degradative pathway of tetralin compounds used in these assays were produced in incubations with *Corynebacterium* sp. strain C125. The *cis*-dihydro diol of tetralin was produced by a mutant strain of *Corynebacterium* sp. strain C125 lacking the *cis*-dihydro diol dehydrogenase (unpublished results).

The dioxygenase type of enzyme was assayed by monitoring the consumption of oxygen with seven different assay substrates. The results presented in Table 2 were obtained with NADPH as the electron donor, although activities were comparable when NADH was used (data not shown). Ex-



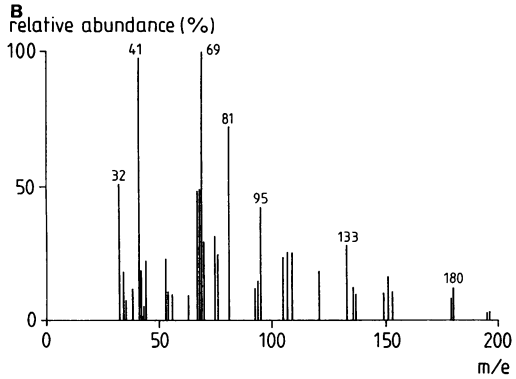


FIG. 3. (A) Relative abundances in the mass spectrum of 5,6,7,8-tetrahydroquinoline formed by derivatization of the ring fission product of tetralin (top) and the authentic compound (bottom). (B) Relative abundances of mass fragments in the spectrum of the ring fission product of tetralin.

tracts of cells grown on *o*-xylene had higher specific activities than extracts of tetralin-grown cells, but in both cases activities were highest with toluene and low with benzene. The *cis*-dihydro diol dehydrogenase was assayed with the commercially available *cis*-dihydro diols of benzene and toluene and the *cis*-dihydro diol of tetralin. All three *cis*-dihydro diols were good substrates for the NAD^+-dependent dehydrogenase. The *trans*-dihydro diol of benzene was not a substrate for the dehydrogenase.

The catechol fission dioxygenase was a *meta* fission enzyme (Table 2). Yellow intermediates, which showed keto-enol tautomerism similar to the compounds observed during growth experiments (Fig. 2), were accumulated from both aromatic hydrocarbons and catechols. The highest activities of the ring fission dioxygenase were observed with 3-methylcatechol and 1,2-dihydroxy-naphthalene, both catechols

TABLE 2. Activities of enzymes involved in the catabolism of tetralin in cell extracts of *Corynebacterium* sp. strain C125 grown on tetralin, *o*-xylene, or succinate

Enzyme	Assay substrate	Enzyme activity (nmol · min^{-1} · mg of $protein^{-1}$)		
		o-Xylene	Tetralin	Succinate
Dioxygenase	*o*-Xylene	22	8	0
	Tetralin	14	6	0
	Naphthalene	9	5	0
	Toluene	30	11	0
	Benzene	2	0	0
	Ethylbenzene	24	8	0
	o-Diethylbenzene	17	6	0
Dehydrogenase	*cis*-Benzene glycol	41	36	7
	trans-Benzene glycol	0	0	0
	cis-Toluene glycol	96	103	12
	1,2,5,6,7,8-Hexahydro-*cis*-1,2-naphthalene diol	84	67	8
2,3-Dioxygenase				
	Catechol	84	52	10
	3-Methylcatechol	202	178	28
	4-Methylcatechol	47	35	8
	1,2-Dihydroxynaphthalene	532	487	47
	2,3-Dihydroxynaphthalene	22	23	6
	5,6,7,8-Tetrahydro-1,2-naphthalene diol	371	346	27
Hydrolase	RFP[a] of catechol	12	9	0
	RFP of 3-methylcatechol	53	37	3
	RFP of 4-methylcatechol	4	3	0
	RFP of 1,2-dihydroxynaphthalene	107	89	4
	RFP of 5,6,7,8-tetrahydro-1,2-naphthalene diol	71	58	6

[a] RFP, ring fission product.

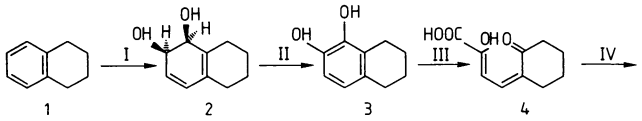


FIG. 4. Proposed pathway for the degradation of tetralin by *Corynebacterium* sp. strain C125. (1) Tetralin; (2) 1,2,5,6,7,8-hexahydro-*cis*-1,2-naphthalene diol; (3) 5,6,7,8-tetrahydro-1,2-naphthalene diol; (4) 4-(2'-oxocyclohexane)-2-hydroxy-buta-2,4-dienoic acid. I, dioxygenase; II, *cis*-dihydro diol dehydrogenase; III, catechol-2,3-dioxygenase; IV, ring fission product hydrolase.

with the substituent proximal to the hydroxyl groups. No *ortho*-cleaving activity could be detected in any of the extracts. The products of the catechol-2,3-dioxygenase-catalyzed ring opening were further catabolized by a hydrolytic reaction catalyzed by a hydrolase. There was no observance of a dehydrogenase acting on the ring fission product. In the enzyme assays a transient accumulation of the yellow intermediates in the catabolism of 3-methylcatechol, 1,2-dihydroxynaphthalene, and 5,6,7,8-tetrahydro-1,2-naphthalene diol was observed. Interestingly, incubation of 4-methylcatechol with cell extracts resulted in a continuous increase in A_{381}. The resulting ring fission product was apparently not further degraded, even when NAD^+ was added to the incubation mixture.

DISCUSSION

The results obtained in this study show that *Corynebacterium* sp. strain C125 attacks tetralin by an initial oxidation of the aromatic nucleus at positions C-5 and C-6 (Fig. 4). Metabolism of *o*-xylene in this organism (32), and in strains of *Pseudomonas stutzeri* (2) and *Nocardia* sp. (13), also proceeded via initial hydroxylation of the benzene nucleus proximal to a substituent methyl group. Metabolic routes reported in organisms cooxidizing tetralin involved an initial attack of the benzylic carbon (17, 19, 33), leading to cleavage of the alicyclic ring (19).

In cell extracts, the *cis*-dihydro diol dehydrogenase was only active with NAD^+ as the electron acceptor, which is in accordance with observations made with *cis*-dihydro diol dehydrogenases in other bacteria (1, 27). However, exceptions to this hypothesis do exist (29).

The accumulation of yellow intermediates by cells of *Corynebacterium* sp. strain C125 growing on various aromatic compounds, and especially the shift in absorption maxima of these compounds upon a change in pH, is indicative of an extra-diol cleavage of catechols. This was supported by the high activity of the *meta* cleavage dioxygenase in cell extracts and the absence of activity of the *ortho* cleavage enzymes. The catechol-cleaving enzyme showed a marked preference for catechols that posessed a hydrocarbon substituent proximal to the hydroxyl groups. Similar observations have been made for *meta* cleavage enzymes in other bacteria growing on hydrocarbon-substituted aromatic compounds, e.g., biphenyl and alkylbenzenes (18, 38, 39). However, unlike the catechol dioxygenases in *Pseudomonas cruciviae* described by Ishigooka et al. (18) and in *Pseudomonas* sp. strain NCIB 10643 described by Smith and Ratledge (39), the *meta* cleavage enzyme of *Corynebacterium* sp. strain C125 is also able to cleave 3,4-dihydroxy compounds (4-substituted catechols).

The consecutive metabolism of the compounds after fission of the aromatic ring was by a hydrolase. Tetralin-grown cells of *Corynebacterium* sp. strain C125 apparently did not possess a ring fission product dehydrogenase. This was supported by the observed inability of cell extracts of *Corynebacterium* sp. strain C125 to catabolize the ring fission product of 4-methylcatechol, which is believed to be exclusively catabolized by the dehydrogenase type of enzyme (31, 44). Also, in the metabolism of *o*-xylene by *Corynebacterium* sp. strain C125, a hydrolase was involved (32). However, the substituted cyclohexane compounds, derived from 4-(2'-oxocyclohexane)-2-hydroxy-buta-2,4-dienoic acid by hydrolysis, could not be identified. Therefore, the products of the hydrolase-catalyzed reaction are not known. The inability to demonstrate the formation of pyruvic acid indicated that the metabolism of the ring fission product of tetralin differed from the routes observed for naphthalene (6) and *o*-xylene (13). The observation that *Corynebacterium* sp. strain C125 formed acetate in the hydrolase-catalyzed metabolism of the *o*-xylene ring fission product indicated that this organism possesses a different type of hydrolase (32).

The enzymes that catalyze the initial steps of the metabolic pathway are induced by the presence of aromatic substrates. This can be concluded from the absence of oxygen consumption (Table 1) and the activities of these enzymes (Table 2) in cells grown on succinate.

On the basis of the above results, we propose an inducible degradation pathway (Fig. 4) for tetralin (step 1) that starts with hydroxylation of the aromatic moiety at the C-5 and C-6 positions. The resulting *cis*-dihydro diol (step 2) is oxidized by a *cis*-dihydro diol dehydrogenase, which yields the 5,6,7,8-tetrahydro-1,2-naphthalene diol (step 3). This catechol is subsequently cleaved by a *meta*-cleaving catechol dioxygenase, resulting in the formation of 4-(2'-oxo-cyclohexane)-2-hydroxy-buta-2,4-dienoic acid (step 4). This compound was further metabolized by a hydrolytic enzyme. The reaction products of this step were not identified nor were the subsequent reactions investigated. These results show that the metabolism of tetralin initially proceeds via a pathway analogous to the route described for naphthalene in pseudomonads (6).

The metabolism of tetralin in *Corynebacterium* sp. strain C125 as presented here is the first report on the catabolism of tetralin by an initial attack at the aromatic moiety (37). The presence of a specifically dioxygenating enzyme system which, moreover, is able to attack a broad range of aromatic substrates makes *Corynebacterium* sp. strain C125 an excellent catalyst for the specific production of special *cis*-dihydro diols (28). Compounds attacked by dioxygenases include alkylbenzenes, haloaromatics, and benzoic acids, though to our knowledge the dioxygenation of tetralin has not been reported (21, 28). Since tetralin derivatives have an enormous potential in the pharmaceutical (25, 42) and flavoring (23) industries, methods to optimize the oxo-functionalization of this compound are of interest.

ACKNOWLEDGMENTS

This research was supported by the Dutch Programme Committee for Industrial Biotechnology (P.C.I.B.).

We gratefully acknowledge Mark Smith for stimulating discussions and critical reading of the manuscript. Henk Schaap (Quest International, Naarden, The Netherlands) and Kees Teunis (Wageningen Agricultural University) are acknowledged for recording mass spectra.

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