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Dissimilation of Aromatic Compounds by *Alcaligenes eutrophus*

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The range of aromatic compounds that support the growth of *Alcaligenes eutrophus* has been determined, and the pathways used for the dissimilation of these substrates have been explored, largely by enzymatic analyses. The β-ketoadipate pathway operates in the dissimilation of benzoate and *p*-hydroxybenzoate; the gentisate pathway, in the dissimilation of *m*-hydroxybenzoate; and the *meta* cleavage pathway, in the dissimilation of phenol and *p*-cresol. L-Tryptophan is oxidized via anthranilate; but the metabolic fate of anthranilate was not established. The metabolism of the three stereoisomers of muconic acid was also examined.

Like other hydrogen bacteria, *Alcaligenes* (*Hydrogenomonas*) *eutrophus* is a versatile chemoorganotroph. The first detailed nutritional characterization of this species (D. H. Davis, Ph. D. thesis, Univ. of California, Berkeley, 1967) showed that it can use several aromatic compounds as sole carbon and energy sources. These included benzoic acid and its *m*- and *p*-hydroxy derivatives. Preliminary qualitative evidence suggested that benzoic and *p*-hydroxybenzoic acids are metabolized through the parallel branches of the β-ketoadipate pathway and that *m*-hydroxybenzoic acid is metabolized through the gentisate pathway (7). In the course of an analysis of the physiological role and regulatory control of the β-ketoadipate pathway in *A. eutrophus*, we further explored the pathways for the dissimilation of aromatic compounds and of some metabolically related nonaromatic compounds by this bacterial species. The results of this survey will be described here.

MATERIALS AND METHODS

Biological materials. The strains of *A. eutrophus* examined in this study are maintained in the Berkeley culture collection under the following strain designations: strain 335 (ATCC 17697), strain 336 (ATCC 17698), strain 337 (ATCC 17699), strain 338 (ATCC 17700), strain 339 (ATCC 17701), strain 340 (ATCC 17702), strain 341 (ATCC 17703), strain 342 (ATCC 17704), strain 345 (ATCC 17707), strain 346 (ATCC 17708), and strain 373. In most of our work, the type strain (355) was used.

Media and conditions of cultivation. All media were prepared in a mineral base of the following composition: 200 mg of nitriloacetic acid; $MgSO_4 \cdot 7H_2O$, 580 mg; $CaCl_2 \cdot 2H_2O$, 67 mg; $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, 0.2 mg; $FeSO_4 \cdot 7H_2O$, 2.0 mg; Hutner's "Metals 44" (5), 1.0 ml; $(NH_4)_2SO_4$, 1.0 g; KH_2PO_4, 2.72 g; $Na_2HPO_4 \cdot 7H_2O$, 5.36 g; and distilled water to a final volume of 1.0 liter. The final *p*H of this medium was 6.8. Carbon sources were separately sterilized in concentrated stock solutions and added aseptically to the sterile mineral base. Solid media were prepared by the addition of an Oxoid Ionagar No. 2 solution (separately sterilized at double strength) to solutions of the basal medium (likewise sterilized at double strength). Unless otherwise noted, the concentration of carbon sources was 5 mM in liquid media and 2.5 mM in solid media.

Liquid cultures were incubated in flasks on a rotary shaker at 30 C. Growth was measured turbidimetrically in flasks with tubular side arms by using a Klett-Summerson colorimeter fitted with a number 66 filter. Cultures were harvested while still in the course of exponential growth, at population densities of approximately 5×10^8 cells/ml.

Preparation of cell-free extracts. Cultures were harvested by centrifugation in the cold (4 C). The cells were washed once with 20 mM tris(hydroxymethyl) aminomethane-hydrochloride, *p*H 8.0, containing 10 μM MgK_2 ethylenediaminetetraacetic acid, and resuspended at approximately 100 times the original culture density in the same buffer supplemented with 1 mM β-mercaptoethanol. The addition of β-mercaptoethanol is necessary to preserve *p*-hydroxybenzoate hydroxylase activity in cell-free extracts. The resuspended pellets were either extracted immediately or stored overnight at −15 C before extraction. Extracts were prepared by sonic treatment (2 min) in a 20-kHz, 60-w probe-type sonic disintegrator (Measuring and Scientific Equipment Co.). The probe was prechilled, and the cell suspension was kept during treatment in an NaCl-ice-water bath. The sonically treated preparation was centrifuged for 15 min at 35,000 × *g*, and the supernatant fluid was used for enzyme assays. Extracts were stored at 0 to 2 C before assay.

Enzymological methods and assays. All assays are performed at 25 C by using spectrophotometric methods with silica cuvettes (1.0-cm pathlength) containing a total volume of 3 ml. Measurements were

made in a Gilford model 2000 recording spectrophotometer.

The following enzymes were assayed by published methods: catechol-1,2-oxygenase (EC 1.13.1.1, catechol:oxygen 1,2-oxidoreductase; reference 4), *cis,cis*-muconate lactonizing enzyme [EC 5.5.1.1, 4-carboxymethyl-4-hydroxyisocrotonolactone lyase (decyclizing); reference 4], muconolactone isomerase (14), *p*-hydroxybenzoate hydroxylase (4), protocatechuate oxygenase (EC 1.13.1.3, protocatechuate: oxygen 3,4-oxidoreductase; reference 20), β-carboxymuconate lactonizing enzyme (13), β-carboxymuconolactone decarboxylase (13), β-ketoadipate enollactone hydrolase (EC 3.1.1.16, incorrectly listed as 4-carboxymethyl-4-hydroxyisocrotonolactone hydrolase; reference 13), β-ketoadipate succinyl-CoA transferase (EC 2.8.3.6, 3-ketoadipate succinyl-CoA transferase; reference 4). Cathechol-2,3-oxygenase (EC 1.99.2a, catechol:oxygen 2,3-oxidoreductase) and 2-hydroxymuconic semialdehyde hydrolyase were measured by slight modifications of the methods of Feist and Hegeman (9). In the former assay, 30 mg of bovine serum albumin (Armour, fraction V) was added to the assay mixture to stabilize the oxygenase. In the latter assay, the disappearance of 0.1 μmole of 2-hydroxymuconic semialdehyde was measured in the presence of 0.3 μmole of nicotinamide adenine dinucleotide.

Tryptophan pyrrolase (EC 1.13.1.12, L-tryptophan: oxygen oxidoreductase) could not be assayed by the method of Palleroni and Stanier (16) because of its sensitivity to semicarbazide. Since extracts of tryptophan-grown cells convert L-tryptophan quantitatively to anthranilate, and since the *N*-formyl-L-kynurenine formamidase and kynureninase activity are present at high levels in induced cells relative to pyrrolase activity, tryptophan pyrrolase was assayed by measuring the decrement in optical density at 280 nm, which reflects the conversion of L-tryptophan to anthranilate. The reaction was performed in 0.066 M phosphate buffer (*p*H 7.2) at an L-tryptophan concentration of 0.166 mM; when using a cuvette with a light path of 1 cm, a decrement of 0.165 absorbance units corresponded to the conversion of 0.1 μmole of L-tryptophan. *N*-formyl-L-kynurenine formamidase (EC 3.5.1.9, aryl-formylamine amidohydrolase) was assayed by the procedure of Palleroni and Stanier (16), and kynureninase (EC 3.7.1.3, L-kynurenine hydrolase) was assayed by the procedure of Hayaishi and Stanier (11). Gentisate oxygenase (EC 1.13.1.4, gentisate:oxygen oxidoreductase) and maleylpyruvate isomerase (EC 5.2.14, 3-maleylpyruvate *cis,trans*-isomerase) were assayed by the procedure of Wheelis et al. (21).

With the exception of β-ketoadipate succinyl-coenzyme A (CoA) transferase, a unit of enzymatic activity is defined as the amount of enzyme required to cause the disappearance of 1 μmole of substrate per minute under the conditions of assay. In the case of β-ketoadipate succinyl-CoA transferase, the extinction coefficient of the product measured in this reaction (β-ketoadipyl-CoA) is not known, and the unit is defined arbitrarily as the amount of enzyme necessary to cause a change of 1.0 absorbance unit per min under the conditions of the assay. Specific activities are expressed as units of enzymatic activity per milligram of protein. Protein was determined by the procedure of Lowry et al. (12), with bovine serum albumin fraction V (Armour Co.) used as a standard.

Manometry. A Gilson GRP20 differential respirometer was used for all manometric experiments. Flasks contained 2 ml of cells at a turbidity of 200 Klett units (0.17 mg dry weight per ml) that had been washed and suspended in a mineral base medium without $(NH_4)_2SO_4$. The center well contained 0.2 ml of 2 M NaOH and the side arm contained 2.5 μmoles of the substrate dissolved in buffer of the same composition as that in which cells were suspended. Oxygen uptake was measured at 30 C and corrected for endogenous activity.

Chemical and enzymatic reagents. Chemicals employed were obtained from commercial sources with the following exceptions. β-Carboxy-*cis,cis*-muconate and (+)-muconolactone were prepared enzymatically by the procedure of Ornston and Stanier (15). *cis,cis*-Muconic acid, synthesized by the procedure of Riegel and Lilienfeld (17), were kindly provided by M. Robert-Gero. Succinyl-CoA was synthesized chemically by the procedure of Stadtman (18). *cis,trans*-Muconic acid was prepared from the *cis,cis* isomer as described by Elvidge et al. (8).

Purified preparations of β-ketoadipate enol-lactone hydrolase, (+)-muconolactone isomerase, *cis,cis*-muconate lactonizing enzyme, and β-carboxy-*cis,cis*-muconate lactonizing enzyme, required for enzyme assays, were prepared by the procedures of Ornston (13, 14).

RESULTS

Utilization of aromatic compounds for growth by A. eutrophus. The aromatic compounds that support growth by most strains of *A. eutrophus* include: benzoate, *m*-hydroxybenzoate, *p*-hydroxybenzoate, phenol, *p*-cresol, and L-tryptophan (Table 1). The hydroaromatic acids, shikimate and quinate, are not utilized. Every strain grows readily with the following nonaromatic intermediates of the β-ketoadipate pathway: *cis,cis*-muconate, muconolactone, and β-ketoadipate. The *cis,trans* isomer of muconic acid also supports growth. Only one strain could grow immediately with *trans,trans*-muconate, but all strains examined acquired the ability to use this compound by spontaneous mutation. Among the aromatic compounds metabolically related to tryptophan, anthranilate, and kynurenate support growth of all strains. DL-Kynurenine is initially unutilizable, but all strains yield mutants able to grow at its expense.

The type strain of *A. eutrophus* (strain 335) was employed in all further experiments. The generation times of this strain at 30 C in synthetic medium with various aromatic and nonaromatic carbon sources are shown on Table 2.

The attack on aromatic substrates by strain 335 is strictly inducible. This is shown by the failure of succinate-grown cells to respire aromatic compounds at a significant initial rate, as

TABLE 1 *Ability of aromatic and certain nonaromatic compounds to support the growth of 14 strains of Alcaligenes eutrophus*[a]

Substrates tested	No. of strains tested	No. of positive strains	Negative strains
Benzoate	14	14	None
p-Hydroxybenzoate	14	14	None
m-Hydroxybenzoate	14	14	None
o-Hydroxybenzoate	14	0	All
Shikimate	12	1	All except strain 345
Quinate	14	0	All
Phenol	14	14	None
p-Cresol	12	11	Strain 345
m-Cresol	12	0	All
o-Cresol	12	0	All
L-Tryptophan	14	14	None
DL-Kynurenine	12	0	All[b]
Anthranilate	12	11	Strain 345[b]
Kynurenine	14	14	None
Nicotinate	14	14	None
L-Mandelate	14	0	All
Benzoylformate	14	14	None
cis,cis-Muconate	12	12	None
cis,trans-Muconate	12	12	None
*trans,trans*Muconate	12	1	All[b] except strain 345
Muconolactone	12	12	None
β-Ketoadipate	12	12	None
Adipate	14	14	None

[a] Data taken in part from Davis (6).

[b] All negative strains gave rise to spontaneous mutants capable of utilizing the compound for growth.

well as by the pronounced growth lags that occur when cells growing exponentially with succinate are transferred to media containing an aromatic compound as sole carbon and energy source.

Role of the β-ketoadipate pathway in A. eutrophus. Table 3 shows the specific activities of enzymes of the β-ketoadipate pathway in extracts of *A. eutrophus* grown with succinate, benzoate, and *p*-hydroxybenzoate. All enzymes are either undetectable or present at very low levels in succinate-grown cells. The two enzymes that mediate terminal common step-reactions (β-ketoadipate enol-lactone hydrolase and β-ketoadipate succinyl-CoA transferase) are induced to roughly the same extent in cells grown with either aromatic acid. High levels of enzymes specifically operative in the catechol branch of the pathway occur only in benzoate-grown cells, and high levels of those operative in the protocatechuate branch occur only in *p*-hydroxybenzoate-grown cells. The specific activities of catechol-1,2-oxygenase, muconate lactonizing enzyme, and muconolactone isomerase are slightly elevated in *p*-hydroxybenzoate-grown cells. It should be noted, however, that these cells contain very high levels of activity of the enzymes that catalyze the three analogous reactions in the protocatechuate branch. Since the substrate specificities of these enzymes are not known, it is possible that they can attack the analogous substrates of the catechol branch at very low rates. In this event, the observed effect would reflect nonspecificity of enzyme action rather than nonspecificity of induction. The enzymological data in Table 3 confirm the role of the β-ketoadipate pathway in the metabolism of benzoate and *p*-hydroxybenzoate by *A. eutrophus*.

Pathways for oxidation of monophenols. In cells of *A. eutrophus* grown with either phenol or *p*-cresol, there is no measurable induction of catechol-1,2-oxygenase, muconate lactonizing enzyme, and β-ketoadipate succinyl-CoA transferase (Table 4). However, cells grown with these substrates contain high levels of two other inducible enzymes, catechol-2,3-oxygenase and 2-hydroxymuconate semialdehyde hydrolyase, which operate in the so-called *meta*-cleavage pathway for the dissimilation of catechols (1, 6, 9). The enzymological data accordingly suggest that *A. eutrophus* oxidizes phenol and *p*-cresol via the corresponding catechols, which then undergo *meta*-cleavage.

As shown in Table 4, catechol-2,3-oxygenase is not measurably induced in *A. eutrophus* by growth with 2.5 mM benzoate, even though catechol is an intermediate common to the oxida-

TABLE 2. *Generation times of Alcaligenes eutrophus 335 grown in a synthetic medium at 30 C in shaking liquid cultures*

Growth substrates	Generation time (min per doubling of culture turbidity)
2.5 mM Fructose	85
6 mM Acetate	90
2.5 mM Succinate	60
2.5 mM Adipate	120
2.5 mM *cis,cis*-Muconate	110
2.5 mM *cis,trans*-Muconate	120
2.5 mM *trans,trans*-Muconate	125[a]
2.5 mM (+)-Muconolactone	390
2.5 mM β-Ketoadipate	95
2.5 mM Benzoate	65
2.5 mM *p*-Hydroxybenzoate	85
2.5 mM Protocatechuate[b]	70
2.5 mM Phenol	100
2.5 mM *p*-Cresol	135
2.5 mM L-Tryptophan	160
2.5 mM Gentisate	80
2.5 mM Kynurenate	90

[a] Generation time of *trans,trans*-muconate-positive strains of *A. eutrophus* 335.

[b] Substrate added in 0.5-mM increments to minimize the effect of substrate toxicity.

TABLE 3. *Specific activities of enzymes of the β-ketoadipate pathway in cell-free extracts prepared from cultures of Alcaligenes eutrophus 335 grown with succinate (5 mM), benzoate (5 mM), and p-hydroxybenzoate (5 mM)*[a]

Enzymes	Succinate	Benzoate		*p*-Hydroxybenzoate	
	Specific activity	Specific activity	Magnitude of induction[b]	Specific activity	Magnitude of induction[b]
Catechol branch					
Catechol-1,2-oxygenase	<0.0001	9.19	>1,900	0.005	50
Muconate lactonizing enzyme	<0.0001	0.65	>6,500	0.0005	5
Muconolactone isomerase	0.006	2.68	4,000	0.14	23
Protocatechuate branch					
p-Hydroxybenzoate hydroxylase	<0.0001	<0.0001	1	0.22	>2,200
Protocatechuate oxygenase	0.001	<0.0002	1	0.84	840
β-Carboxy-*cis*,*cis*-muconate lactonizing enzyme	0.003	0.006	2	2.31	770
γ-Carboxymuconolactone decarboxylase	0.021	0.03	1.5	2.46	120
Common terminal steps					
β-Ketoadipate enol-lactone hydrolase	0.006	1.30	220	1.53	250
β-Ketoadipate succinyl-CoA transferase	0.009	0.62	68	0.66	73

[a] Specific activities are expressed as units per milligram of protein.
[b] Specific activity relative to that in an extract of succinate-grown cells.

TABLE 4. *Induction of enzymes of the ortho- and meta-cleavage pathways for the dissimilation of catechol in cells of Alcaligenes eutrophus 335 grown with phenols and with benzoate*[a]

Enzymes	Growth substrates				
	5 mM Succinate	2.5 mM Phenol	2.5 mM *p*-Cresol	2.5 mM Benzoate	5 mM[b] Catechol
ortho Cleavage pathway					
Catechol-1,2-oxygenase	<0.0002	<0.0002	<0.0002	0.19	<0.001
Muconate lactonizing enzyme	<0.0001	<0.0001	<0.0001	0.65	0.006
β-Ketoadipate succinyl-CoA transferase	0.009	0.015	0.007	0.62	0.04
meta Cleavage pathway					
Catechol-2,3-oxygenase	0.0003	0.30	0.32	0.0002	0.075
2-Hydroxymuconate semialdehyde hydrolyase	<0.0001	0.043	0.049		0.019

[a] Specific activities are expressed as units per milligram of protein.
[b] *A. eutrophus* was grown to substrate exhaustion with succinate; catechol was then added in five successive increments of 1 mM. The culture was harvested after approximately one doubling.

tion of both phenol and benzoate. This type of metabolic channeling with respect to catechol metabolism has been observed in other aerobic bacteria capable of oxidizing both phenol and benzoate via catechol. As shown by Feist and Hegeman (9), the metabolic divergence at the level of catechol is determined by the way in which the syntheses of the two catechol oxygenases are regulated. In the *Pseudomonas* strains which they studied, catechol-2,3-oxygenase is specifically induced by phenol, whereas catechol-1,2-oxygenase is product-induced by *cis*,*cis*-muconate.

In *A. eutrophus* we have observed that cultures prepared with relatively high initial concentrations of benzoate (>5 mM) become bright yellow in the later stages of growth, and the absorption spectrum of the supernatant medium indicates that the yellow material is 2-hydroxymuconate semialdehyde, the immediate product of the *meta* cleavage of catechol. Under these conditions, accordingly, some oxidation of catechol via the *meta* cleavage pathway does take place. Catechol can be detected in the medium in such cultures. The formation of 2-hydroxymuconate semialdehyde is therefore probably attributable to a weak induction of catechol-2,3-oxygenase by its substrate, which accumulates in benzoate-grown cultures when the level of benzoate is sufficiently high. This interpretation cannot be readily tested directly by growing *A. eutrophus* with catechol as a carbon source, since catechol is extremely toxic. However, if small successive increments of catechol are added to a suspension of uninduced cells, grown at the expense of succinate to a high density, a slight but significant induction of catechol-2,3-oxygenase and 2-hydroxymuconate semialdehyde hydrolyase can be demonstrated (Table 4, last column). The absence of these enzymes in cells grown with relatively low initial

concentrations of benzoate is therefore attributable to the fact that the steady-state level of catechol under these growth conditions always remains too low to induce detectable synthesis of enzymes operative in the *meta* cleavage pathway.

Pathway for the oxidation of L-tryptophan. After growth at the expense of L-tryptophan, cells of *A. eutrophus* respire L-tryptophan, L-kynurenine, and anthranilate at high and essentially identical rates but are unable to respire catechol, kynurenate, or gentisate at significant rates (Fig. 1). Tryptophan pyrrolase, formylkynurenine formamidase, and kynureninase are present at high levels in L-tryptophan-grown cells but are barely detectable in succinate-grown cells (Table 5). The respirometric and enzymatic data both indicate that L-tryptophan is converted via L-kynurenine to anthranilate, a sequence of reactions also characteristic of many *Pseudomonas* species (19). In the *Pseudomonas* species that dissimilate tryptophan via anthranilate, the latter compound is further metabolized by the catechol branch of the β-ketoadipate pathway (10). Consequently, certain enzymes of the β-ketoadipate pathway are fully induced in tryptophan-grown cells (16). In *A. eutrophus*, however, catechol-1,2-oxygenase, muconate-lactonizing enzyme, and β-ketoadipate succinyl-CoA transferase were not measurably induced in tryptophan-grown cells (Table 5), an observation that excludes a dissimilation of anthranilate through the reactions of the β-ketoadipate pathway. Since catechol-2,3-oxygenase is likewise not induced by growth of *A. eutrophus* with L-tryptophan, it seems probable that anthranilate is not converted to catechol by this organism.

Crude, cell-free extracts of tryptophan-grown cells convert L-tryptophan rapidly and with stoichiometric yield to anthranilate, which is not further attacked. We have been unable to determine the metabolic fate of anthranilate. It is not dissimilated via gentisate, a pathway for the oxidation of anthranilate demonstrated by Cain (3) in *Nocardia opaca:* gentisate oxygenase is not induced in tryptophan-grown cells of *A. eutrophus* (Table 6). The operation of the quinoline pathway (2) from the level of kynurenine is likewise excluded, since extracts of tryptophan-grown cells of *A. eutrophus* do not contain detectable kynurenine transaminase activity.

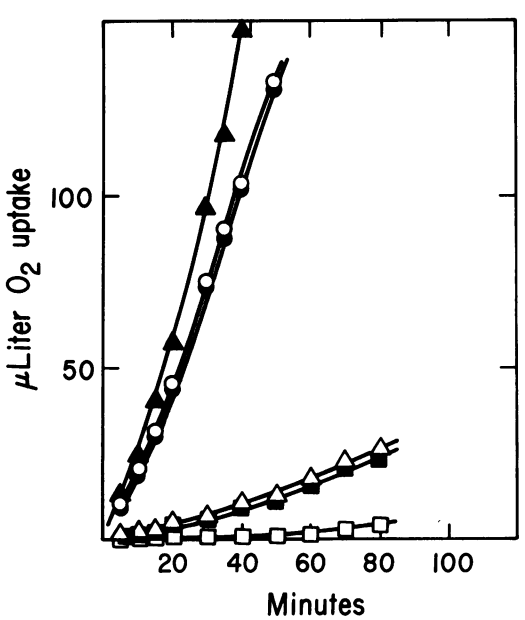

FIG. 1. *Oxygen consumption by tryptophan-grown cells of Alcaligenes eutrophus 335 with 2.5 μmoles of tryptophan and of certain metabolically related aromatic compounds. The cells were treated with chloramphenicol (250 μg/ml) to prevent enzyme synthesis during the course of the experiment. Symbols: tryptophan (▲), L-kynurenine (○), anthranilate (●), kynurenate (■), catechol (□), and gentisate (△).*

TABLE 5. *Enzymes induced in Alcaligenes eutrophus 335 as a result of growth with L-tryptophan*

Enzymes	Growth substrates[a]	
	5 mM Succinate	5 mM L-Tryptophan
Tryptophan oxidation		
Tryptophan pyrrolase	<0.0001	0.076
Formylkynurenine formamidase	0.015	0.31
Kynureninase	0.0006	0.082
β-Ketoadipate pathway (*ortho* cleavage)		
Catechol-1,2-oxygenase ..	<0.0001	<0.0002
Muconate lactonizing enzyme	<0.0001	0.0080
β-Ketoadipate succinyl-CoA transferase	0.009	0.016
Phenol pathway (*meta* cleavage)		
Catechol-2,3-oxygenase ..	0.0003	0.0006

[a] Specific activities are expressed as units per milligram of protein.

TABLE 6. *Specific activity of gentisate oxygenase and maleylpyruvate isomerase in Alcaligenes eutrophus 335 grown with succinate, L-tryptophan, and gentisate*[a]

Enzymes	Growth substrates		
	5 mM Succinate	5 mM L-Tryptophan	5 mM Gentisate
Gentisate oxygenase	0.001	0.001	0.76
Maleylpyruvate isomerase ..			2.4

[a] Specific activities are expressed as units per milligram of protein.

A. eutrophus 335 can grow readily at the expense of gentisate, and, as shown in Table 6, high levels of gentisate oxygenase and maleylpyruvate isomerase are present in extracts of such cells. These observations confirm the existence of the gentisate pathway in *A. eutrophus* and are in accord with the earlier report (7) that *m*-hydroxybenzoate is dissimilated via gentisate.

Metabolism of cis,cis- and cis,trans-muconate by A. eutrophus. Table 7 shows that the growth of *A. eutrophus* 335 with *cis,cis*-muconate causes a significant induction of the four enzymes that convert this compound to β-ketoadipyl-CoA: all these specific activities are increased at least 50-fold over those in extracts of succinate-grown cells. Extracts of cells grown with *cis,trans*-muconate contain the same level of β-ketoadipate succinyl-CoA transferase, but the other three enzymes are induced to a considerably lesser extent. This suggests that the *cis, trans* isomer is not metabolized through the same pathway, although one of the four enzymes, namely β-ketoadipate succinyl-CoA transferase, may also be operative in its dissimilation.

Metabolism of trans,trans-muconate by spontaneous mutants of A. eutrophus. Spontaneous mutants of *A. eutrophus* able to use *trans,trans*-muconate for growth can be readily selected on media containing this compound as sole carbon source. Such mutants fall into two classes with respect to enzymatic constitution (Table 8). In one class, exemplified by ttM.1, the basal levels in succinate-grown cells of the four enzymes assayed are similar to those of the wild type. In *trans,trans*-muconate-grown cells, the specific activity of β-ketoadipate succinyl-CoA transferase is increased 80-fold, the specific activities of muconolactone isomerase and β-ketoadipate enol-lactone hydrolase are increased 15-fold, and that of muconate lactonizing enzyme remains unchanged. Mutants of the other class, exemplified by ttM.2, synthesize β-ketoadipate succinyl-CoA transferase constitutively; its level in succinate-grown cells is almost equal to that in fully induced cells of the wild type. Basal levels of muconolactone isomerase and β-ketoadipate enol-lactone hydrolase are also somewhat elevated over those characteristic of the wild type. The enzymatic constitution of ttM.2 changes little as a result of growth with *trans,trans*-muconate: the levels of these three enzymes increase only two- to three-fold. Muconate lactonizing enzyme remains at basal level in both succinate- and *trans,trans*-muconate-grown cells of strain ttM.2.

These data show that muconate lactonizing enzyme is not operative in the dissimilation of *trans,trans*-muconate but suggest that β-ketoadipate succinyl-CoA transferase does play a role in its dissimilation. The point at which the metabolism of the *cis,trans* and *trans,trans* isomers of muconic acid converge with that of the *cis,cis* isomer remains unclear. We were unable to establish the pathway (or pathways) by which *A. eutrophus* dissimilates *cis,trans*- and *trans,trans*-muconate, since no disappearance of these compounds could be demonstrated in extracts prepared from cells grown at their expense.

DISCUSSION

The pathways for the dissimilation of aromatic compounds by *A. eutrophus* are schematized in

TABLE 7. *Specific activities of four enzymes of the β-ketoadipate pathway in cells of Alcaligenes eutrophus 335 grown at the expense of cis,cis- and cis,trans-muconate*[a]

Enzymes	Growth substrates		
	5 mM Succinate	5 mM *cis,cis*-Muconate	5 mM *cis,trans*-Muconate
Muconate lactonizing enzyme	<0.0001	0.61	0.002
Muconolactone isomerase	0.006	0.64	0.09
β-Ketoadipate enol-lactone hydrolase	0.006	0.32	0.09
β-Ketoadipate succinyl CoA transferase	0.009	0.47	0.52

[a] Specific activities are expressed as units per milligram of protein.

TABLE 8. *Specific activities of four enzymes of the β-ketoadipate pathway in two spontaneous mutants of A. eutrophus 335, selected for the ability to grow with trans, trans-muconate*

Enzymes	Mutant ttM.1[a]		Mutant ttM.2[a]	
	5 mM Succinate	5 mM *trans,trans*-Muconate	5 mM Succinate	5 mM *trans,trans*-Muconate
Muconate lactonizing enzyme	<0.0001	<0.0001	<0.0001	<0.0001
Muconolactone isomerase	0.01	0.18	0.07	0.23
β-Ketoadipate enol-lactone hydrolase	0.01	0.16	0.06	0.19
β-Ketoadipate succinyl-CoA transferase	0.01	0.86	0.41	0.85

[a] Specific activities are expressed as units per milligram of protein.

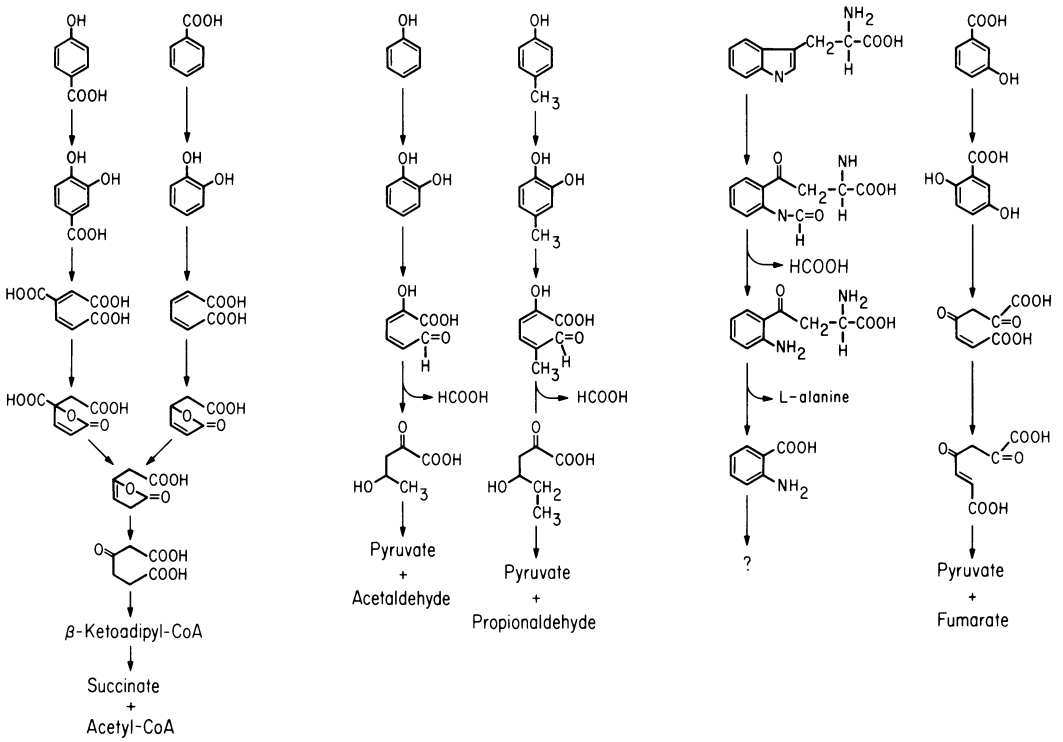

FIG. 2. *The five pathways used by Alcaligenes eutrophus for the oxidation of aromatic compounds.*

Fig. 2. Only benzoate and *p*-hydroxybenzoate appear to be dissimilated through the β-ketoadipate pathway. The catechol branch of this pathway also serves for the dissimilation of *cis,cis*-muconate, utilizable as an exogenous substrate by *A. eutrophus*. Although the *cis,trans* and *trans,trans* isomers of muconic acid can also be utilized by this species (the latter substrate after mutation), neither isomer appears to be metabolized through the catechol branch of the β-ketoadipate pathway, although our data suggest that terminal enzymes of the pathway, especially β-ketoadipate succinyl-CoA transferase, may be operative in their metabolism.

The two monophenols utilizable for growth by *A. eutrophus*, phenol and *p*-cresol, are metabolized via catechols through *meta* cleavage pathways. *A. eutrophus* can also synthesize the key enzymes of the gentisate pathway, and data of Davis et al. (7) indicate that this pathway is employed for the dissimilation of *m*-hydroxybenzoate. L-Tryptophan is initially oxidized via L-kynurenine to anthranilate, but the subsequent metabolic fate of this intermediate remains unknown.

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