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Fermentative and Oxidative Transformation of Ferulate by a Facultatively Anaerobic Bacterium Isolated from Sewage Sludge

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A facultatively anaerobic, gram-negative, non-sporeforming, motile rod-shaped bacterium was isolated from methanogenic consortia degrading 3-methoxy-4-hydroxycinnamate (ferulate). Consortia were originally enriched from a laboratory anaerobic digester fed sewage sludge. In the absence of exogenous electron acceptors and with the addition of 0.1% yeast extract, the isolated bacterium transformed ferulate under strictly anaerobic conditions (N_2-CO_2 gas phase). Ferulate (1.55 mM) was demethoxylated and dehydroxylated with subsequent reduction of the side chain, resulting in production of phenylpropinate and phenylacetate. Under aerobic conditions, the substrate was completely degraded, with transient appearance of caffeate as the first aromatic intermediate and beta-ketoadipate as an aliphatic intermediate. The pure culture has been tentatively assigned to the genus *Enterobacter* with the type strain DG-6 (ATCC 35929). Tentative pathways for both fermentative and oxidative degradation of ferulate are now proposed.

The anaerobic transformation of methoxylated aromatic compounds which are released during the aerobic catabolism of lignin has recently received increased attention (4). Some mechanisms of anaerobic degradation of monoaromatic lignin derivatives by mixed bacterial populations have been proposed (2, 9–12, 17), and some pure cultures of strictly anaerobic microorganisms which are capable of transforming such substrates have been isolated. Bache and Pfennig (1) observed the demethoxylation of aromatic acids by *Acetobacterium woodii*, and Frazer and Young (8) recently isolated a similar organism from methanogenic consortia which degraded ferulate. Krumholz and Bryant (Abstr. Annu. Meet. Am. Soc. Microbiol. 1985, R1, p. 210) reported the isolation of a *Clostridium* sp. and another unidentified bacterium from the rumen; these bacteria utilized syringate and vanillate. Taylor (23) recently demonstrated that *Pseudomonas* sp. strain PN-1 degraded several methoxylated aromatic acids aerobically as well as anaerobically with nitrate as an electron acceptor. In this study, a fermentative facultatively anaerobic bacterium capable of degrading 3-methoxy-4-hydroxycinnamate (ferulate) is described. This organism degrades ferulate completely under aerobic conditions and partially transforms it under strictly anaerobic conditions, without the addition of exogenous electron acceptors.

MATERIALS AND METHODS

Source of the organism. A facultatively anaerobic microorganism was isolated from methanogenic enrichment cultures provided ferulate as the sole carbon and energy source (D. Grbić-Galić, Abstr. Annu. Meet. Am. Soc. Microbiol. 1984, O45, p. 196). The enrichments were originally obtained from sewage sludge, as described previously (10).

Media and conditions of cultivation. The microorganism was cultivated under both aerobic and anaerobic conditions at 35°C in the dark. For anaerobic incubations, techniques modified from those of Hungate (14) and Miller and Wolin (18) were used. The cultures were grown in 250-ml serum bottles containing 150 ml of medium and a 30% CO_2–70% N_2 atmosphere and closed with butyl rubber stoppers. For aerobic cultivation, 250-ml Erlenmeyer flasks plugged with foam plugs were used. The basal medium, which was also used for isolation, had the following composition (values in grams per liter of deionized water): $(NH_4)_2HPO_4$, 0.04; NH_4Cl, 0.2; $MgCl_2 \cdot 6H_2O$, 1.8; KCl, 1.3; $MnCl_2 \cdot 4H_2O$, 0.02; $CoCl_2 \cdot 6H_2O$, 0.03; H_3BO_3, 0.0057; $CaCl_2 \cdot 2H_2O$ 0.0027; $Na_2MoO_4 \cdot 2H_2O$, 0.0025; $ZnCl_2$, 0.0021; and $NaHCO_3$, 2.64 (11). A vitamin solution (1% vol/vol) (26) was added. The medium was buffered at pH 7.0 with bicarbonate and under anaerobic conditions with a bicarbonate-CO_2 system with a gas atmosphere of 30% CO_2–70% N_2. For anaerobic incubations, the following ingredients were added (in grams per liter): resazurin (as an indicator of redox conditions), 0.001; $FeCl_2 \cdot 4H_2O$, 0.368; and $Na_2S \cdot 9H_2O$, 0.5 (reducing agents). For experiments in which the optical density (OD) of cultures was measured, $FeCl_2 \cdot 4H_2O$ was omitted to avoid formation of a black precipitate. Agar (2% wt/vol) was added to the mineral salts solution if solid media were needed. Ferulate was added to a final concentration of 0.3 g/liter (1.55 mM). Since growth in this medium was slow, 0.1% (wt/vol) yeast extract was added. In experiments designed to determine the effect of an additional substrate on the metabolism of ferulate, glucose (0.3% wt/vol) was added to the medium. The vitamin solution, reducing agents, sodium bicarbonate, and substrates were filter sterilized and added to the medium after autoclaving. Care was taken to maintain a C/N/P molar ratio of 100:15:1 after addition of substrates.

The stock cultures were maintained on slants of the solid basal medium plus ferulate and 0.1% (wt/vol) yeast extract, as described above, and on Bacto Nutrient Agar (Difco Laboratories, Detroit, Mich.) with 0.5% (wt/vol) glucose. Peptone-yeast extract-glucose (PYG) medium as described by Holdeman and Moore (13) was used to determine fermentation products from glucose under anaerobic conditions.

Culture purity. Cultures were routinely checked for purity by examining wet mounts and Gram stains of the isolate grown under either aerobic or anaerobic conditions. In addition, the Minitek (BBL Microbiology Systems, Cockeysville, Md.) series of biochemical tests were repeated periodically to make sure that no changes in the culture characteristics occurred.

Characterization of the microorganism. Wet mounts and

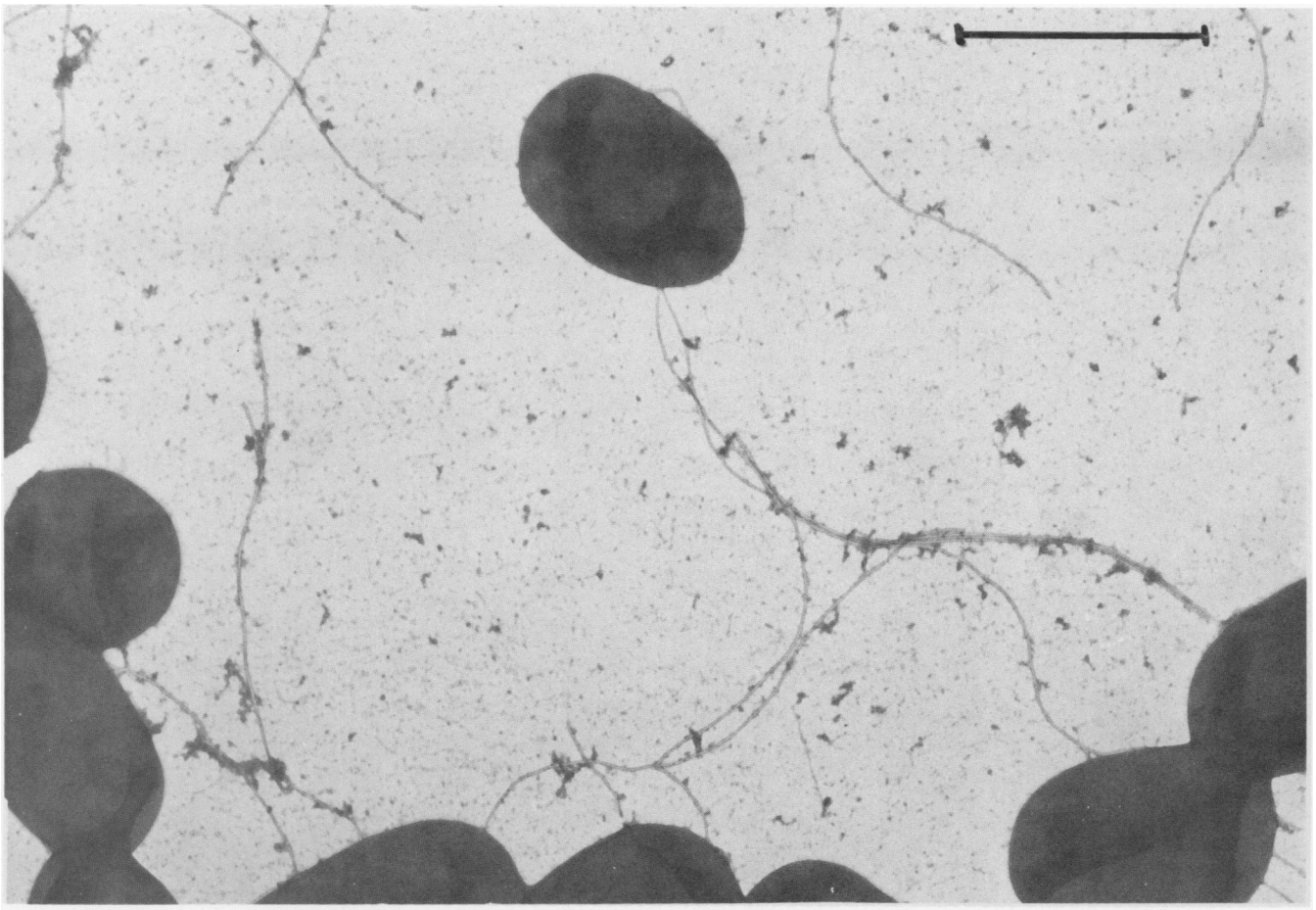


FIG. 1. Transmission electron micrograph of strain DG-6 showing partial subpolar flagellation. The specimen was stained with 1% uranyl acetate. Bar represents 0.5 μm.

Gram-stained smears were examined with an Olympus-Vanox microscope (Olympus Optical Co., Ltd., Tokyo, Japan). The type of flagellation was determined by the staining procedure of Leifson, as described by Janke and Dickscheit (15). Specimens were prepared for transmission electron microscopy by staining with 1% uranyl acetate and examined with a Hitachi HU-11E-1 transmission electron microscope operated at 75 kV.

The biochemical characteristics of the strain were determined with the Minitek Miniaturized Microorganism Differentiation Systsm (BBL). The incubations were aerobic, although in most cases parallel anaerobic incubations were also made.

Substrate utilization and transformation: analytical techniques. The OD_{630} of the cultures was determined spectrophotometrically (Spectronic 600; Bausch and Lomb, Rochester, N.Y.). Generation times were calculated from the linear region of the growth curve by measuring the time needed for the OD of the culture to double. Growth on methanol, formate, formaldehyde, CH_4, CO_2 plus H_2, and acetate was followed by measuring the OD_{630}. The disappearance of ferulate was quantitatively monitored by UV spectrophotometry. Absorption between 210 and 315 nm was scanned, since ferulate absorbs maximally at 310 and 290 nm. Changes in absorption maxima suggested that substrate transformations had occurred. The ability of the isolate to transform various aromatic substrates, including syringate, 3,4-dihydroxycinnamate (caffeate), cinnamate, cinnamaldehyde, phenylpropionate, phenylacetate, and benzoate, was also determined spectrophotometrically. Samples of culture fluid were centrifuged, and the supernatant liquid was appropriately diluted with basal medium.

The concentration of substrate was also determined by high-pressure liquid chromatography (HPLC). HPLC was also used to monitor and identify aromatic intermediates produced during anaerobic and aerobic transformation of ferulate. An HPLC system (Waters Associates, Inc., Milford, Mass.) with a model 440 absorbance detector and WISP 710 B automatic injector was used. The supernatant liquid of the centrifuged samples (10 μl) was injected onto a reverse-phase column (250 mm Spherisorb ODS 10 μm; Alltech Associates, Inc., Deerfield, Ill). The mobile phase consisted of acetonitrile and 0.01 N perchloric acid, operated in a linear gradient of 10 to 60% acetonitrile. The flow rate was 1.2 ml/min, and the run time was 20 min. The UV detector was operated at both 280 and 254 nm. An external standard procedure was used for peak identification, and the following standard compounds were examined: ferulate, vanillate, caffeate, 3,4-dihydroxyhydrocinnamate, *p*-hydroxycinnamate, *p*-hydroxyhydrocinnamate, *trans*-cinnamate, phenylpropionate, phenylacetate, benzoate, protocatechuate, and catechol. For each sample run, a minimum of three replicate injections were made at each of the two wavelengths.

Aliphatic intermediates of ferulate transformation were identified by gas chromatographic analysis. A Hewlett-Packard 5730A gas chromatograph (Hewlett-Packard Co., Avondale, Pa.) was fitted with a 198-cm glass column packed with 10% FFAP and 100-120 Chromosorb WAW. The injector temperature was 250°C, the column temperature was 150°C, and the flame ionization detector temperature was 300°C. The carrier gas was helium, set at a flow rate of 60 ml/min. The nonvolatile organic acids were qualitatively determined as methyl esters (13). Volatile fatty acids were determined under the same operating conditions as already described, but with a different preparative procedure (12). The composition of gas produced from glucose under anaerobic conditions was determined by gas partitioning chromatography (Fisher-Hamilton, model 25V; Fisher Scientific Co., Pittsburgh, Pa.).

RESULTS

Isolation of the microorganism. The pure culture was obtained by diluting the original cultures (10^{-3} to 10^{-7}) and inoculating plates of solid mineral salts medium which contained 1.55 mM ferulate. The plates were incubated anaerobically in a 30% CO_2–70% N_2 atmosphere in a glove box at 35°C. Isolated colonies were repeatedly transferred to the same minimal medium and, alternatively, to Bacto Nutrient Agar plus 0.5% (wt/vol) glucose for purification. Several strains with similar characteristics were isolated and grew equally well under both aerobic and anaerobic conditions. One isolate was chosen for more detailed examination and was designated DG-6.

Morphology. Cells of strain DG-6 were rod-shaped with rounded ends, 0.3 μm long and 0.5 to 1.6 μm wide. They occurred singly or in pairs and stained gram-negative. The cells were actively motile. Electron microscopic examination of the bacterium revealed laterally inserted flagella (Fig. 1), and so did the flagellum staining and light microscopy. Strain DG-6 showed no sporulation in any of the growth media used.

Growth rates. ODs of cultures growing in basal medium plus ferulate and yeast extract and in the same medium plus glucose were measured under both aerobic and anaerobic conditions (Fig. 2). In the defined medium with ferulate and yeast extract, the generation times under aerobic and anaerobic conditions were 8.5 h (67.5 h without yeast extract) and 20 h, respectively. Figure 2 shows the correlation between culture growth and transformation of ferulate. Aerobic incubations in the aromatic medium with glucose added resulted in a generation time of 5.5 h compared with 14 h for the same medium without yeast extract. Both glucose and yeast extract apparently increased the growth rate.

Physiology and nutrition. As shown with help of the BBL Minitek Miniaturized Microorganism Differentiation System, strain DG-6 degraded glucose, arabinose, maltose, rhamnose, sucrose, trehalose, xylose, cellobiose, mannose, raffinose, mannitol, malonate, citrate, adonitol, and sorbitol both aerobically and anaerobically and glycerol and salicin only aerobically (lactose degradation data were inconclusive). It did not degrade melezitose or inositol either aerobically or anaerobically. According to OD measurements, DG-6 grew on acetate, formate, and CO_2-H_2 with the addition of yeast extract under aerobic conditions. The generation time was 33.6 h on acetate, 19.2 h on formate, and 64.8 h on H_2-CO_2. DG-6 did not grow on methane, formaldehyde, or methanol (there was no difference in OD between these cultures and control cultures with yeast extract as the only substrate).

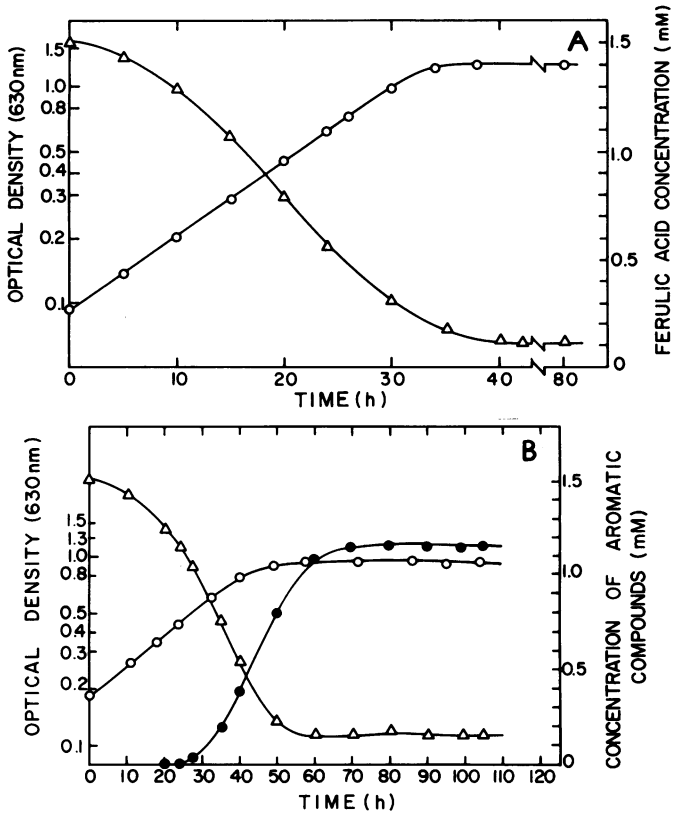
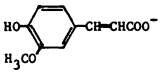
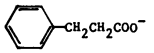
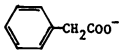
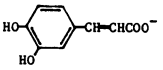
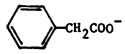
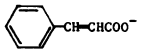
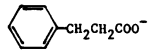
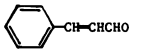
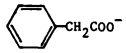
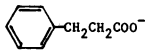
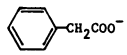


FIG. 2. Growth of strain DG-6 and its degradation of ferulate in basal medium plus 0.1% (wt/vol) yeast extract under aerobic (A) and anaerobic (B) conditions. The buildup of phenylpropionate, the aromatic intermediate produced during ferulate transformation, is also shown in panel B. Values are means of triplicate measurements. Symbols: ○, cell density; △, ferulate concentrations; ●, phenylpropionate concentration. Note the difference in time scale in panels A and B.

Strain DG-6 hydrolyzed esculin and reduced nitrate. It was catalase, beta-galactosidase, ornithine decarboxylase, arginine dihydrolase, and Voges-Proskauer positive; it was lysine decarboxylase negative. DG-6 produced gas (CO_2, H_2, and CO) from glucose anaerobically. No indole was produced from tryptophan. There was no phenylalanine deamination, no H_2S production from thiosulfate, and no gelatin or urea hydrolysis. Under anaerobic conditions DG-6 was a typical fermentative bacterium which did not require nitrate or any other exogenous electron acceptor. Also, it was cytochrome-oxidase and oxidase negative. It transformed ferulate without a lag under both aerobic and anaerobic conditions. With glucose as an additional energy source generation time was reduced, but the rate and extent of the anaerobic ferulate degradation did not change. Aerobically, the addition of glucose slowed ferulate utilization, since glucose was a preferred substrate.

Of the eight aromatic compounds tested in the presence of yeast extract or yeast extract-glucose, DG-6 transformed four, ferulate, caffeate, phenylpropionate, and cinnamaldehyde, under both aerobic and anaerobic conditions. According to UV-spectrophotometry and HPLC data, these compounds were completely mineralized aerobically, yet only partially chemically transformed anaerobically (no deg-

TABLE 1. Anaerobic transformation of various aromatic compounds by the isolated bacterium[a]

Aromatic substrate (0.3 g/liter; 0.1% [wt/vol] yeast extract present (concn [mM])	Chemical structure	Concn (mM) of substrate after 15 days incubation[b]	Residual aromatic compounds detected after 15 days (concn [mM])	Chemical structure
Ferulate (1.55)		0	Phenylpropionate (1.15)[a]	
			Phenylacetate (0.25)	
Caffeate (1.67)		0.20	Phenylacetate (0.87)	
Cinnamate (2.03)		1.93	Phenylpropionate (0.05)	
Cinnamaldehyde (2.27)		1.20	Phenylacetate (0.51)	
Phenylpropionate (2.0)		1.60	Phenylacetate (0.17)	

[a] The nature of the residual aromatic compounds was determined from their UV absorption spectra and HPLC chromatograms of the culture fluid.
[b] The results are the mean values of measurements for three parallel cultures.

radation of the ring). Syringate, phenylacetate, and benzoate were completely degraded aerobically, but were not attacked anaerobically. Cinnamate was transformed under anaerobic conditions, but the rate and extent of transformtion were extremely low; the product, phenylpropionate, became detectable after 15 days and only 5% of the substrate was degraded. Cinnamate was not degraded under aerobic conditions. Table 1 shows the aromatic compounds released during anaerobic transformation of the five aromatic substrates, as well as the chemical structures of both the substrates and intermediates.

In PYG medium under anaerobic conditions, strain DG-6 fermented glucose (55.56 mM), producing the following compounds: hexanol (2.00 mM), acetate (17.50 mM), isobutyrate (6.82 mM), butyrate (2.60 mM), lactate (8.00 mM), succinate (2.00 mM), CO_2 (33.2 mM), and low concentrations of propionate (1.63 mM), isovalerate (1.20 mM), butanol (1.85 mM), and H_2 and CO (not quantitated).

The temperature optimum for growth was between 25 and 37°C. No growth occurred above 39°C or below 15°C.

Aromatic substrate transformations. Under strictly anaerobic conditions, without any exogenous electron acceptors but CO_2, DG-6 catabolized ferulate (1.55 mM) as far as phenylacetate. With cultures transferred several times with ferulate medium, complete transformation required about 60 h. After 50 h, with 78% substrate degraded, the extracellular intermediates detected in the culture fluid were caffeate (0.07 mM), *p*-hydroxycinnamate (0.04 mM), cinnamate (0.30 mM), phenylpropionate (1.15 mM), and phenylacetate (0.14 mM). The results are the mean values of measurements for three parallel cultures. Aliphatic compounds were also found: succinate (0.24 mM), maleate (0.25 mM), and traces of pimelate (0.01 mM) and lactate (0.02 mM). It is important to emphasize that hydrocaffeate, *p*-hydroxyhydrocinnamate, and acetate were not found. If glucose was added to the medium, malate (0.03 mM) and acetate (2.50 mM), CO_2 (12.50 mM), and traces of adipate (0.01 mM) and pyruvate (0.02 mM) were formed; the amount of lactate formed (2.80 mM) was considerably larger.

Under aerobic conditions, ferulate (1.55 mM) was completely degraded in 40 h. With ferulate as the sole energy and carbon source, caffeate (0.53 mM) and protocatechuate (0.33 mM) were the only aromatic intermediates detected. Vanillate was not found. In addition, maleate (0.49 mM), succinate (0.12 mM), and malate (0.10 mM), as well as traces of lactate (0.05 mM) and malonate (0.07 mM) were found. If glucose was also present, all the above intermediates were again detected. In addition, high levels of beta-ketoadipate (0.28 mM) and acetate (0.33 mM) appeared in the gas chromatography chromatogram. Traces of pyruvate (0.06 mM) were found as well.

DISCUSSION

Strain DG-6 is a facultatively anaerobic, gram-negative, oxidase-negative, motile rod-shaped bacterium, which carries out both oxidative and fermentative metabolism. Its characteristics are similar to those of the family *Enterobacteriaceae* (3) and the genus *Enterobacter*, although flagellation seems to be subpolar (lateral). The compunds produced from glucose anaerobically (high concentrations of acetate, isobutyrate, lactate, succinate, hexanol, and CO_2 and lower amounts of other products) are comparable to the fermentation pattern of *Enterobacter aerogenes* ATCC 13048 and *E. cloacae* ATCC 13047, as checked in our laboratory. Therefore, DG-6 is tentatively placed into the genus *Enterobacter*. The type strain, DG-6^T, is deposited in the American Type Culture Collection with the accession number ATCC 35929. Further investigations on its complete characterization and classification, including DNA and plasmid studies, are in progress.

Concerning the transformation of ferulate under anaerobic conditions, strain DG-6 exhibits the ability to demethoxylate via hydrolysis, which involves cleavage of a phenylether bond. This reaction results in the formation of caffeate. Such anaerobic demethoxylations by bacteria were first apparent in *A. woodii* (1). Although DG-6 utilized a broad range of organic substrates, it could not utilize methanol, formaldehyde, or methane; it grew on acetate, formate, and CO_2-H_2,

with the addition of yeast extract, but only aerobically. Furthermore, no acetate was detected in the culture fluid when ferulate was the main substrate, which is different from fermentation by *A. woodii*. Since no acetate was produced, it must be assumed that the electrons resulting from demethoxylation and C_1-compound oxidation were used elsewhere. Indeed, in addition to reduction of the double bond in the acrylic side chain, which is comparable to the metabolism of *A. woodii* (25), DG-6 also dehydroxylated the intermediates formed after demethoxylation (caffeate to p-hydroxycinnamate to cinnamate). Since no hydrocaffeate or p-hydroxyhydrocinnamate were found, it may be concluded that the dehydroxylations occurred before reductions of the propenoate side chain. Reductive dehydroxylation of caffeate was documented earlier (20). The proposed reactions are summarized in Fig. 3. Thermodynamic reasoning favors the dehydroxylations over acetate formation, as shown in the following reactions (the free energy changes at standard conditions calculated by the method of Parks and Huffman [19]):

(i) Homoacetate fermentation in *A. woodii*; ferulate is demethoxylated and the double bond in the side chain is reduced (1):

$$4C_{10}H_9O_4^- + 4H^+ + 4H_2O \rightarrow \underset{\text{hydrocaffeate}}{4C_9H_9O_4^-} + 2CH_3COO^- + 6H^+$$

$$\Delta G^{\circ\prime} = -457.28 \text{ kJ per reaction}$$

(ii) Ferulate is demethoxylated and dehydroxylated, and the double bond in the side chain is reduced by DG-6; no acetate is formed:

$$4C_{10}H_9O_4^- + 4H^+ + 4H_2O \rightarrow 4HCO_3^- + 4C_9H_9O_2^- + 8H^+$$

phenylpropionate

$$\Delta G^{\circ\prime} = -697.24 \text{ kJ per reaction}$$

DG-6 decarboxylated phenylpropionate to phenylacetate, but at a low rate (only 20% of phenylpropionate fed to the culture was transformed in 15 days of incubation—see Table 1). This reaction remains chemically unexplained. Peppercorn and Goldman (20) showed that similar decarboxylations occurred aerobically with bacteria from rumen, but with p-hydroxylated substrates only.

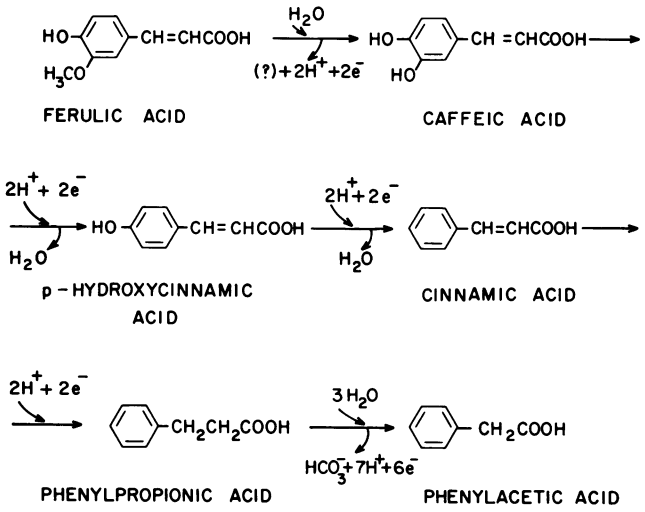


FIG. 3. Proposed pathway for the anaerobic transformation of ferulate by strain DG-6. All aromatic compounds shown were detected in the culture fluid.

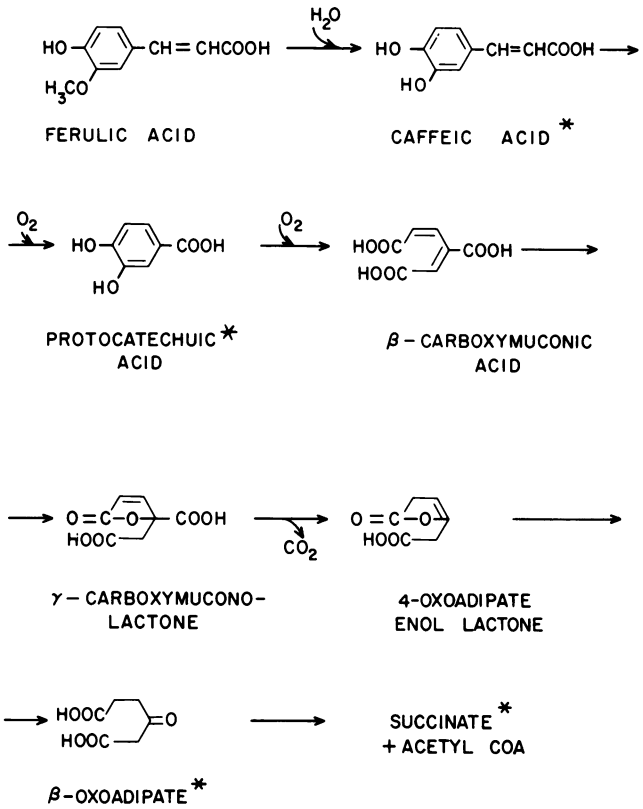


FIG. 4. Proposed pathway for the aerobic degradation of ferulate by strain DG-6. The ring cleavage steps were taken from Dagley (6). Asterisk denotes the compounds which were detected in the culture fluid in this study.

Strain DG-6 decarboxylated phenylpropionate to phenylacetate, but at a low rate (only 20% of phenylpropionate fed to the culture was transformed in 15 days of incubation [Table 1]). This reaction remains chemically unexplained. Peppercorn and Goldman (20) showed that similar decarboxylations occurred aerobically with bacteria from rumen, but with *p*-hydroxylated substrates only.

Under aerobic conditions, strain DG-6 degraded ferulate completely. It was capable of oxygenative ring cleavage, which, from the few intermediates found, probably goes through the *ortho* (intradiol) pathway (5, 6). The degradation was rapid, but the addition of glucose, which was preferentially utilized, facilitated the detection of intermediates. A theoretical scheme for the suggested pathway is shown in Fig. 4. Since caffeate was found, it is conceivable that demethoxylation occurred before side-chain transformation. This is different from the pathways used by *Pseudomonas acidovorans* (24) and *Rhodococcus erythropolis* (7), in which ferulate was first converted to vanillate. After demethoxylation, the aromatic ring is already dihydroxylated and ready for fission. However, the first reaction to occur is the transformation of the side chain, which yields protocatechuate. This aromatic intermediate is reported as the substrate for ring opening in all the aerobic ferulate degradation experiments performed so far (7, 23, 24). The finding of β-ketoadipate and succinate suggests that the beta-ketoadipate pathway (22) is used.

If comparisons between strain DG-6 and another described facultatively anaerobic ferulate degrader, *Pseudomonas* sp. strain PN-1 (23) are to be made, it must be emphasized that the *Pseudomonas* sp. is capable of respiratory metabolism only and therefore requires an alternate electron acceptor in the absence of oxygen. Also, *Pseudomonas* sp. strain PN-1 degrades the aromatic ring under both conditions. On the contrary, DG-6 is fermentative under anaerobic conditions and does not require an external electron acceptor. It is not capable of the anaerobic ring cleavage. The initiation of the ferulate transformation under both anaerobic and aerobic conditions is the same—a demethoxylation. However, the mechanisms may be different. DG-6 is the first fermentative facultative anaerobe reported so far to be capable of transforming aromatic compounds both aerobically and anaerobically. Both DG-6 and *Pseudomonas* sp. strain PN-1 appear to have demethoxylating functions in aerobic and anaerobic environments where lignin derivatives are present.

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LITERATURE CITED

1. **Bache, R., and N. Pfennig.** 1981. Selective isolation of *Acetobacterium woodii* on methoxylated aromatic acids and determination of growth yields. Arch. Microbiol. **130**:255–261.
2. **Boyd, S. A., D. R. Shelton, D. Berry, and J. M. Tiedje.** 1983. Anaerobic biodegradation of phenolic compounds in digested sludge. Appl. Environ. Microbiol. **46**:50–54.
3. **Brenner, D. J.** 1984. Enterobacteriaceae, p. 408–420. *In* N. R. Krieg and J. G. Holt (ed.), Bergey's manual of systematic bacteriology, vol 1. The Williams & Wilkins Co., Baltimore.
4. **Cain, R. B.** 1980. The uptake and catabolism of lignin-related aromatic compounds and their regulation in microorganisms, p. 21–60. *In* T. K. Kirk, T. Higuchi, and H. Chang (ed.), Lignin biodegradation: microbiology, chemistry and potential applications, vol 1. CRC Press, Inc., Boca Raton, Fla.
5. **Chapman, P. J.** 1972. An outline of reaction sequences used for the bacterial degradation of phenolic compounds, p. 17–55. *In* Degradation of synthetic organic molecules in the biosphere. Proceedings of a Conference, San Francisco, Calif. June 12–13, 1971. National Academy of Sciences, Washington, D.C.
6. **Dagley, S.** 1971. Catabolism of aromatic compounds by microorganisms. Adv. Microb. Physiol. **6**:1–46.
7. **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.
8. **Frazer, A. C., and L. Y. Young.** 1985. A gram-negative anaerobic bacterium that utilizes *O*-methyl substituents of aromatic acids. Appl. Environ. Microbiol. **49**:1345–1347.
9. **Grbić-Galić D.** 1983. Anaerobic degradation of coniferyl alcohol by methanogenic consortia. Appl. Envrion. Microbiol. **46**:1442–1446.
10. **Grbić-Galić, D., and L. Y. Young.** 1985. Methane fermentation of ferulate and benzoate: anaerobic degradation pathways. Appl. Environ. Microbiol. **50**:292–297.
11. **Healy, J. B., Jr., and L. Y. Young.** 1979. Anaerobic biodegradation of eleven aromatic compounds to methane. Appl. Environ. Microbiol. **38**:84–89.
12. **Healy, J. B., Jr., L. Y. Young, and M. Reinhard.** 1980. Methanogenic decomposition of ferulic acid, a model lignin derivative. Appl. Environ. Microbiol. **39**:436–444.
13. **Holdeman, L. V., and W. E. C. Moore (ed.).** 1975. Anaerobe laboratory manual. Virginia Poytechnic and State University, Blacksburg.
14. **Hungate, R. E.** 1969. A roll tube method for cultivation of strict anaerobes, p. 117–132. *In* J. R. Norris and D. Ribbons (ed.), Methods in microbiology, vol 3B. Academic Press, Inc. (London), Ltd., London.
15. **Janke, A., and R. Dickscheit.** 1967. Handbuch der mikrobiologischen Laboratoriumstechnik. Verlag Theodor Steinkopff, Dresden, German Democratic Republic.
16. **Kaiser, J. P., and K. W. Hanselmann.** 1982. Fermentative metabolism of substituted monoaromatic compounds by a bacterial community from anaerobic sediments. Arch. Microbiol. **133**:185–194.
17. **Kaiser, J. P., and K. W. Hanselmann.** 1982. Aromatic chemicals through anaerobic microbial conversion of lignin monomers. Experientia **38**:167–176.
18. **Miller, T. C., and M. J. Wolin.** 1974. A serum bottle modification of the Hungate technique for cultivating obligate anaerobes. Appl. Microbiol. **27**:985–987.
19. **Parks, G. S., and H. M. Huffman.** 1932. The free energies of some organic compounds. American Chemical Society Monograph Series no. 60. The Chemical Catalog Co., Inc., New York.
20. **Peppercorn, M., and P. Goldman.** 1971. Caffeic acid metabolism by bacteria of the human gastrointestinal tract. J. Bacteriol. **108**:996–1000.
21. **Richard, C.** 1984. Enterobacter, p. 465–466. *In* N. R. Krieg and J. G. Holt (ed.), Bergey's manual of systematic bacteriology, vol 1. The Williams & Wilkins Co., Baltimore.
22. **Stanier, R. Y., and L. N. Ornston.** 1973. The beta-ketoadipate pathway. Adv. Microb. Physiol. **9**:89–149.
23. **Taylor, B. F.** 1983. Aerobic and anaerobic catabolism of vanillic acid and some other methoxy-aromatic compounds by *Pseudomonas* sp, strain PN-1. Appl. Environ. Microbiol. **46**:1286–1292.
24. **Toms, A., and J. M. Wood.** 1970. The degradation of trans-ferulic acid by *Pseudomonas acidovorans*. Biochemistry **9**:337–343.
25. **Tschech, A., and N. Pfennig.** 1984. Growth yield increase linked to caffeate reduction in *Acetobacterium woodii*. Arch. Microbiol. **137**:163–167.
26. **Wolin, E. A., M. J. Wolin, and R. S. Wolfe.** 1963. Formation of methane by bacterial extracts. J. Biol. Chem. **238**:2882–2886.