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Fermentation of Trihydroxybenzenes by *Pelobacter acidigallici* gen. nov. sp. nov., a New Strictly Anaerobic, Non-Sporeforming Bacterium

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Abstract. Five strains of rod-shaped, Gram-negative, non-sporing, strictly anaerobic bacteria were isolated from limnic and marine mud samples with gallic acid or phloroglucinol as sole substrate. All strains grew in defined mineral media without any growth factors; marine isolates required salt concentrations higher than 1 % for growth, two freshwater strains only thrived in freshwater medium. Gallic acid, pyrogallol, 2,4,6-trihydroxybenzoic acid, and phloroglucinol were the only substrates utilized and were fermented stoichiometrically to 3 mol acetate (and 1 mol CO_2) per mol with a growth yield of 10 g cell dry weight per mol of substrate. Neither sulfate, sulfur, nor nitrate were reduced. The DNA base ratio was 51.8 % guanine plus cytosine. A marine isolate, Ma Gal 2, is described as type strain of a new genus and species, *Pelobacter acidigallici* gen. nov. sp. nov., in the family Bacteroidaceae. In coculture with *Acetobacterium woodii*, the new isolates converted also syringic acid completely to acetate. Cocultures with *Methanosarcina barkeri* converted the respective substrates completely to methane and carbon dioxide.

Key words: *Pelobacter acidigallici* gen. nov. sp. nov. – Genus and species description – Phenolic compounds – Gallic acid – Pyrogallol – Phloroglucin – Anaerobic degradation – Acetate – Methanogenic cultures

The complete anaerobic degradation of lignin derivatives including ferulic acid, vanillic acid and other methoxylated phenolic compounds by mixed bacterial populations was reported already by Tarvin and Buswell (1934) and was again proven recently in more refined studies (Healy and Young 1978, 1979; Healy et al. 1980). The organisms involved in these processes have not yet been identified. From anaerobic enrichments on methoxylated phenol derivatives recently *Acetobacterium woodii* was isolated. This organism fermented the methoxyl groups to acetate and did not attack the aromatic ring (Bache and Pfennig 1981). In the present study pure cultures of new organisms are described which degrade two trihydroxybenzene isomers fermentatively and produce exclusively acetate and CO_2 as products.

Materials and Methods

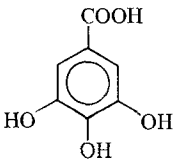
Source of Organisms

The following strains were isolated in pure culture from enrichment cultures inoculated with mud samples:

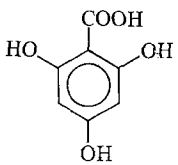
1. Strains Ma Gal 1, Ma Gal 2 and Ma Phl 1 from black, anaerobic mud of Rio Marin, a channel about 2.5 m wide and 70 cm deep located in the city of Venice, Italy. Similar isolates were also obtained from a black mud sample of the Canale Grande in Venice, Italy, and from marine black mud taken near Cuxhaven, W. Germany.

2. Strains Ott Gal 1 and Ott Gal 2 from black, anaerobic mud of a pasture creek near Hannover, W. Germany. Similar isolates were also obtained from another freshwateer creek mud near Konstanz, W. Germany, and also from anaerobic digestor sludge of sewage plants.

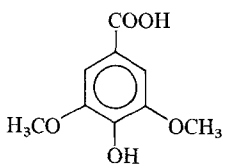
Methanospirillum hungatei strain M 1 h was isolated from digested sludge of the sewage plant of Göttingen, W. Ger-



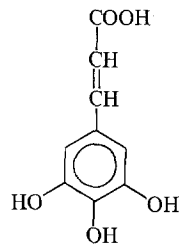
Gallic acid



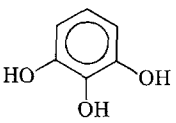
2.4.6–Trihydroxy-benzoic acid



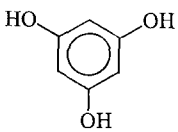
Syringic acid



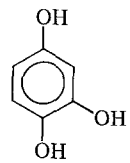
3.4.5–Trihydroxy-cinnamic acid



Pyrogallol



Phloroglucinol



Hydroxy-hydroquinone

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many. *Methanosarcina barkeri* strain Fusaro (DSM 804) was obtained from the Deutsche Sammlung für Mikroorganismen, Göttingen. *Desulfovibrio vulgaris* strain Marburg was kindly provided by Prof. Dr. R. K. Thauer, Marburg.

Media and Growth Conditions

The basal medium had the following composition (values in g/l): KH_2PO_4, 0.2; NH_4Cl, 0.25; KCl, 0.5; $CaCl_2 \cdot 2H_2O$, 0.15. Freshwater medium in addition contained 1.0 g NaCl and 0.4 g $MgCl_2 \cdot 2H_2O$, marine medium 20.0 g NaCl and 3.0 g $MgCl_2 \cdot 2H_2O$ per liter. Sodium bicarbonate, sodium sulfide, trace element solution SL 7 and vitamin solution (Pfennig 1978) were added to the autoclaved, cooled medium from stock solutions as described in detail (Widdel and Pfennig 1981). The pH was adjusted to 7.2–7.3. For enrichment cultures, the medium was dispensed à 50 ml into 100 ml serum bottles gassed with N_2/CO_2 mixture (80 %/20 %) and sealed with butyl rubber stoppers or, for pure cultures, into rubber-sealed 50 ml screw cap bottles or 20 ml screw cap tubes which were completely filled. Substrates were added 5 mM from sterile stock solutions before inoculation. Phenolic substrates were filter-sterilized as 0.1 M or 0.2 M (NaOH-neutralized) solutions and stored under nitrogen gas. Growth of pure cultures was followed in 20 ml tubes in a Bausch and Lomb-Spectronic 70 spectrophotometer. In tests for syntrophic growth excess of pure cultures of *Methanospirillum hungatei*, *Methanosarcina barkeri* or *Desulfovibrio vulgaris* was added, the latter with additional sodium sulfate (20 mM final concentration added from sterile 1 M stock solution). For further characterization, also commercial media systems (API 20 A, BioMerieux, Nürtingen, W. Germany) were applied. Aerobic growth was tested in agar shake gradient cultures under air. All growth tests were carried out at least in duplicates at 28° C.

Isolation

Pure cultures were obtained by repeated application of the agar shake culture method as described by Pfennig (1978). Tubes were gassed with N_2/CO_2 mixture (80/20) and sealed with butyl rubber stoppers. Purity was checked microscopically and also by growth test in complex medium (AC-medium, Difco-Laboratories, Detroit, Michigan, USA).

Gram-staining was carried out according to Magee et al. (1975) as modified by Widdel (1980) without counterstaining. *Acetobacterium woodii* and *Escherichia coli* were used as control strains.

Chemical Analyses

Sulfide formation from sulfur or sulfate was analyzed by the methylene blue method (Pachmayr 1960). Formation of nitrite from nitrate was assayed by azo dye formation with sulfanilic acid and α-naphthylamine. Phenolic substrates were quantified by absorption spectra taken at 200–350 nm wavelength in a Gilford model 250 spectrophotometer.

Lactate was assayed photometrically with D- and L-lactate dehydrogenase according to Bergmeyer (1965).

Acetate, other volatile fatty acids, and alcohols were assayed by gas chromatography on Porapak QS, 100–120 mesh, Column length 2.0 m, 1/4″ diameter, in a Perkin-Elmer Sigma 3 B gas chromatograph with flame ionization detector, injector and detector temperature 250° C, oven temperature 220° C, carrier gas nitrogen, 30 ml/min. Samples were acidified prior to injection with formic acid from 10 M stock solution to 0.5 M final concentration.

Methane was quantified in a Perkin-Elmer Sigma 4 B gas chromatograph equipped with a molecular sieve column (5 Å, 60/80 mesh, 1.8 m, 1/8″) and a flame ionization detector.

DNA Base Composition

The mole per cent guanine plus cytosine of the DNA was determined with the thermal denaturation method according to De Ley (1970) after extraction as described by Marmur (1961).

Results

Enrichment, Isolation, and Enumeration

50 ml – enrichments in freshwater medium with 5 mM gallic acid or 5 mM phloroglucinol were inoculated with 3–5 ml of anaerobic freshwater mud from various creeks or of sludge from sewage plants. Marine medium was used in the same manner for enrichments from marine mud samples from Venice and Cuxhaven. Gas production started after 5–12 days and ceased after 3–4 weeks. In subcultures on the same media turbidity developed within 2–3 days but gas production was small and the pH dropped from 7.2 to 6.3–6.5. Further subcultures were run alternatively with and without high background populations of *Methanospirillum hungatei* in order to promote a possible electron transfer towards methanogenesis. Independently of added methanogens the same type of rod-shaped bacteria developed in all subcultures. By fluorescence microscopy no methanogenic bacteria other than those added could be detected. Isolation was attempted in agar shake series with and without *Desulfovibrio vulgaris* added as a hydrogen sink. In both cases the same yellowish, lens-shaped colonies developed which were picked with sterile Pasteur pipettes, resuspended in small amounts of anaerobic mineral medium, and again diluted in shake series. The resulting colonies were checked for purity by microscopic control and by inoculation into AC-medium. Finally, 5 strains were chosen for further characterization.

Enumeration of gallate-degrading bacteria was carried out in two mud samples by the three-tube most probable number technique (American Public Health Association, 1969). In fresh water creek mud from Hannover, the MPN index of gallate degraders was 23 cells per ml; in mud of Rio Marin, Venice, 240 cells per ml were found.

Morphology

Strains isolated from freshwater or marine mud did not differ markedly in morphology. Cells of marine isolated (Ma Gal 1, Ma Gal 2, Ma Phl 1) were short rods, 0.5–0.7 × 1.0–2.5 µm, with rounded ends (Fig. 1 a). Cells of freshwater isolates (Ott Gal 1, Ott Gal 2) were often longer, 0.7 × 1.5–4.0 µm in size, and tended to form chains (Fig. 1 b). Cell length varied; often cells of different length occurred in one single chain, and also coccoid minicells were observed (arrow). Dark granular inclusions appeared in the polar regions. Motility was occasionally observed in young cultures growing on gallic acid. Cells moved in a tumbling manner indicating subpolar or peritrichous flagellation. Motility, however, was lost early and could not be reproduced reliably.

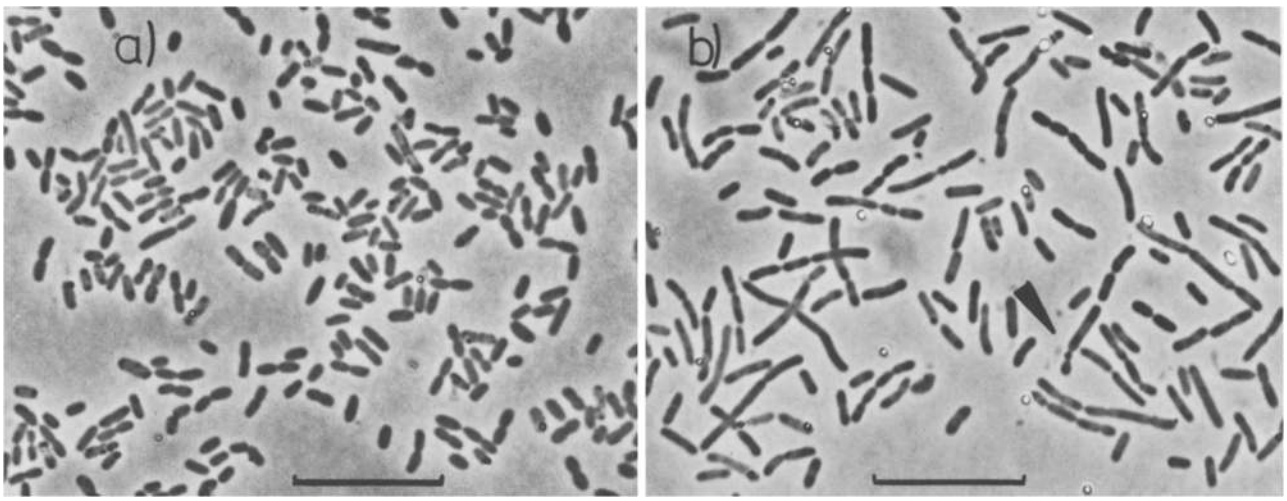

Fig. 1 a, b. Phase contrast photomicrographs of isolates on gallic acid. **a** Marine isolate Ma Gal 2. **b** Freshwater isolate Ott Gal 1. Arrow points at a cell chain with cells of different lengths. Bar equals 10 µm for both prints. Refractile particles are sulfur droplets in the medium

Cells of strain Ma Gal 1 tended to form clumps in all growth phases whereas the other strains clumped only in old cultures. All strains stained Gram-negative, and a typical Gram-negative cell wall structure was also proven by electron microscopic examination of ultrathin sections.

Spore formation could never be detected, neither in mineral medium nor in the sporulation media of Duncan and Strong (1968) or Hollaus and Sleytr (1972) nor in combinations of either two or all three. Enrichments from pasteurized mud samples (15 min at 80° C) failed to degrade gallic acid.

Physiological and Nutritional Properties

All five isolated strains grew well in their respective isolation media, however, the two freshwater isolates often exhibited lag phases of several days after transfer. Although marine and fresh water isolates were similar with respect to all morphological and physiological properties they differed by their salinity requirements: Whereas the freshwater isolates Ott Gal 1 and Ott Gal 2 did not grow in marine medium or brackish water medium (containing half of the salt concentration of marine medium), the marine isolates did not grow in freshwater medium and only slowly with diminished yield in brackish water medium. No growth was found under aerobic or microaerobic conditions. Phosphate concentrations higher than 5 mM retarded growth for several days; no growth was found at phosphate concentrations higher than 20 mM.

Vitamins as well as the trace elements selenium, molybdenum and tungsten were present in the isolation medium but were not required by pure cultures for at least five subsequent transfers. Yeast extract or other undefined additions were never needed by any isolate and did not enhance growth yields at all. Phloroglucinol, pyrogallol, gallic acid, and 2,4,6-trihydroxybenzoic acid were the only substrates utilized. No mono- or dihydroxybenzenes were degraded, neither in the presence nor absence of *Desulfovibrio vulgaris* added as a hydrogen sink. No degradation of these phenols was either observed in the presence of other reducing agents like ascorbate, cysteine, or dithionite; neither growth nor changes of absorption spectra of these substrates could be detected.

Table 1. Substrates tested for degradation by strains Ma Gal 1, Ma Gal 2, Ma Phl 1, and Ott Gal 2

Substrates degraded:
Gallic acid, 10 mM; Phloroglucinol, 10 mM; Pyrogallol, 5 mM; 2,4,6-trihydroxybenzoic acid, 5 mM.
Substrates not degraded:
2 mM: Phenol, o-Cresol, Fructose, Glucose.
5 mM: Catechol, Resorcinol, p-Hydroquinone, Hydroxyhydroquinone, Salicylic acid, Protocatechuic acid, Cyclohexane carboxylic acid, Benzoic acid, Trihydroxycinnamic acid, Chinaic acid, meso-Inositol, Syringic acid, 3,4,5-Trimethoxybenzoic acid.
10 mM: Methanol, Ethanol, Glycerol, Formate, Glyoxylate, Glycolate, Pyruvate, Lactate, β-Hydroxybutyrate, Malonate, Succinate, Malate, L-Tartrate, Citrate, Fumarate, Nicotinate, Urate.
Mannitol, Lactose, Saccharose, Maltose, Salicin, Xylose, Arabinose, Cellobiose, Mannose, Melezitose, Raffinose, Sorbit, Rhamnose, Trehalose not fermented.
No formation of indole from tryptophan, no hydrolysis of urea, gelatine, or esculin. No catalase activity

The same was true for the third trihydroxybenzene isomer, hydroxyhydroquinone, for 3,4,5-trihydroxycynnamic acid or for methoxylated gallic acid derivatives like syringic acid or 3,4,5-trimethoxybenzoic acid. None out a of great variety of further substrates including sugars, organic mono- and dicarboxylic acids and alcohols was utilized. Neither nitrate or sulfate nor sulfur was reduced during degradation of gallic acid, pyrogallol, or phloroglucinol. The results of all substrate tests are summarized in Table 1. The only product formed was acetic acid. No other fatty acids nor lactate or alcohols could be detected. No gas other than carbon dioxide was produced.

The correlation between growth, substrate decomposition, and acetic acid formation is shown in Fig. 2 a for strain Ma Gal 2 grown on gallic acid. No qualitative change was found in absorption spectra of the culture supernatant as shown in Fig. 2 b indicating that no unsaturated intermediates were excreted. The maximum growth rate was $0.347\,h^{-1}$, (minimum doubling time 2.0 h) at 35° C. No growth occurred at 9° C and at 40° C. The pH limits were pH 5.3 and 8.2, the

Table 2. Growth yields and stoichiometry of fermentation by strain Ma Gal 2

Substrate	Amount of substrate degraded (mmol)	Cell dry weight formed (mg)	Acetate assimilated[a] (mmol)	Acetate produced (mmol)	Growth yield: mg per mol substrate utilized	Carbon recovery %
Gallic acid	2.5	25.38	0.522	7.0	10.1	101.0
Gallic acid	5.0	51.18	1.05	13.9	10.2	99.7
Phloroglucinol	2.5	24.72	0.509	6.9	9.9	98.8
Pyrogallol	2.5	24.85	0.512	7.1	9.9	102.1
2,4,6-Trihydrobenzoic acid	2.5	24.07	0.496	6.9	9.6	98.6

[a] Assimilated cell material was calculated from acetate as substrate by the equation: $17CH_3COOH \rightarrow 8\ (C_4H_7O_3) + 2CO_2 + 6H_2O$; thus, 0.0206 mmol acetate are required for 1.0 mg of cell dry weight. All figures are means of at least two independent assays. Cell dry weights were determined in 500 ml cultures

optimum being at 6.5–7.0. The maximum substrate concentrations tolerated were 20 mM gallic acid and phloroglucinol, and 5 mM pyrogallol.

Growth Yield, Stoichiometry of Fermentation, and Coculture Experiments

The yields of acetate and cell dry matter obtained with strain Ma Gal 2 in marine medium are summarized in Table 2. With all substrates, about 10 g cell dry weight per mol of substrate was formed. The yield was the same with strains Ma Gal 1 and Ma Phl 1, but was about 20 % less with the freshwater isolates Ott Gal 1 and Ott Gal 2 although the same amounts of acetate were formed.

The total fermentation equation for gallic acid and 2,4,6-trihydroxybenzoic acid reads as follows:

$$C_7H_5O_5^- + 4H_2O \rightarrow 3CH_3COO^- + HCO_3^- + 3H^+$$

for phloroglucinol and pyrogallol,

$$C_6H_6O_3 + 3H_2O \rightarrow 3CH_3COO^- + 3H^+.$$

Cocultures of *Methanosarcina barkeri* and strain Ott Gal 1 degraded gallic acid stoichiometrically to methane and CO_2. Acetate was an intermediary product. The overall equation of methane formation from gallic acid can be written as follows:

$$C_7H_5O_5 + 7H_2O \rightarrow 3CH_4 + 4HCO_3^- + 3H^+.$$

In our experiment, 250 µmol gallic acid was transformed to 743 µmol methane; the remaining acetate in the medium was < 7 µmol after 22 days of incubation.

In cocultures of *Acetobacterium woodii* and strain Ma Gal 2, syringic acid was completely degraded to acetate; 250 µmol syringic acid was converted to 1013 µmol acetate; the theoretical value would be 1095 µmol after subtraction of substrate assimilated into cell material.

Pigments and DNA Base Composition

Crude cell extracts of strain Ma Gal 2 with a protein content of about 10 mg/ml were subjected to spectrophotometric analysis. No pigments absorbing in the range of 400 to 650 nm could be detected, neither in air-oxidized nor dithionite-reduced extracts. Redox difference spectra of dithionite-reduced minus air-oxidized or minus ferricyanide-oxidized extracts did not give any indications for the presence of cytochromes either.

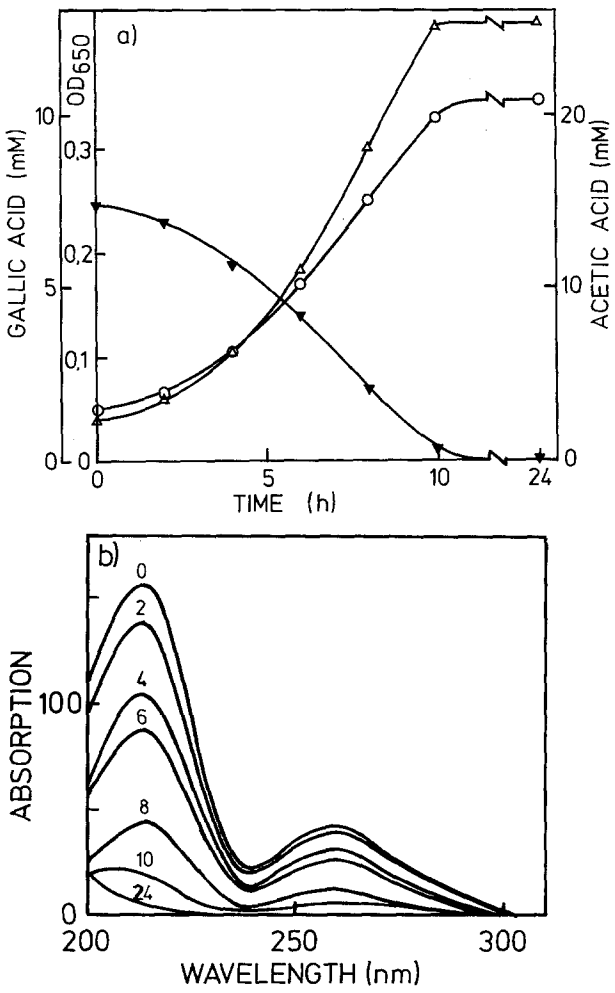

Fig. 2a. Fermentation time course of strain Ma Gal 2 on gallic acid. Experiments were performed at 30° C in 20 ml tubes sealed with a Bellco rubber septum. Samples were removed by a syringe at times indicated and the headspace was flushed with N_2/CO_2 gas mixture. (○) Cell density, (△) acetic acid, (▼) gallic acid. **b** Absorption spectra of the growth medium in the same experiment. Numbers indicate the sampling times (in h). Samples were diluted 1:2000 for measurement

The guanine + cytosine content of the DNA of strain Ma Gal 2 as determined by thermal denaturation was 51.8 ± 0.4 mol % guanine + cytosine.

Discussion

Physiology of Substrate Degradation

Aerobic degradation of aromatic compounds is well understood and involves a dioxygenase reaction as common step of ring cleavage (Ornston and Stanier 1966; Dagley 1975). In contrast, only few information exists on anaerobic degradation. Anaerobic photometabolism of benzoate was observed with *Rhodopseudomonas palustris* (Dutton and Evans 1969), *Rhodospirillum fulvum* (Pfennig et al. 1965) and *Rhodocyclus purpureus* (Pfennig 1978) and occurs via saturation of the benzene nucleus and subsequent hydrolytic cleavage (Dutton and Evans 1969; Guyer and Hegeman 1969; Whittle et al. 1976). A similar pathway is used by *Pseudomonas sp.* and *Moraxella sp.* in anaerobic, nitrate-dependent benzoate degradation (Taylor and Heeb 1972; Williams and Evans 1975) and is probably also active in benzoate degradation by sulfate-reducing bacteria (Widdel 1980). In the absence of external electron acceptors or light, methanogenic degradation of benzoate is possible and proceeds again via ring saturation (Fina et al. 1978; Keith et al. 1978); however, degradation was so far only observed in obligately syntrophic microbiologically undefined mixed cultures (Ferry and Wolfe 1976). The latter is also true for the anaerobic degradation of hydroxylated and methoxylated benzoic acid compounds derived from lignin (Healy and Young 1978, 1979); degradation via saturation of the aromatic ring is again assumed (Healy et al. 1980).

Anaerobic photoassimilation of phloroglucinol by pure cultures of *Rhodopseudomonas gelatinosa* was studied in detail (Whittle et al. 1976; Evans 1977). The degradation mechanism includes an initial NADPH-dependent reduction to dihydrophloroglucinol followed by enol-ketone-tautomerization, saturative hydratization and subsequent hydrolytic cleavage into pyruvate and a malonyl residue which both are assimilated into the intermediary metabolism.

Fermentative degradation of phloroglucinol was reported for pure cultures of *Streptococcus bovis* and *Coprococcus sp.* (Tsai and Jones 1975; Tsai et al. 1976). Ring cleavage started again with a NADPH-dependent reduction to dihydrophloroglucinol and followed probably the mechanism observed with *R. gelatinosa* (Patel et al. 1981). However, the fermentation balance given (2 mol acetate and 2 mol CO_2 per mol of phloroglucinol; Tsai et al. 1976) is not equilibrated since 8 electrons are not accounted for.

In the present paper we report on a new bacterium which was able to degrade four trihydroxybenzene isomers in defined mineral medium by fermentation to acetate (3 mol/mol of substrate). To our knowledge, this is the first report on a completely fermentative degradation of phenolic compounds in pure cultures of bacteria. No substrates other than gallic acid, pyrogallol, phloroglucinol, and 2,4,6-trihydroxybenzoic acid were degraded, neither in pure culture nor in mixed culture with a sulfate-reducing bacterium. Pyrogallol and phloroglucinol are on the same redox level as sugars or acetic acid, and gallic acid and 2,4,6-trihydroxybenzoic acid should be easily convertible to pyrogallol and phloroglucinol by decarboxylation.

Only speculations are so far possible on the mechanism of degradation of the substrates utilized. Using the methods of Patel et al. (1981) we were able to measure with strain Ma Gal 2 a NADPH-dependent reduction of phloroglucinol with simultaneous increase of absorption at 278 nm. This indicated the formation of dihydrophloroglucinol and suggests the same initial step in phloroglucinol degradation to occur in our organism as observed in *R. gelatinosa* and *Coprococcus sp.* However, this reaction was not observed with pyrogallol, gallic acid, or 2,4,6-trihydroxybenzoic acid as electron acceptors. Either these substrates are degraded by a different pathway or they have first to be transformed to phloroglucinol in a reaction not detectable under our assay conditions. In the case of pyrogallol, this transformation would need a para-transhydroxylation, either intra- or intermolecularly, a reaction not yet observed to occur in nature. It is of interest in this context that the third trihydroxybenzene isomer, hydroxyhydroquinone, which is not degraded by our isolates, cannot be altered by a para-transhydroxylation. On the other hand, this is the only trihydroxybenzene isomer not occuring in nature and thus no evolutionary pressure existed to develop a degradative system for it.

The high yield of cell dry matter (10 g/mol) obtained with our isolates on all substrates utilized suggests basically similar degradation pathways for all of them. Since yields on mineral media with acetate as carbon source are usually around 10 g/Mol ATP (Stouthamer 1979) the net yield of ATP in our case is probably in the range of 1 – 2 mol ATP/mol substrate.

Ecological Considerations

The question arises how an organism so specialized on a few "unusual" substrates can survive in nature and is present in the mud in modest numbers. Lignin as the main source of aromatic derivatives in nature is not degraded in its native state under anaerobic conditions (Zeikus 1980). Thus, only a small amount of monomers released during aerobic lignin depolymerization may reach anaerobic muds. It is of importance that during aerobic degradation the methoxyl groups are released only late after depolymerization (Donnelly et al. 1981). So the hydroxyl groups are protected from oxidation and phenol radical-catalyzed polymerization in aerobic environments. Oxidation products of e.g. pyrogallol or gallic acid were not metabolized by our organisms; if oxidized brownish substrate stock solutions were used for growth experiments only the "colourless" substrate monomers were fermented and the yellowish color remained unchanged. In the anoxic mud a metabiotic association of *Acetobacterium woodii* and our isolates may be able to demethoxylate and completely ferment the monomeric compounds. Acetate as the only fermentation product can then be easily converted to methane as shown in our experiments. A mixed culture system of *A. woodii*, our isolates, and *M. barkeri* or *M. soehngenii* would transform methoxylated gallic acid derivatives completely to methane and CO_2 and thus could be the constituents of the undefined culture systems studied by Healy and Young 1978, 1979; Healy et al. 1980.

Low molecular weight phenols are important constituents of plant tissue, either as precursors of more complex phenolic compounds (lignins, phytoalexins) or in glycosylated or methoxylated form as easy-to-release antibacterial protectants (Pridham 1965). Dead plant material contains considerable amounts of these substances. Moreover, at least phloroglucinol is an important intermediary product of anaerobic degradation of bioflavonoids (Simpson et al. 1969), and pyrogallol is formed during aerobic resorcinol degradation (Groseclose and Ribbons 1981); however, the ecological importance of the latter process for anoxic environments has still to be examined.

Taxonomy

The new isolates irrespective of their origin and salt requirements are similar with respect to substrate utilization, physiology, and cytological characteristics. As obligately anaerobic, Gram-negative, non-sporeforming rods they should be assigned to the Family Bacteroidaceae (Buchanan and Gibbons 1974). Due to their unique metabolism, our isolates cannot be assigned to any of the existing genera of the Bacteriodaceae (*Bacteroides*, *Fusobacterium* of *Leptotrichia*) which all ferment carbohydrates and produce a complex mixture of butyrate, lactate and other organic acids. For the same reason, assignment to the affiliated genera *Butyrivibrio*, *Succinivibrio*, *Succinomonas*, *Lachnospira*, and *Selenomonas* is not possible either. It appears necessary, therefore, to establish a new genus in the family Bacteroidaceae for our metabolically unusual, Gram-negative rods. We propose the name *Pelobacter acidigallici* for the new trihydroxybenzene derivatives-fermenting bacteria.

Genus Pelobacter gen. nov. Pe. lo. bac'ter. Gr. masc. n. *pelos* mud; *bacter* masc. equivalent of Gr. fem. n. *bacteria* rod or staff; M. L. masc. n. *Pelobacter* a mud-inhabiting rod.

Rod-shaped cells with rounded ends, single, in pairs or in chains. Motile forms occur. Gram-negative. Endospores not formed.

Chemoorganotrophic, metabolism fermentative. Strict anaerobes. Carbohydrates are not utilized. Media containing a reductant are necessary for growth; marine forms require NaCl concentrations higher than 1%.

Habitats: anaerobic muds of limnic or marine origin.

Pelobacter acidigallici sp. nov. a.ci.di.gal' li.ci. M. L. neutr. n. *acidum gallicum* gallic acid, gen. *acidigallici* of gallic acid.

Rod-shaped cells, 0.5–0.8 by 1.5–3.5 µm in size, with rounded ends, single, in pairs or in chains. Motile in young cultures, however, motility may be lost early. No spore formation. Gram-negative.

Strictly anaerobic chemoorganotroph. Gallic acid, pyrogallol, 2,4,6-trihydroxybenzoic acid, and phloroglucinol are the only fermentable substrates, and acetate and CO_2 are the only fermentation products. No other phenolic compounds, fatty acids, dicarbocylic acids, or alcohols metabolized. Sulfate, sulfur, or nitrate not reduced. Growth requires mineral media with a reductant. The marine type strain requires at least 10 g NaCl and 1.5 g Mg Cl_2 per l, whereas freshwater isolates do not need enhanced salt concentrations. No growth factors or vitamins are needed. Indole not formed, gelatine or urea not hydrolyzed. Selective enrichment with gallic acid or phloroglucinol as substrates.

pH range: 5.3–8.2, optimum at 6.5–7.0,
Temperatur range: 10°C–37°C, optimum at 35°C.
No cytochrome detectable.
DNA base ratio: 51.8% G + C (thermal denaturation)
Habitats: anaerobic muds of freshwater or marine origin.
Type strain: Ma Gal 2, DSM 2377, deposited in: Deutsche Sammlung von Mikroorganismen, Göttingen.

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