Arch Microbiol (1986) 144:8–14

Archives of
Microbiology

Eubacterium oxidoreducens sp. nov. requiring H_2 or formate to degrade gallate, pyrogallol, phloroglucinol and quercetin

Lee R. Krumholz[1] and M. P. Bryant[1,2]

Departments of [1]Dairy Science and [2]Microbiology, University of Illinois, Urbana, IL 61801, USA

Abstract. Based on most probable number (MPN) estimates of rumen fluid from a hay-fed steer, 10 mM gallate was decarboxylated by 9.3×10^6 bacteria per ml. It was decarboxylated and reductively dehydroxylated by 9.3×10^5 bacteria per ml and was further catabolized to non-aromatic products by 4.3×10^3 bacteria per ml. Resorcinol was not further degraded and, with 0.1 ml of inoculum, catechol was not degraded. Strain G41 was isolated from a pyrogallol-medium roll tube inoculated with 1 µl of rumen fluid and, with slight modifications of the generic description, was named *Eubacterium oxidoreducens* sp. nov. It was an anaerobic, nonmotile, curved, Gram-positive, small rod with rounded ends and required H_2 or formate to degrade gallate, pyrogallol, phloroglucinol or quercetin to acetate and butyrate and, in the case of quercetin, to 3,4-dihydroxyphenylacetate. Crotonate was catabolized to acetate and butyrate and no electron donor was required. No other compounds were degraded with or without an electron donor or with *Desulfovibrio* sp. plus sulfate as a possible electron acceptor system. *E. oxidoreducens* grew well in a chemically-defined culture medium containing usable energy source, minerals, B-vitamins, cysteine and CO_2-HCO_3^- buffer, pH 7.2.

Key words: Anaerobic gallate degrader – Quercetin – Rumen – Butyrate production – H_2:CO_2 – Crotonate – *Eubacterium oxidoreducens*

Trihydroxybenzenoids such as gallate and phloroglucinol are present in free or combined form in a large variety of compounds within the plant kingdom. These include flavonoids, tannins, lignin precursors and their intermediate degradation products. As a result they are common constituents of the ruminant diet. There has been recent interest in the products of flavonoid catabolism in the gut ecosystem due to their potent mutagenicity (Hardigree and Epler 1978; Hirono et al. 1981).

Bacteria, isolated from the rumen, which degrade phloroglucinol include *Streptococcus bovis* and *Coprococcus* sp. (Tsai and Jones 1975). The major products of phloroglucinol catabolism are acetate and carbon dioxide. *Coprococcus* sp. at least partially degrades the flavonols quercetin and rhamnetin (Tsai et al. 1976).

Offprint requests to: M. P. Bryant

Abbreviations. G + C, Guanine plus cytosine; MPN, most probable number; OD, optical density; TLC, thin layer chromatography

A recently described species, *Pelobacter acidigallici* (Schink and Pfennig 1982), has been isolated from sewage and anaerobic aquatic sediments, and it degrades a number of trihydroxybenzenoids with acetate as the major product. An organism as versatile as this has not been observed in the rumen.

We report here the isolation and features of the predominant rumen bacterium catabolizing gallate and pyrogallol.

Materials and methods

Anaerobic methods and media. The anaerobic techniques were those of Hungate (1969) as modified by Bryant (1972) except in cases where changes in the gas phase were monitored. For this purpose serum tubes were used (Balch and Wolfe 1976) with incubations on a shaker.

Basal medium containing 5% (v/v) rumen fluid was prepared with 4:1 N_2:CO_2 gas phase as described by Genthner et al. (1981) except that the vitamin solution contained (per l), 200 mg each of lipoic acid, Ca-D-pantothenate, thiamine-HCl, nicotinamide, riboflavin, pyridoxine-HCl and 10 mg each of *p*-aminobenzoic acid, biotin, folic acid and cyanocobalamin. Basal medium used for pure cultures contained 0.2% Casitone (Difco) and lacked rumen fluid.

Benzenoids and tannic acid were filter sterilized except where indicated. Flavonoids were autoclaved in the medium. No effect on the flavonoids was observed due to autoclaving.

All incubations were at 39° C unless otherwise indicated.

Isolation of pure cultures were done as previously described (Genthner et al. 1981) with pyrogallol as the energy source. They were maintained in stabbed slants of basal medium with 30-mM sodium gallate, 30-mM sodium formate and 1% Bacto agar (Difco) added. When slants were maintained at room temperature after growth, weekly transfer was required for survival of the culture. For long-term storage, cultures were frozen after the addition of glycerol to 20% final concentration (Teather 1982).

Oxygen sensitivity was determined in crotonate stab slants as previously described (Bryant and Burkey 1953).

Most probable number (MPN) estimations. Three-tube MPN estimations were as described by Genthner et al. (1981) with the addition of 20 mM acetate and 100 µM each *iso*butyrate, valerate, *iso*valerate and DL-2-methylbutyrate. Positive results were based on growth above that in tubes lacking substrate and on substrate disappearance.

Inoculum for pyrogallol and catechol MPN cultures was rumen contents collected from a steer fed a diet of 30% grain

and 70% forage and then strained through cheese cloth. Gallate MPN's were inoculated with contents from a steer fed 100% bromegrass-alfalfa hay.

Nutrition and growth parameters. All nutritional requirements were determined by monitoring growth on basal medium minus rumen fluid and containing 30 mM each sodium gallate and formate which were autoclaved with the medium. Partial conversion of the gallate to pyrogallol was observed on autoclaving but this did not influence growth. The requirement for CO_2 was determined by comparing growth rates on the above medium with increasing levels of $NaHCO_3^-$ between 0 and 83 mM. The pH was adjusted to 7.0 by the addition or deletion of appropriate levels of CO_2 to the gas phase. The temperature range was determined by comparing growth rates at temperatures between 21 and 50° C at 2 to 3° C intervals. The pH range was determined in a similar manner in basal medium by adjusting the pH from 6.1 to 7.8 with $NaHCO_3$. The bicarbonate level could not be adjusted any lower than 17 mM because of a bicarbonate requirement of the isolates. Capacity to use different energy sources was as described (Genthner et al. 1981).

The test for arginine deamination was done in peptone-yeast extract medium (Holdeman et al. 1977) containing formate and gallate. Ammonia was determined with the phenol hypochlorite method (Chaney and Marback 1962). The basal medium with 6% gelatin added was used for the gelatin liquefaction test. The test for nitrate reduction was done in basal medium containing 20-mM $NaNO_3$ along with 5-mM gallate and 30-mM formate. Nitrite formation was determined qualitatively as described by Barnes and Folkard (1951).

Analytical methods. Thin layer chromatography (TLC) was used to identify end products of anaerobic benzenoid metabolism and as a means of qualitatively measuring their disappearance. Cellulose coated TLC plates were used (Macherey, Nagel & Co.) with two solvent systems, 20% aqueous KCl and benzene: glacial acetic acid: water (2:3:1). Benzenoids were extracted from the culture supernatant fluids with ethyl acetate and then run on TLC plates. Spots were visualized by spraying with a 1% methanolic $FeCl_3$ solution to color ortho-, di- and vicinal trihydroxybenzenoids and diazotized *p*-nitroaniline to color meta, di- and trihydroxybenzenoids. Representative R_f values for a number of benzenoids with the above solvents have been published (Jangaard 1979; Humbel 1970).

The ultraviolet spectra of culture extracts were determined by diluting extracts to about 50 µM benzenoid with water and scanning between 350 and 205 nm with a Varian model 635 or a Gilford Response spectrophotometer.

Gallic acid and pyrogallol were determined by the molybdate method with samples containing between 75 and 1,500 nmol benzenoid (Swain and Goldstein 1964). Phloroglucinol, resorcinol and pyrogallol were determined by the vanillin method with samples containing between 2.5 and 25-nmol benzenoid. To quantitate the amounts of two of the above compounds in a single culture, both procedures were used; and individual concentrations were determined by subtraction based on relative extinction coefficients. Quercetin was determined by the formation of a colored complex with aluminum chloride (Dowd 1959).

Growth was determined by measuring changes in optical density (OD) at 600 nm (13 × 100 mm tubes) in a Bausch and Lomb Spectronic 70. Generation times were calculated from the slope of the linear portion of the growth curve, with OD values above 0.3 corrected for deviation from Beer's law (Toennies and Gallant 1949).

Production or use of CO_2 or other gases was determined as follows: 10 ml cultures in serum tubes were acidified with 1 ml of 30% phosphoric acid. Total gas volume was determined by displacement of the plunger of a glass piston syringe. Gases were analyzed by gas chromatography (McInerney et al. 1979) and CO_2 levels were corrected for solubility (Umbreit et al. 1951).

Volatile fatty acids, lactate and succinate were determined by analysis of butyl esters by gas chromatography (Salanitro and Muirhead 1975) and methanol, ethanol, and crotonate were determined by gas chromatography (Genthner et al. 1981).

Protein content was determined by the Lowry procedure (Lowry et al. 1951) after solubilization of washed cell material by boiling it in 0.1 M NaOH for 10 min. Bovine serum albumin was the standard.

Fermentation balances were calculated as previously described (Mountfort and Bryant 1982).

DNA was purified from 1 l of log phase culture by the method of Breznak and Canale-Parola (1975), except that 50 µg of pronase per ml was added to the sodium deoxycholate mixture which was then incubated for 2 h at 40° C.

The percent guanine plus cytosine of the DNA (G + C) was determined by the thermal denaturation method of Mandel and Marmur (1968) using a Beckman DU-8 spectrophotometer and calculated with the equation of DeLey (1970).

Electron microscopy. Mid-exponential growth phase cells were prepared for negative staining and thin sectioning as described by Murray et al. (1983), except that specimens were dehydrated in acetone rather than ethanol. Cells were treated with polymyxin B and then negatively stained with 3% uranyl acetate to determine the Gram type (Wiegel and Quandt 1982). Electron micrographs were taken on a JOEL 100C electron microscope at 80 kV and recorded on Kodak 4489 electron image film.

Results

Enumeration. Pyrogallol (5 mM) MPN's monitored for growth with substrate versus growth with no added substrate indicated 460 cells per ml of rumen fluid and 1,500 cells per ml of sewage anaerobic digester sludge. Where growth occurred in pyrogallol MPN cultures, it was initiated within about 4 days. Catechol (5 mM) MPN's using rumen fluid inoculum indicated no growth and no degradation of catechol even with 0.1 ml of rumen fluid incubated for 30 days.

With gallate (10 mM) MPN's inoculated with rumen fluid and incubated for 40 days (Table 1), 9.3×10^6 bacteria per ml decarboxylated gallate with either pyrogallol or resorcinol or both being produced. Some resorcinol was produced from gallate by 9.3×10^5 bacteria per ml and 4.3×10^3 converted gallate completely to non-benzenoid products.

Isolation of G41. A 5-mM pyrogallol MPN tube culture with inoculum of 1-µl rumen fluid was transferred several times at 2 to 3 day intervals. This enrichment was serially diluted

Table 1. Concentration (mM) of individual benzenoids in rumen 10 mM gallate MPN culture supernatants. Samples were quantitated after an incubation period of 40 days

Benzenoid	Tube	Concentration at each dilution (−log)						
		2	3	4	5	6	7	8
Gallate	1	−[a]	−	−	−	−	8.57	9.53
	2	−	−	−	−	−	−	9.57
	3	−	−	−	−	−	10.01	9.38
Pyrogallol	1	−	−	4.23	2.38	9.29	−	−
	2	−	−	2.57	8.22	−	9.43	−
	3	−	−	4.07	5.08	7.16	−	−
Resorcinol	1	−	−	4.36	6.29	−	−	−
	2	−	2.86	4.56	1.60	8.88	−	−
	3	−	−	4.48	4.25	1.84	−	−

[a] Less than 0.2 mM detected were indicated as −

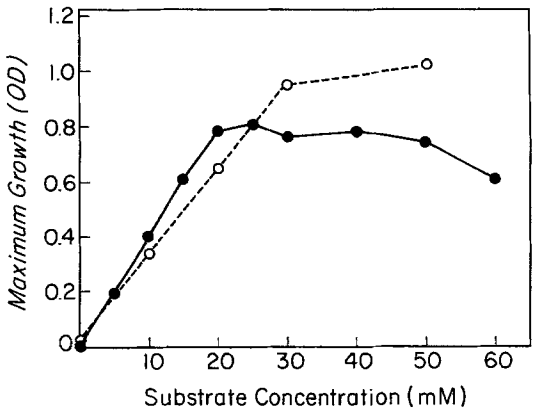

Fig. 1. Maximum growth of G41 with different levels of formate (○-----○) and gallate (●-----●). Media contained 40 mM gallate and 25 mM formate, respectively

and inoculated into pyrogallol roll tubes. After about three-weeks incubation, isolated colonies were picked to pyrogallol liquid medium but only one degraded pyrogallol and it contained a variety of morphotypes. After several repeated isolation attempts from pyrogallol roll tubes, strains G41 and G44 were isolated in pure culture. Strain G44 produced the same amount of growth in the enrichment medium with or without pyrogallol. Strain G41 exhibited only slightly visible growth in stabbed slants with pyrogallol and no visible growth in liquid pyrogallol enrichment medium. Prior to isolation, the G41 morphotype exhibited relatively large (3 mm), smooth, entire colonies only when closely associated with colonies of G44 in pyrogallol roll tubes. This suggested a metabolic interaction between the two strains.

Subsequent studies showed that G41, grown in medium containing 5-mM pyrogallol and autoclaved supernatant fluids of either anaerobically glucose-grown G44 or *Escherichia coli*, grew very well and rapidly degraded the pyrogallol. Formate or H_2, but not lactate, succinate, methanol, ethanol, 30% (v/v) of sterile rumen fluid, or 0.2% yeast extract, replaced the G44 or *E. coli* culture supernatant fluid.

G41 grew on and catabolized pyrogallol, gallate, and phloroglucinol in the presence of formate or H_2 and produced mainly acetate, butyrate and CO_2 (Fig. 1, Table 2). No other carboxylic acids, nor ethanol or methanol were detected. Pyrogallol, at a concentration of 10 mM or above, inhibited growth (generation time 6.4 h compared to 2.9 h at 5 mM) and little growth was observed at 20 mM or above. Gallate and phloroglucinol were not inhibitory up to 50 mM with 50-mM formate.

Crotonate was used as sole energy source without a formate or H_2 requirement. Acetate and butyrate were the major products. At levels of crotonate below about 50 mM, the growth rate was depressed. Growth yields (OD) were linear with crotonate concentration up to at least 50 mM and increases in the amount of growth were observed up to 150-mM crotonate.

Table 2. Fermentation balances, cell protein yield and minimum generation times for growth of G41 on selected substrates

	Substrate						
	Crotonate	Pyrogallol		Gallate		Phloroglucinol	
Main energy source used (mM)	28.8[a]	5.0	4.9 9.9	10.0 20.0	10.0 17.0	10.0 19.3	9.9 18.9
Mol/100 mol main energy source							
Formate used	−	94.0	−	102	−	80.1	−
H_2 used	−	−	153	−	128	−	132
Products formed							
Acetate	90.2	154.0	158.0	149.9	161.1	139.5	135.8
Butyrate	27.5	21.6	21.7	24.9	27.0	23.4	25.7
H_2	0.4	1.6	−	1.2	−	1.4	−
CO_2	3.5	138	51.0	200.0	105	99	23
C Recovery (%)[b]	79	85	85	82	82	76	74
H Recovery (%)[b,c]	76	74	71	75	75	69	67
Y protein (g/mol main energy source used)	3.13	7.98	7.24	7.69	5.04	5.53	6.06
Generation time (h)	8.4	3.9	2.9	1.8	3.2	2.4	2.0

[a] All values are means of triplicate samples at one or two different concentrations

[b] Determined by the method of Barker (1936) and corrected for cell carbon based on the assumption that the cells are 50% protein and the cell composition is $C_{4.86}H_{8.85}O_{2.41}N$ and 7.4% ash (Lynd et al. 1982)

[c] Corrected for cellular electron equivalents (Harris and Adams 1979)

G41 grew slowly with 20-mM quercetin as energy source but only with formate added (H_2 not studied). Quercetin is highly insoluble; therefore, growth was determined by end product production or chemical analysis of quercetin disappearance. After 9 days, 4.3-mM acetate, 1.4-mM butyrate and significant levels of 3,4-dihydroxyphenylacetate were detected. With 5-mM quercetin and 30-mM formate present, 3-mM quercetin was degraded within 7 days and degradation continued until almost all of the quercetin was broken down (about 3 weeks).

Photomicrographs of G41 are shown in Figs. 2 and 3.

Data showing the requirements for small amounts of CO_2 and bicarbonate are shown in Table 3.

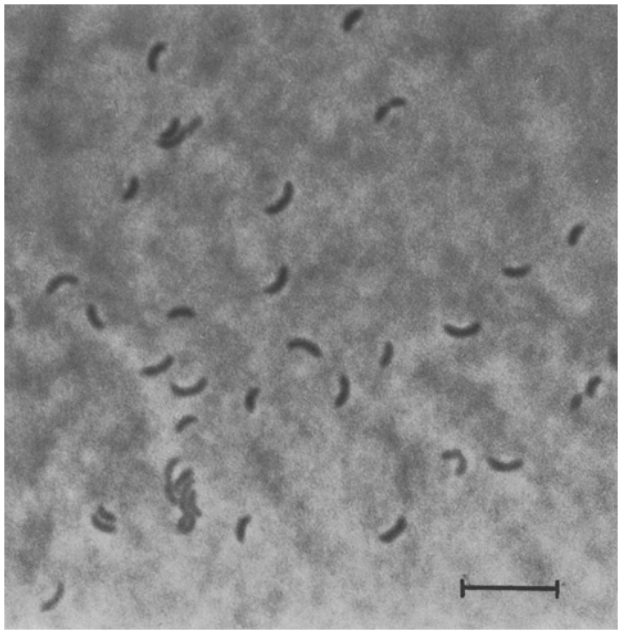

Fig. 2. Phase contrast micrograph of strain G41. Bar equal 5 μm

Table 3. Specific growth rate of strain G41 grown in the gallate-formate medium with different levels of CO_2 and bicarbonate present in the medium

$NaHCO_3$ level (mM)	% CO_2 in gas phase	Initial pH	Specific growth rate (h^{-1})
0	0	7.40	0*
5	4	7.27	[a]
11	9	7.20	0.081**
17	14	7.10	0.127** ***
23	18	7.09	0.136** ***
29	23	7.15	0.169***
42	34	7.10	0.124** ***
83	66	7.07	0.155***

[a] The growth rate was not determined but slow growth did occur
* ** *** Values with identical superscripts are not significantly different ($P < 0.05$) as determined by the F test

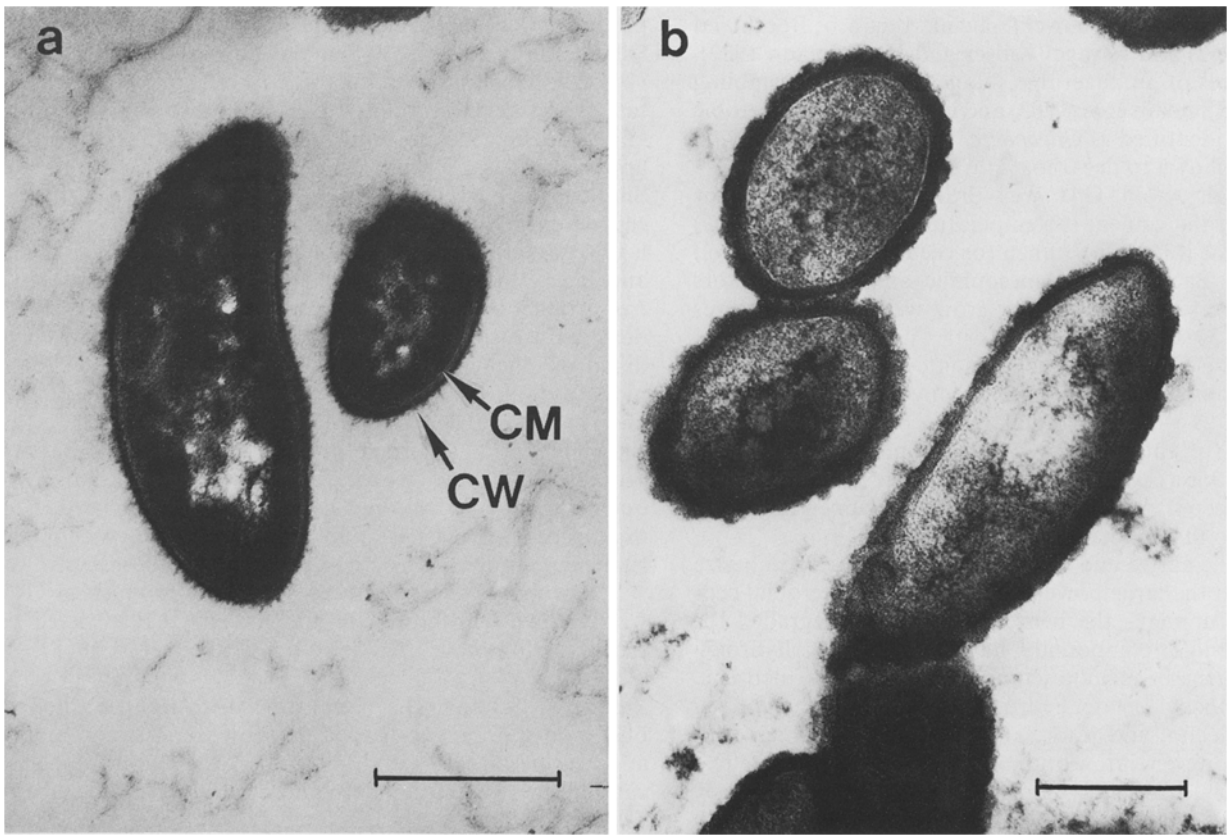

Fig. 3a,b. Electron micrographs of thin sections of strain G41. **a** Note the thin Gram-positive type cell wall (*CW*) with cytoplasmic membrane (*CM*) resolved. **b** These cells were stained with ruthenium red. The outer layer exhibits amorphous material, presumably allowing cells to stick together in culture. Bars equal 250 nm

Table 4. Theoretical stoichiometric equations, change in free energies and growth yields for catabolism of energy sources by strain G41 and other bacteria carrying out similar reactions

Reaction	ΔG^0 (kJ)	Molar Y g cells
1. Gallate$^-$ + 4 $H_2O \rightleftharpoons$ 3 acetate$^-$ + HCO_3^- + 3 H^+ [a]	−160 [c]	10.1 [b]
2. Gallate$^-$ + H_2 + 3 $H_2O \rightleftharpoons$ 2 acetate$^-$ + 0.5 butyrate$^-$ + HCO_3^- + 2.5 H^+ [a]	−184 [c,g]	10−15 [a,f]
3. Pyrogallol + 3 $H_2O \rightleftharpoons$ 3 acetate$^-$ + 3 H^+ [b]	−159 [c]	9.9 [b]
4. Pyrogallol + H_2 + 2 $H_2O \rightleftharpoons$ 2 acetate$^-$ + 0.5 butyrate$^-$ + 2.5 H^+ [a]	−183 [c,g]	14−16 [a,f]
5. Phloroglucinol + 3 $H_2O \rightleftharpoons$ 3 acetate$^-$ + H^+ [b]	−159 [c]	9.9 [b]
6. Phloroglucinol + H_2 + 2 $H_2O \rightleftharpoons$ 2 acetate + 0.5 butyrate$^-$ + 2.5 H^+ [a]	−183 [f,g]	11−12 [a,f]
7. Quercetin + H_2 + 5 $H_2O \rightleftharpoons$ 2 acetate$^-$ + 0.5 butyrate$^-$ + HCO_3^- + 3,4-dihydroxyphenylacetate$^-$ + 4.5 H^+ [e]	−	−
8. Crotonate$^-$ + $H_2O \rightleftharpoons$ acetate$^-$ + 0.5 butyrate$^-$ + 0.5 H^+ [a,d]	− 51 [g]	6.3 [a,f] 4.8 [d]

[a] See data Table 5
[b] Schink and Pfennig 1982
[c] Kaiser and Hanselmann 1982
[d] Thauer et al. 1968
[e] See text
[f] Assuming cells are 50% protein
[g] Thauer et al. 1977

Discussion

Strain G41 appeared to be the predominant organism (4.3×10^3 per ml) in the rumen capable of anaerobic gallate and pyrogallol degradation to non-aromatic products. Based on the MPN estimations, there were unknown species capable of decarboxylation and reductive dehydroxylation of gallate to resorcinol. Anaerobic decarboxylation of phenolic acids has been previously documented for bacteria in the cecum and large bowel (Scheline 1966a, b; Booth and William 1963) and sewage (Kaiser and Hanselmann 1982). Pure cultures of an anaerobic sludge bacterium resembling *Pelobacter* (Samain et al. 1983) and a facultatively anaerobic soil isolate identified as *Citrobacter* sp. (Yoshida et al. 1982) have been shown to decarboxylate phenolic acids.

Although strain G41 was present in relatively low numbers in the rumen, its temperature range (30 to 43° C) suggests that it is a true rumen (or cecum and large bowel) bacterium. Bacteria from mesophilic anaerobic digestors and aquatic sediments usually grow well at temperatures well below 30° C.

It is not clear whether the main fate of gallate within the rumen is complete degradation or conversion to resorcinol. Resorcinol as well as pyrogallol have been observed after incubation of rat cecal or colonic contents with gallate (Scheline 1966a). Also, these products are observed in the urine of sheep if gallate is infused into the rumen but not if infused into the abomasum (Martin 1982).

From the above and other information, we hypothesize that, within the large bowel, cecal or rumen microbial ecosystems of animals, the benzene ring is not degraded for monobenzenoid compounds having 2 or less hydroxyl functions (these include compounds such as benzene, benzoate, phenylacetate, hydrocinnamate, protocatechuate, phenol, catechol and resorcinol). Even in the rumen with relatively long retention times of 50 to 60 h for forage residues and 8 to 24 h for fluids (Hungate 1966; McInerney et al. 1979), bacteria degrading the benzene ring of such compounds are likely to be washed out. These bacteria, have long generation times, and have not been successfully enriched from 10% inoculum of bovine rumen fluid or from other gastrointestinal tract ecosystems (Barik et al. 1985; Mountfort and Bryant 1982).

To our knowledge, a requirement for exogenous electron donors such as H_2 or formate (in approximately a 1:1 ratio) for catabolism of certain organic compounds has not been previously reported and Strain G41 differed from trihydroxybenzenoid-degrading bacteria heretofore studied in this aspect and in production of both acetate and butyrate. These other bacteria produce acetate as the only organic product and do not require H_2 or formate (Tsai et al. 1976; Schink and Pfennig 1982; Samain et al. 1983) (Table 4).

On administration of quercetin or related compounds to rats or humans, flavonoids are broken down with a number of phenolic acids, including 3,4-dihydroxyphenylacetate, appearing in the urine (Booth et al. 1956; Griffiths and Smith 1972; Baba et al. 1981). This catabolism is probably carried out by the bacterial flora as little or no degradation is observed in germ-free or antibiotic-treated rats (Griffiths and Barrow 1972; Nakagawa et al. 1965). Mixed bacteria from rumen contents also degrade quercetin and products detected are similar or identical to protocatechuate, 3,4-dihydroxyphenylacetate and 3,4-dihydroxyphenylpropionate (Simpson et al. 1969). A rumen *Coprococcus* sp. (Tsai et al. 1976) degrades both quercetin and rhamnetin, as shown by partial clearing of the medium during incubation, but does not need H_2 or formate. The degradation of quercetin observed during in vivo studies may be due to organisms such as G41 and *Coprococcus* sp. within the rumen and other anaerobic gastrointestinal ecosystems.

Clostridium kluyveri produces the same products as G41 when grown with crotonate and the enzymes involved in these reactions have been described (Thauer et al. 1968).

Taxonomy. Strain G41 is a Gram-positive, obligately anaerobic, non-sporing rod-shaped bacterium and is non-motile and produces acetate and butyrate as the end products of catabolism. Therefore, it might be placed in the genus *Eubacterium* (Moore and Holdeman 1985). However, based on its inability to grow with carbohydrates or in peptone-yeast extract broth, and requirement for formate or H_2 for growth, except with crotonate, it does not fit into any of the

described species of *Eubacterium*. We place G41 in the genus *Eubacterium*. This presently requires only a slight change in the genus description (Moore and Holdeman 1985) to include some species that use crotonate and trihydroxybenzenoids plus formate as energy source but do not use sugars, lactate, amino acids, or peptides. The new species is described below:

Eubacterium oxidoreducens sp. nov. ox.i.do.re.du' cens. *oxido* combining form of modern chemical term, oxide, L. part. adj. *reducens* reducing; N. L. adj. *oxidoreducens* reducing compounds (containing) oxygen.

Rod-shaped curved cells, 0.45 by 1.5–2.2 μm in size, with rounded ends, singles, or in pairs or in small clumps. Nonmotile. No spore formation (pasteurized cultures are not viable). Gram positive.

Strictly anaerobic chemoorganotroph. Requires formate or hydrogen as electron donor to catabolize approximately equimolar gallate, pyrogallol, phloroglucinol, or quercetin to acetate, butyrate, and sometimes CO_2. No exogenous electron donor is required for catabolism (fermentation) of crotonate (growth rate much faster with 60 mM than with 30 mM or less) to acetate and butyrate. No other compounds are used as energy sources with or without formate present. These include rutin, hesperidin, monobenzenoids with or without methoxyl groups, fatty acids, citrate, acrylate, lactate, pyruvate, dicarboxylic acids, alcohols, sugars, amino acids and peptides. It also does not grow in co-culture with *Desulfovibrio* sp. (with sulfate and with or without formate) with butyrate, protocatechuate, 3,5-dihydroxybenzoate, benzoate, phenol, 4-hydroxybenzoate, hydroquinone or caffeate as substrate. Sulfate does not serve as a dissimilatory electron acceptor with formate in the medium. Nitrate is not reduced. It grows well in defined medium containing utilizable energy sources, minerals, including NH_4Cl, CO_2-bicarbonate (required), B-vitamins, sulfide and cysteine. Gelatin is not hydrolyzed, ammonia is not produced from arginine or Casitone, but ammonia is essential as the main nitrogen source.

pH range: 6.9 to 7.8, optimum at 7.4.

Temperature range: 30 to 43°C, optimum 39 to 41°C.

DNA base ratio: 35.7% G + C (thermal denaturation).

Habitat: Rumen of cattle.

Type strain: G41 (= DSM 3217). DSM Göttingen.

Acknowledgements. We thank R. B. Hespell for pertinent discussions, I. C. Gunsalus for use of the DU8 spectrophotometer, Thomas McAdoo and L. V. Holdeman for the proposed nomenclature and the Center for Electron Microscopy of the University of Illinois for use of their facilities. The research was supported by the US Department of Energy contract no. DE-AC02-81ER10874, the Agricultural Experiment Station, University of Illinois, Urbana-Champaign, and L. R. K. was supported by a scholarship from the Natural Sciences and Engineering Research Council of Canada.

References

Baba S, Furuta T, Horie M, Nakagawa H (1981) Studies on drug metabolism by the use of isotopes. XXVI. Determination of the urinary metabolites of rutin in humans. J Pharm Sci 70:780–782

Balch WE, Wolfe RS (1976) New approach to the cultivation of methanogenic bacteria: 2-Mercaptoethanesulfonic acid (HS-CoM)-dependent growth of *Methanobacterium ruminantium* in a pressurized atmosphere. Appl Environ Microbiol 32:781–791

Barik S, Brulla WJ, Bryant MP (1985) PA-1, a versatile anaerobe obtained in pure culture, catabolizes benzenoids and other compounds in syntrophy with hydrogenotrophs, and P-2 plus *Wolinella* sp. degrades benzenoids. Appl Environ Microbiol 50:304–310

Barker HA (1936) On the fermentation of some dibasic C_4 acids by *Aerobacter aerogenes*. K Akad Wet Amsterdam Proc 39:674-683

Barnes H, Folkard AR (1951) The determination of nitrites. Analyst 76:599–603

Booth AN, Williams RT (1963) Dehydroxylation of catechol acids by intestinal contents. Biochem J 88:66P–67P

Booth AN, Murray CW, Jones FT, DeEds F (1956) The metabolic fate of rutin and quercetin in the animal body. J Biol Chem 223:251–257

Breznak JA, Canale-Parola E (1975) Morphology and physiology of *Spirochaeta aurantia* strains isolated from aquatic habitats. Arch Microbiol 105:1–12

Bryant MP (1972) Commentary on the Hungate technique for culture of anaerobic bacteria. Amer J Clin Nutr 25:1324–1328

Bryant MP, Burkey LA (1953) Cultural methods and some characteristics of some of the more numerous groups of bacteria in the bovine rumen. J Dairy Sci 36:205–217

Chaney AL, Marbach EP (1962) Modified reagents for determination of urea and ammonia. Clin Chem 8:130–132

DeLey J (1970) Reexamination of the association between melting point, buoyant density and chemical base composition of deoxyribonucleic acid. J Bacteriol 101:738–754

Dowd LE (1959) Spectrophotometric determination of quercetin. Anal Chem 31:1184–1187

Genthner BRS, Davis CL, Bryant MP (1981) Features of rumen and sewage sludge strains of *Eubacterium limosum*, a methanol- and H_2-CO_2-utilizing species. Appl Environ Microbiol 42:12-19

Griffiths LA, Barrow A (1972) Metabolism of flavonoid compounds in germ-free rats. Biochem J 130:1161–1162

Griffiths LA, Smith GE (1972) Metabolism of myricetin and related compounds in the rat: metabolite formation in vivo and by the intestinal microflora in vitro. Biochem J 130:141–151

Hardigree AA, Epler JL (1978) Comparative mutagenesis of plant flavonoids in microbial systems. Mutation Res 58:231–239

Harris RF, Adams SS (1979) Determination of the carbon and electron composition of microbial cells and metabolites by dichromate oxidation. Appl Environ Microbiol 37:237–243

Hirono I, Ueno I, Hosaka S, Takanashi H, Matsushima T, Sugimura T, Natori S (1981) Carcinogenicity examination of quercetin and rutin in ACI rats. Cancer Lett 13:15–21

Holdeman LV, Cato EP, Moore WEC (1977) Anaerobe laboratory manual, 4th ed, Virginia Polytechnic Institute and State University, Blacksburg, Virginia

Humbel R (1970) La chromatographie sur couche mince des acides phenoliques urinaires, techniques et applications cliniques. Rev Roum Biochim 7:45–51

Hungate RE (1966) The rumen and its microbes. Academic Press, New York

Hungate RE (1969) A roll tube method for cultivation of strict anaerobes. In: Norris JR, Ribbons DW (eds) Methods in microbiology, vol 3B. Academic Press, Inc, New York, pp 117–132

Jangaard NO (1970) Thin-layer chromatography of some plant phenolics. J Chromatog 50:146–148

Kaiser JP, Hanselmann KW (1982) Fermentative metabolism of substituted monoaromatic compounds by a bacterial community from anaerobic sediments. Arch Microbiol 133:185–194

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the Folin phenol reagent. J Biol Chem 193:265–275

Lynd L, Kerby R, Zeikus JG (1982) Carbon monoxide metabolism of the methylotrophic acidogen *Butyribacterium methylotrophicum*. J Bacteriol 149:255–263

Mandel M, Marmur J (1968) Use of ultraviolet absorbance temperature profile for determining the guanine plus cytosine content

of DNA. In: Grossman L, Moldave K (eds) Methods in enzymology, vol 12, part B. Academic Press, Inc, New York, pp 195–206

Martin AK (1982) The origin of urinary aromatic compounds excreted by ruminants. 3. The metabolism of phenolic compounds to simple phenols. Br J Nutr 48:497–507

McInerney MJ, Bryant MP, Pfennig N (1979) Anaerobic bacterium that degrades fatty acids in syntrophic association with methanogens. Arch Microbiol 122:129–135

Moore WEC, Holdeman LV (1986) *Eubacterium*. In: Sneath P (ed) Bergey's manual of systematic bacteriology. Williams and Wilkins Co, Baltimore, MD, vol. 2 (in press)

Mountfort DO, Bryant MP (1982) Isolation and characterization of an anaerobic syntrophic benzoate-degrading bacterium from sewage sludge. Arch Microbiol 133:249–256

Murray RGE, Hall M, Thompson BG (1983) Cell division in *Deinococcus radiodurans* and a method for displaying septa. Can J Microbiol 29:1412–1423

Nakagawa Y, Shetlar MR, Wender SH (1965) Urinary products from quercetin in neomycin treated rats. Biochim Biophys Acta 97:233–241

Salanitro JP, Muirhead PA (1975) Quantitative method for gas chromatographic analysis of short chain monocarboxylic and dicarboxylic acids in fermentation media. Appl Microbiol 29:374–381

Samain E, Albagnac G, Dubourguier HC (1983) Fermentation homo-acetique des composes aromatiques trihydroxyles par une souche anaerobie stricte AG2. Pasteur Biosciences Inst Pasteur, Paris, France

Scheline RR (1966a) Decarboxylation of some phenolic acids by the rat. Acta Pharmacol Toxicol 24:275–285

Scheline RR (1966b) Decarboxylation and demethylation of some phenolic benzoic acid derivatives by rat cecal contents. J Phar Pharmac 18:664–669

Schink B, Pfennig N (1982) Fermentation of trihydroxybenzenes by *Pelobacter acidigallici* gen. nov. sp. nov., a new strictly anaerobic, non-sporeforming bacterium. Arch Microbiol 133:195–201

Simpson FJ, Jones GA, Wolin EA (1969) Anaerobic degradation of some bioflavonoids by microflora of the rumen. Can J Microbiol 15:972–974

Swain T, Goldstein JL (1964) The quantitative analysis of phenolic compounds. In: Pridham JB (ed) Methods in polyphenol chemistry. Pergamon Press, New York, pp 131–146

Teather RM (1982) Maintenance of laboratory strains of obligately anaerobic rumen bacteria. Appl Environ Microbiol 44:499–501

Thauer RK, Jungermann K, Henninger H, Wenning J, Decker K (1968) The energy metabolism of *Clostridium kluyveri*. European J Biochem 4:173–180

Thauer RK, Jungermann K, Decker K (1977) Energy conservation in chemotrophic anaerobic bacteria. Bacteriol Rev 41:100–180

Toennies G, Gallant DL (1949) The relation between photometric turbidity and bacterial concentration (bacterimetric studies IV). Growth 13:7–20

Tsai CG, Jones GA (1975) Isolation and identification of rumen bacteria capable of anaerobic phloroglucinol degradation. Can J Microbiol 21:749–801

Tsai CG, Gates DM, Ingledew WM, Jones GA (1976) Products of anaerobic phloroglucinol degradation by *Coprococcus* sp. $Pe_1 5$. Can J Microbiol 22:159–164

Umbreit WW, Burris RH, Stauffer JF (1951) Manometric techniques and tissue metabolism. Burgess Publishing Co, Minneapolis, Minnesota

Wiegel J, Quandt L (1982) Determination of the Gram type using the reaction between polymyxin B and lipopolysaccharides of the outer cell wall of whole bacteria. J Gen Microbiol 128:2261–2270

Yoshida H, Tani Y, Yamada H (1982) Isolation and identification of a pyrogallol producing bacterium from soil. Agric Biol Chem 46:2539–2546

Received February 24, 1985/ Accepted October 1, 1985