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Oxalobacter formigenes gen. nov., sp. nov.: oxalate-degrading anaerobes that inhabit the gastrointestinal tract

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Abstract. This report describes a new group of anaerobic bacteria that degrade oxalic acid. The new genus and species, *Oxalobacter formigenes*, are inhabitants of the rumen and also of the large bowel of man and other animals where their actions in destruction of oxalic acid may be of considerable importance to the host. Isolates from the rumen of a sheep, the cecum of a pig, and from human feces were all similar Gram-negative, obligately anaerobic rods, but differences between isolates in cellular fatty acid composition and in serologic reaction were noted. Measurements made with type strain OxB indicated that 1 mol of protons was consumed per mol of oxalate degraded to produce approximately 1 mol of CO_2 and 0.9 mol of formate. Substances that replaced oxalate as a growth substrate were not found.

Key words: Oxalic acid – *Oxalobacter formigenes* – Oxalate degradation – Anaerobes – Gastrointestinal bacteria – Rumen bacteria – Taxonomy

Salts of oxalic acid are widely distributed in the diets of man and animals, and ingestion of plants that contain high concentrations of oxalate may lead to intoxication. Oxalate is, however, degraded by ruminal microbes (Morris and Garcia-Rivera 1955; Watts 1957), and by mixed bacterial populations in the large intestines of other herbivores (Allison and Cook 1981; Shirley and Schmidt-Nielsen 1967). While there is evidence for oxalate degradation by bacteria in human feces (Barber and Gallimore 1940; Hagmaier et al. 1981), information concerning the nature of oxalate degraders in the human bowel or of their significance is lacking.

Dawson et al. (1980a) described an anaerobic oxalate-degrading bacterium that was isolated by enrichment culture techniques from rumen contents. The isolate, OxB, grew in media containing oxalate as the sole substrate. It produced both CO_2 and formate from oxalate, and failed to grow with any of a number of substrates that were tested as replacements for oxalate. This and other information about strain OxB support the concept that organisms similar to strain OxB are responsible for degradation, and thus detoxication, of oxalate in the rumen. Substrate-based selections of increased concentrations of such organisms appear to be responsible for acquired tolerance to oxalate by ruminants when amounts of dietary oxalate are gradually increased (Allison et al. 1977; Allison and Reddy 1984).

In this paper, we further describe isolate OxB as well as similar anaerobic oxalate-degrading bacteria isolated from human feces and from cecal contents of a pig. We propose that these organisms are a unique group and that a new genus and species, *Oxalobacter formigenes*, be established to accommodate them.

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Materials and methods

Culture methods

Unless stated otherwise, the anaerobic culture methods originally developed by Hungate, as further described and modified (Bryant 1972; Holdeman et al. 1977), were employed.

Strain OxB was isolated from sheep rumen contents and the enrichment culture techniques used to achieve this have been described (Dawson et al. 1980a, b). This strain was then used in tests to develop a method to facilitate isolation of other oxalate-degrading anaerobes. This method involved use of a medium (e.g. medium A) that was somewhat opaque due to the presence of calcium oxalate so that colonies of oxalate-degrading bacteria could be detected based upon the formation of cleared zones around colonies.

Medium A contained (g/l: KH_2PO_4, 0.25; K_2HPO_4, 0.25; trace metals solution (Pfennig and Lippert 1966), 20; sodium acetate, 0.082, resazurin, 0.001; yeast extract (Difco Laboratories, Detroit, MI, USA), 0.5; $CaCl_2 \cdot 2H_2O$, 1.0; ammonium oxalate, 5.68; Na_2CO_3, 4.0; cysteine $\cdot$ HCl $\cdot H_2O$, 0.33; $Na_2S \cdot 9H_2O$, 0.33; and agar (Inolex Corporation, Glenwood, IL, USA), 22. Ingredients other than the last four were mixed and pH was adjusted to 6.8. The mixture was boiled and after it had cooled, sodium carbonate was added and 7.5 ml of the mixture was placed in 18 × 150-mm tubes that contained agar. The boiling step and all subsequent steps were conducted under an atmosphere of CO_2 that had been passed through a hot reduced copper column to remove traces of contaminating O_2 (Bryant 1972). The medium was anaerobically dispensed into tubes that were closed with black rubber stoppers. Tubes were held in a press while they were sterilized by autoclaving. A solution containing cysteine and sodium sulfide was added to tubes just prior to melting the agar medium for use.

Cells used for studies of DNA, lipids and antigens were grown in medium B, which contained (g/l): K_2HPO_4, 0.25;

KH_2PO_4, 0.25; $(NH_4)_2SO_4$, 0.5; $MgSO_4 \cdot 7\,H_2O$, 0.025; trace metals solution (Pfennig and Lippert 1966), 20; sodium acetate, 0.82; resazurin, 0.001; yeast extract, 1; sodium oxalate, 10; Na_2CO_3, 4.0; and cysteine $HCl \cdot H_2O$, 0.5. The medium was maintained under a CO_2 gas phase and initial pH was approximately 6.8. A similar medium plus 10% (v/v) clarified rumen fluid was used to grow cells used for preparation of cell-free extracts. Medium C contained (g/l): KH_2PO_4, 0.9; $(NH_4)_2SO_4$, 0.9; trace metals solution (Pfennig and Lippert 1966), 20; K_2 oxalate, 18.4; resazurin, 0.001; Na_2CO_3, 4; cysteine $HCl \cdot H_2O$, 0.5; menadione, 0.0005; and hemin, 0.0025. Cultures were grown at 37° C in test tubes, in 300-ml flasks with an 18-mm side arm to permit absorbance measurements during growth, or in 2- or 14-l glass fermenter vessels. Cells grown in 14-l fermenter vessels were harvested with a continuous flow centrifuge.

Cell densities were estimated as optical density at 600 nm (A_{600}) using a Spectronic 70 colorimeter (Bausch and Lomb, Rochester, NY, USA) in 18-mm cuvettes, unless otherwise indicated, and estimates of the densities of concentrated cell suspensions were based on measurements with diluted samples. Suspensions of OxB cells when corrected to A_{600} 1.0 had a dry weight of 0.19 g/l.

Fecal samples to be cultured were weighed and then diluted in anaerobic dilution solution (Bryant and Burkey 1953). The first dilution (1:4−1:10, w/v) was prepared by shaking the sample with glass beads for 2 min. Melted medium (45° C) was inoculated; roll tubes were immediately prepared and were subsequently incubated at 37° C. Isolates from colonies that produced clear zones were streaked for reisolation on roll tubes of medium A and were cultured in broth medium B or in a similar medium to test for oxalate-degrading capabilities.

Cell extracts

Cells used for preparation of cell-free extracts were suspended in three volumes of anaerobic dilution and were ruptured by ultrasonic disintegration (Measuring and Scientific Equipment, Ltd., London, UK) during three 3-min periods, separated by a 1-min cooling interval, while gassing with CO_2. The supernatant obtained after 20 min of centrifugation (23,500 × *g*), was treated with $(NH_4)_2SO_4$ to 80% saturation, and the precipitate after centrifugation (23,500 × *g*) was dissolved in 0.5 M phosphate buffer, pH 6.9. All of the above operations were at 0−4° C. Extract was stored for as long as 40 days at −42° C without marked loss of activity. Protein was estimated with a dye-binding assay (BioRad Laboratories, Richmond, CA, USA) with bovine albumin as the reference standard.

Oxalate degradation by washed cell preparations and by cell-free extracts was estimated from measurements of [^{14}C]carbon dioxide produced during incubation in rubber-stoppered test tubes with [^{14}C]oxalate (Allison et al. 1981). Production of 0.5 mol of CO_2 per mol of oxalate degraded (Dawson et al. 1980a) was assumed for calculations of oxalate degradation by pure cultures or by cell-free extracts.

Other methods

DNA was isolated and the guanine plus cytosine (G + C) content of the DNA was determined by the thermal denaturation method (Marmur and Doty 1962) with DNA from *Escherichia coli* strain B ($t_m = 90.5$) as reference standard. Measurements were made with a Gilford spectrophotometer equipped with a thermoprogrammer.

Antibodies to whole cells of strain OxB were prepared by injection of formalin-killed whole cells (resuspended to OD 1.0 at 575 nm in 0.145 M NaCl) into a rabbit. The injection schedule was that used for production of capsular antibodies against *Klebsiella pneumoniae* (Edwards and Ewing 1955). Cell suspensions, 0.4 g in 4 ml of Dulbeccos phosphate-buffered saline plus 0.3% phenol were used in slide agglutination and in slide agar gel double diffusion tests (Heddleston et al. 1972).

Concentrations of oxalate and other short-chain organic acids were measured by gas-liquid chromatography of butyl esters (Allison et al. 1977). A calcium precipitation test for the presence of oxalate in broth media (Dawson 1980b) was also used. Cells used for API-Zyme (Rapid-Zyme, DMA Laboratories Inc., Flemington, NJ, USA) were grown in medium B, harvested by centrifugation, and suspended in 0.145 M NaCl at A_{600} of 1.5 for tests which were evaluated after incubation for 4 h at 38° C (Lankhorst et al. 1979). Amino acids were analyzed by ion-exchange chromatography with an amino acid analyzer (Durrum Instrument Corp., Sunnyvale, CA, USA) using norleucine as internal standard.

Cellular fatty acids

Wet cell pellets were hydrolyzed at 100° C for 12 h in 5 ml of 2 N HCl. Hydrolysates were partitioned with 5 ml chloroform. The chloroform extracts were heated at 55° C for 30 min after addition of 3 ml of methanolic HCl (1 M in acid). The reaction mixtures were washed free of methanol and HCl by three washings with 5 ml water. The esterified extracts were evaporated to dryness under N_2. The samples were analyzed without derivatization, and as the trimethylsilyl (TMS) and trifluoroacetyl (TFA) derivatives (Mayberry 1980). The samples were also subjected to brief (5 min) and long-term (15 h) bromination by exposure to a few drops of an elemental bromine solution. Derivatizing reagents were removed under N_2 and samples were reconstituted in chloroform for analysis.

Gas-liquid chromatographic analysis with flame ionization detection (Model 5840A, Hewlett Packard, Avondale, PA, USA) was accomplished using a 50-meter fused silica capillary column coated with nonpolar polydimethoxysilane OV-1 under temperature-programmed conditions (Mayberry 1981). Fatty acid esters were tentatively identified by their equivalent chain length values and identities were confirmed by co-chromatography with authentic standards under various conditions of derivatization (Mayberry 1981). The bromination reactions served to further confirm identification of the unsaturated acids (those brominated within 5 min) and the cyclopropane (cyc) acids (those which were not brominated within 5 min, but were brominated after 15 h exposure to the reagent).

Results

Isolation

Oxalate-degrading anaerobes were isolated from colonies that produced clear zones in medium A that had been inoculated directly with 10^{-5} and 10^{-6} g of feces from apparently healthy humans. Isolates HC-3 and BA-2 that are

described here are representatives of such isolations. The highest colony count of organisms producing such clear zones in calcium oxalate medium was 2.6×10^7 per g (wet wt) of feces. This count was 0.03% of the colony count from this sample on medium 10 (Caldwell and Bryant 1966). Other studies indicate that further modifications of medium A are needed to maximize colony counts of oxalate-degrading bacteria from rumen contents (S. Daniel, unpublished results), and this may also be true for oxalate degraders in human feces.

Enrichment culture techniques similar to those used for isolation of strain OxB (Dawson 1980a, b) were used to isolate strains POX-C and OxK from cecal contents of a pig and from human feces, respectively.

Morphology

The oxalate-degrading isolates are all Gram-negative, nonmotile, nonsporeforming, rod-shaped cells, typically with dimensions of $0.4-0.6\ \mu m \times 1.2-2.5\ \mu m$ (estimated from scanning electron micrographs). Cells were often curved and occasionally chains of cells of strain OxB were observed as spiral or coiled filaments, but no relationship was found between curvature or cell length and culture conditions. The membrane arrangement and cell envelope structure observed in electron micrographs of thin-section preparations of OxB cells (unpublished results) resembled those observed with similar preparations from other Gram-negative bacteria.

Nutrition

As previous studies (Dawson et al. 1980a) indicated that strain OxB required growth factor(s) present in rumen fluid or yeast extract, experiments were conducted to identify the essential nutrient(s). Defined medium C supplemented with 10 ml/l of a solution that contained B-vitamins and amino acids (MEM mixture, Grand Island Biol. Co., Grand Island, NY, USA) did not support growth of strain OxB, but when this medium was further supplemented with a mixture of volatile fatty acids (0.3% of the VFA mixture, Holdeman et al. 1977), good growth was observed. Subsequent tests indicated that acetate was the component in the fatty acid mixture that was required for growth, and that vitamins, hemin, or amino acids were not required. The growth response of strain OxB to different concentrations of acetate is shown in Fig. 1. A similar growth response to acetate was observed in an experiment with strain POX-C, where maximal growth was obtained with acetate concentrations at and above 0.5 mM. Strain OxB grew in the defined medium plus acetate when sodium sulfide (0.24 g/l) was used as reducing agent instead of cysteine, but did not grow when dithiothreitol (0.1 g/l) was used.

All pure cultures of oxalate degraders failed to grow in commonly used anaerobic media such as PYG (Holdeman et al. 1977) or CCA (Allison et al. 1979) unless oxalate was added. This property was used as a frequent check for culture contamination.

Dawson et al. (1980a) reported that growth of strain OxB was proportional to oxalate concentrations up to 111 mM and that none of a number of other materials substituted for oxalate as a growth substrate. We used both strains OxB and OxK in tests for growth based on utilization of substrates added to a medium that was like medium B except the oxalate concentration was limiting for growth (20 mM). Growth was not enhanced by addition of any of the following: acetaldehyde, acrylate, adipate, benzoate, butyrate, citrate, formate, fumarate, glyoxal, glycolate, glyoxylate, glycerol, glyceraldehyde, isocitrate, lactate, maleate, malate, malonate, methanol, oxamate, oxalacetate, pyruvate, phenylpyruvate, propionate, succinate, or tartarate (at 20 mM concentrations). Nor was growth supported by any of a number of mono-, di- and polysaccharides, alanine, aspartate, glycine, glutamate, serine, or casein hydrolysate. Filter sterilized dimethyl oxalate (Eastman Kodak Co.) and parabanic acid (oxalylurea, Sigma) supported growth, but with both of these substances it seems probable that oxalate released from the compound was used for growth.

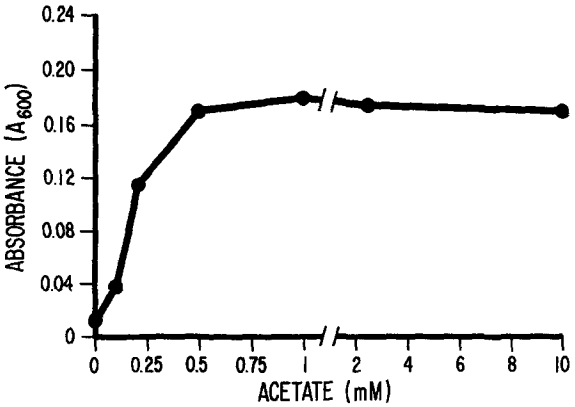

Fig. 1. Growth of strain OxB in defined medium C plus varying amounts of sodium acetate, with growth measured as absorbance at 600 nm in 18-mm tubes using the Spectronic 20 colorimeter. These values (means of highest A_{600} reached in triplicate growth tubes) are equivalent to 0.64 of values obtained with the Spectronic 70 colorimeter

Physiology and metabolism

Dawson et al. (1980a) found that the yield of cells of strain OxB was 1.1 g (dry wt) per mol of oxalate degraded, and that after growth in the presence of [^{14}C]oxalate, 49% of the ^{14}C in the culture medium was recovered as CO_2 and 44% was recovered as formate. No other labeled extracellular products were detected (Dawson et al. 1980a). All of the strains in the present study produced similar amounts of formate (measured as butylformate by gas chromatography) during growth on oxalate. Measurements of growth of strain OxB in medium B, of oxalate removal from culture medium, and of [^{14}C]oxalate degradation rates during growth are shown in Fig. 2. The calculated specific growth rate (μ) during the interval 16–21 h was 0.295 h^{-1} and was similar to that observed in other experiments. In a similar experiment, measurements were made of viable cells (colony count) and of oxalate degradation rates from subsamples obtained during log phase growth. Viable cell numbers (when corrected to A_{600} of 1.0) were approximately 2.1×10^8/ml and the oxalate degradation rate when corrected to the same cell density was 0.56 $\mu mol \cdot min^{-1} \cdot ml^{-1}$ or about 2.9 $mmol \cdot min^{-1} \cdot g^{-1}$ cell (dry wt).

Oxalate degradation, by either growing cultures or concentrated cell suspensions of strain OxB, was accompanied by a loss of H^+ and an increase in pH of the system. When

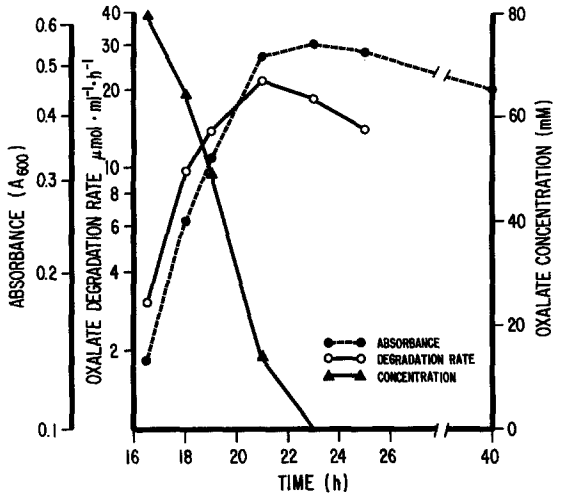

Fig. 2. Measurements of growth of strain OxB in medium B (absorbance at 600 nM), ○; of oxalate concentrations in the culture medium, ▲; and of rates of oxalate degradation by the culture as measured by the conversion of [^{14}C]oxalate to [^{14}C]carbon dioxide, ○

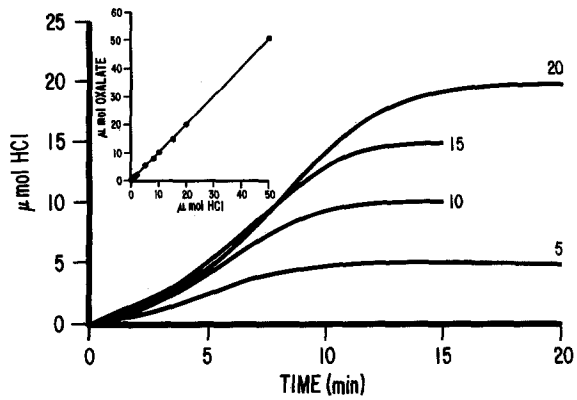

Fig. 3. Titration at pH 6.5 (pH stat) of H^+ uptake by OxB cells as related to amounts of added K_2-oxalate. Reaction at 38°C under N_2 with 5.04 mg of cells per reaction and with amounts of K_2-oxalate (µmol) as designated for each test

a cell suspension (1.65 mg dry wt) was incubated at 38°C for 30 min under N_2 with 0.5–10 µmol of K_2-oxalate, the relationship between µmol of oxalate added and H^+ removed (µmol HCl required to back titrate to pH 4.0) had a slope of 1.18 ($r = 0.996$). In similar experiments where the reaction pH was kept constant (HCl addition with Radiometer TTTl titrator), the relationship between H^+ used and oxalate added was also approximately 1:1 (Fig. 3).

Isolate OxB was cultured in a fermenter (1200 ml) in medium B that was prepared with 10 rather than 100 mM oxalate; however, pH was maintained at 7.0 by automatic addition of a 1:1 mixture of oxalic acid (0.5 M) and K_2-oxalate (0.5 M). After incubation for 20 h, the solution used for pH control was changed to 0.5 M acetic acid. The oxalate concentration in the culture changed from 5.3 to 0.5 mM during the next 1-h time period, and analysis of 20 samples collected during that interval indicated the oxalate was degraded with a K_m of 5.7 mM and a V_{max} of 4.9 mmol of oxalate·min^{-1}·g^{-1} (dry wt) of cells.

Table 1. Requirements for degradation of oxalate by cell-free extracts of *Oxalobacter formigenes*

Reaction[a]	Rate (nmol·min^{-1}·mg^{-1}·protein)
Complete	280
Less thiamin pyrophosphate	220
Less acetate	220
Less succinate	9.2
Less Coenzyme A	8.5
Less ATP	4.6

[a] The complete reaction mixture contained (µmol): phosphate buffer pH 6.9 (100), [^{14}C]oxalate, 0.48 kBq/µmol (16), $MgCl_2$ (10), Na acetate (10), Na succinate (10), ATP (10), Coenzyme A (0.2), and thiamin pyrophosphate (0.1) plus cell extract reconstituted after precipitation with 0–80% saturated ammonium sulfate (0.26 mg protein). The reaction volume was 2 ml and incubation was under CO_2 at 38°C for 30 min. Amounts of [^{14}C]carbon dioxide produced were determined as described in Materials and methods

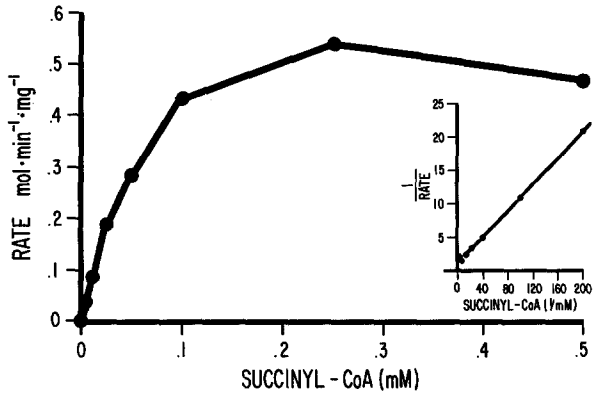

Fig. 4. Oxalate degradation by cell-free extracts from strain OxB as related to amounts of succinyl-CoA in the reaction mixture. Each reaction mixture (2.0 ml) contained (mM): KPO_4, pH 6.9, 50; MgCl, 5; thiamin pyrophosphate, 0.05; ^{14}C-oxalate, 8 (specific activity 0.2 kBq/µmol), and cell extract [0.26 mg of protein from 0–80% $(NH_4)_2SO_4$ precipitate]. The reaction was conducted anaerobically at 38°C for 1 h and [^{14}C]carbon dioxide produced was measured as described in Materials and methods. From a Lineweaver-Burk plot of the data a K_m value of 0.064 mM for succinyl-CoA was calculated

Oxalate degradation during incubation with extracts prepared from cells of strain OxB was dependent upon the presence of succinate, ATP, and coenzyme A in the reaction mixture (Table 1). The requirement for succinate, coenzyme A, and ATP could be replaced by succinyl-CoA and oxalate degradation in relation to amounts of succinyl-CoA in the reaction mixture is illustrated in Fig. 4. The function of succinyl-CoA in this crude system appeared to be catalytic since at a succinyl-CoA concentration of 0.025 mM, 60 nmol of oxalate were degraded per nmol of succinyl-CoA supplied.

No evidence for the presence of any of several glycosidases was found when cells of strains OxB and OxK were tested with the API-Zyme system. Of the 19 tests for hydrolytic enzymes possible with this system, the only strongly positive test was for L-leucyl aminopeptidase (EC 3.4.11.1), while weakly positive tests were obtained for

Table 2. Cellular fatty acid profiles of oxalate-degrading anaerobes

	Group 1 (mol%)		Group 2 (mol%)	
	OxB	HC3	OxK	BA2
Non-hydroxy fatty acids				
12:0[a]	1	1		
14:0	1	tr[b]	2	2
16:1	tr	tr	tr	1
16:0	33	32	28	30
17 cyc	34	32	3	6
18:1	tr	1	11	13
18:0	tr	tr	5	4
19 cyc	14	17	38	32
Hydroxy fatty acids				
3-OH 12:0	6	6	4	4
3-OH 14:0	10	10	8	8

[a] Chain length: number of double bonds; cyc = cyclopropane
[b] Trace, less than 0.5%

esterase-C_4 and C_8 (EC 3.1.1.1), for cystyl-aminopeptidase (EC 3.4.11.3), for acid phosphatase (EC 3.1.3.2), and for phosphoamidase (EC 3.9.1.1). No evidence for nitrate reduction was found with cultures of OxB grown in medium B plus 0.1% KNO_3, and indole was not detected in cultures grown in a medium that contained 1% tryptone (Difco).

Variations among strains

Although profiles of fatty acids from four oxalate-degrading strains were qualitatively similar, strains may be distinguished on the basis of quantitative differences (Table 2). Group 1 contains organisms that have high levels of 16:0 and 17 cyc, a moderate level of 19 cyc, and only trace levels of 18:1 and 18:0 fatty acids. Group 2 contains high levels of 16:0 and 19 cyc, a moderate level of 18:1, and low, but significant, levels of 17 cyc and 18:0. The hydroxy acid component ranged from 12 to 16 mol percent in both groups. No aldehydes were detected.

Differences between these two groups were also apparent from the results of serologic tests. An antiserum prepared against whole cells of strain OxB caused agglutination of cells of strains OxB and HC3, but not OxK and BA-2. With the slide double diffusion test, a single line of identity was observed with antigens from OxB and HC3 and the OxB antiserum, while no precipitin lines were observed between OxK and BA-2 cells and the OxB antiserum.

The G + C ratios (mean ± SD) of DNA from strains OxB, HC3, OxK, and BA-2 were: 49.25 ± 0.74 ($n = 8$), 48.78 ± 0.42 ($n = 7$), 51.01 ± 0.74 ($n = 9$), and 50.52 ± 0.48 ($n = 8$), respectively.

Discussion

We propose that the Gram-negative oxalate degraders described here be assigned to the *Bacteroidaceae* family on the basis of their morphology and anaerobic nature. However, these organisms do not appear to fit into any currently recognized genus. We thus propose here that they be placed in a new genus and species, *Oxalobacter formigenes*. Strain OxB would be the type strain for this new taxonomic group.

Oxalate degradation by ruminal microbes was early recognized as an important function (Talapatra et al. 1948; Morris and Garcia-Rivera 1955). Increased dietary oxalate induces selection of oxalate-degrading ruminal bacteria and make it possible for the animal to tolerate quantities of oxalate that would otherwise be toxic or even lethal (Allison et al. 1977). Similar selections, leading to markedly increased rates of gastrointestinal oxalate degradation when oxalate was added to diets of rabbits, guinea pigs, swine, and a horse (Allison and Cook 1981), support the hypothesis that oxalate is a major substrate for the functional organisms.

Although a variety of aerobic oxalic acid-degrading bacteria have been isolated and studied (Hodgkinson 1977), relatively little is known of microbes responsible for oxalate degradation in anaerobic habitats. Postgate (1963) described isolation of *Desulfovibrio vulgaris* subsp. *oxamicus* from mud enrichment cultures, and both rod and spiral shaped oxalate-degrading anaerobic bacteria have recently been isolated from lake sediments (Smith and Oremland 1983; Smith et al. 1984). The rod-shaped oxalate degraders from sediment appear to be closely related to the organisms we describe here, but the spiral-shaped organisms appear to represent another new group of bacteria. Chandra and Shethna (1975) reported the isolation of an oxalate-decomposing *Clostridium* species from donkey feces, but an adequate description of the latter organism or of its isolation was not given. The first well documented report of isolation of an anaerobic oxalate-degrading organism (strain OxB) from a gastrointestinal habitat is that of Dawson et al. (1980a). It now seems probable that oxalate-degrading anaerobic bacteria similar to strain OxB are widely distributed in gastrointestinal tracts of diverse animals as well as in other anaerobic habitats.

Since little, if any, formate is oxidized by *O. formigenes*, energy-generating mechanisms used by *Pseudomonas oxalaticus* (Quayle and Keech 1959) or *D. vulgaris* ssp. *oxamicus* (Postgate 1963) for growth on oxalate must not be operative. Results obtained here (Table 1, Fig. 4) indicate that oxalate degradation by cell-free extracts from strain OxB requires activated coenzyme A (CoA), and that succinyl-CoA is a good source of this CoA. In this respect, it appears that the oxalate decarboxylase reaction is similar to that found in extracts from *P. oxalaticus* (Quayle et al. 1961), but further work is needed to describe the enzymatic decarboxylation of oxalate by *O. formigenes*.

Growth dependent on energy made available through decarboxylation of oxalate is perhaps analogous to that observed with *Propionigenium modestum*, an organism recently described by Schink and Pfennig (1982) that decarboxylates succinate or oxaloacetate to yield CO_2 and propionate. Growth yields of *P. modestum* were low (2.0–2.4 g/mol of succinate), but slightly higher than the 1.1 g/mol of oxalate measured with *O. formigenes* strain OxB (Dawson et al. 1980b). These low values are in accord with the low free-energy changes of the decarboxylation reactions (Schink and Pfennig 1982). There seems to be little possibility that energy from oxalate degradation by *O. formigenes* is conserved by either substrate-linked or by electron transport-linked processes. Models for linking various decarboxylase reactions to generation of transmembrane proton gradients (Zehnder and Brock 1979) or sodium gradients (Buckel and Semmler 1982; Dimroth 1982; Hilpert and Dimroth 1982) have been proposed. It seems likely that an understanding of the energetics of *O. formigenes* will depend

upon the results of experiments conducted to measure membrane chemiosmotic phenomena.

Description

Genus *Oxalobacter* gen. nov. Ox·al·o·bac'ter. Gr. n. *oxal-* pertaining to oxalate; M.L.n. *bacter* the masculine form of the Gr. neut. n. *bactrum* a rod; M.L. masc. n. *Oxalobacter* an oxalate rod.

Rod-shaped, Gram-negative bacteria ($0.4-0.6 \times 1.2-2.5$ μm) with rounded ends, single, in pairs, or in chains. Cells are often curved. Endospores not found. Flagella not detected. G + C content of DNA is 48 – 51% (t_m).

Chemoorganotrophic metabolism. Strictly anaerobic. Oxalate used as major carbon and energy source. None of a wide variety of other compounds replaced oxalate as growth substrate. Nitrate not reduced. Indole not formed. Temperature range: 14° C – 45° C, optimal growth at 37° C.

Habitats: Various anaerobic environments including: the colon of man and other animals; the rumen; and lake sediments.

Oxalobacter formigenes sp. nov. *form·i·ge'nes* M.L. n. *acidum formicum* formic acid; Gr. v. *gennaio* produce; M.L. adj. formigenes formic acid producing. Description as for the genus. Type species of the genus *Oxalobacter*. The type strain of *Oxalobacter formigenes* is strain OxB. This strain was isolated from the rumen of a sheep and has been deposited with the American Type Culture Collection, where it has been assigned the number ATCC 35274. The description of the type strain is as given above for the genus and species. It is proposed that this organism be placed in the family *Bacteroidaceae*.

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