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Characterization and Description of *Anaeromyxobacter dehalogenans* gen. nov., sp. nov., an Aryl-Halorespiring Facultative Anaerobic Myxobacterium

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Five strains were isolated which form a physiologically and phylogenetically coherent group of chlororespiring microorganisms and represent the first taxon in the *Myxobacteria* capable of anaerobic growth. The strains were enriched and isolated from various soils and sediments based on their ability to grow using acetate as an electron donor and 2-chlorophenol (2-CPh) as an electron acceptor. They are slender gram-negative rods with a bright red pigmentation that exhibit gliding motility and form spore-like structures. These unique chlororespiring myxobacteria also grow with 2,6-dichlorophenol, 2,5-dichlorophenol, 2-bromophenol, nitrate, fumarate, and oxygen as terminal electron acceptors, with optimal growth occurring at low concentrations (<1 mM) of electron acceptor. 2-CPh is reduced by all strains as an electron acceptor in preference to nitrate, which is reduced to ammonium. Acetate, H_2, succinate, pyruvate, formate, and lactate were used as electron donors. None of the strains grew by fermentation. The 16S ribosomal DNA (rDNA) sequences of the five strains form a coherent cluster deeply branching within the family *Myxococcaceae* within the class *Myxobacteria* and are mostly closely associated with the *Myxococcus* subgroup. With the exception of anaerobic growth and lack of a characteristic fruiting body, these strains closely resemble previously characterized myxobacteria and therefore should be considered part of the *Myxococcus* subgroup. The anaerobic growth and 9.0% difference in 16S rDNA sequence from those of other myxobacterial genera are sufficient to place these strains in a new genus and species designated *Anaeromyxobacter dehalogenans*. The type strain is 2CP-1 (ATCC BAA-258).

Myxobacteria have been considered obligately aerobic bacteria (26, 27). The phylogenic placement of this group within the delta-proteobacteria, however, would suggest the possibility of an ancestral anaerobic lifestyle for the myxobacteria (32). The delta-proteobacteria exhibit considerable anaerobic physiological diversity, including sulfate reduction, iron reduction, fermentation, and dehalogenation. Several dechlorinating bacteria in the delta-proteobacteria are able to utilize halogenated compounds as physiological electron acceptors for growth (1, 5, 9, 11, 14–16, 22, 29, 31, 38). Since there are many naturally occurring halogenated compounds in the environment (13) and chlororespiration releases considerable energy for growth (10), greater utilization of this lifestyle might be expected within the delta-proteobacteria.

We used 2-chlorophenol (2-CPh) to search for chlororespiring anaerobes and found a novel group of myxobacteria, the first myxobacteria able to grow anaerobically. The new isolates grow using several ortho-substituted mono- and dichlorinated phenols, nitrate, and fumarate, as well as low concentrations of oxygen, as physiological electron acceptors. Four strains isolated from different environments in this study plus one previous isolate (5) allow a comprehensive characterization of the group. These strains exhibit both phylogenetic and physiological similarities to other myxobacteria but with sufficient differences to represent a new genus and species of myxobacteria.

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MATERIALS AND METHODS

Isolation. Cultures were enriched from anaerobic microcosms containing different soils or sediments and mixtures of monochlorophenols in the presence and absence of nitrate (5, 28, 30). Enrichments were established with the ability to dechlorinate 2-CPh using acetate as the sole carbon and energy source. Isolates were obtained from these enrichments using the agar-shake technique with 10 ml of 2.0% low-melting-point agarose containing 1 mM acetate and 0.2 mM 2-CPh placed in 27-ml anaerobic culture tubes. After 4 to 8 weeks of incubation, colonies were removed using a sterile syringe, with anoxic conditions maintained by continuous flushing of the culture tube with O_2-free N_2. The harvested colonies were transferred to broth culture containing 100 μM 2-CPh and 1 mM acetate. Broth cultures derived from the agar-shake colonies that exhibited dechlorination activity were amended with more 2-CPh once it was consumed. When phenol concentrations exceeded 2 mM, cultures were transferred to fresh medium. These broth cultures were used to inoculate R2A (Difco) agar plates, supplemented with 10 mM fumarate, and deoxygenated for at least 24 h in an anaerobic glove box containing an N_2-H_2 gas mixture of 97:3. Plates were incubated in the anaerobic glove box, and after 2 to 3 weeks of incubation, single colonies were transferred to new agar plates. After at least three transfers, colonies were transferred into chlorophenol or fumarate (5 mM) broth for further characterization. Cultures were subsequently grown anaerobically on plates at 30°C in a gastight glass desiccator with an atmosphere of 10 to 20% CO_2 and the balance being N_2. Previously isolated strain 2CP-1 (5) was included in this study.

Growth medium. Cultures were grown in 160-ml serum bottles with 100 ml of boiled degassed medium or in anaerobic culture tubes with 20 ml of the anaerobic medium and closed with butyl rubber stoppers. The mineral salt medium and vitamin solution were as previously described (30).

The plate medium used for culturing the 2CP strains was R2A (Difco) agar medium supplemented with 10 mM fumarate or 5 mM nitrate as electron acceptor. For anaerobic growth, plates were incubated in an anaerobic glove box.

Electron donors and acceptors. The ability of the isolates to reductively dechlorinate 100 μM 2-CPh with different electron donors was determined in duplicate in 20-ml anaerobic culture tubes incubated at 30°C. The soluble volatile fatty acids (VFAs) acetate, formate, butyrate, succinate, propionate, fumarate, lactate, and pyruvate, as well as H_2, were tested as potential electron donors. The VFAs were added to concentrations of 0.5 or 2.0 mM. H_2 was tested by adding

18 μmol (6.6% [vol/vol]) to the headspace of duplicate culture tubes. An 0.5% inoculum was added to each tube from fresh broth cultures containing the different 2CP strains. The disappearance of 2-CPh and appearance of phenol were monitored by high-pressure liquid chromatography (HPLC). 2-CPh was resupplied at least three times at concentrations of 100 μM to those cultures exhibiting dechlorination activity. VFA utilization was monitored by HPLC, and H_2 was determined by gas chromatography (GC). A positive test was indicated if both dechlorination and depletion of electron donor were observed.

The range of electron acceptors used by the isolates was determined using the same growth conditions as with the electron donor determination. With acetate (1 mM) serving as an electron donor, 100 μM 2,3-dichlorophenol (2,3-DCP), 2,4-DCP, 2,5-DCP, 2,6-DCP, 2-CPh, 3-Cl-4-hydroxybenzoate, 2-fluorophenol, 2-bromophenol, 2-iodophenol, 2,3,5-trichlorophenol (2,3,5-TCP), 2,4,6-TCP, pentachlorophenol, or 3-chloroanisaldehyde was tested as a halogenated electron acceptor. Nitrate, sulfate, sulfite, thiosulfate, and fumarate were also tested at concentrations of 1, 2.5, and 5 mM. Tubes were inoculated with 0.1 to 0.5% concentrations of cultures grown on acetate and 2-CPh. Halogenated substrates were monitored by HPLC and were replenished once depleted. Nitrate and fumarate concentrations were monitored by HPLC. Use of sulfur compounds was determined by monitoring increases in turbidity over those of control cultures with no electron acceptor. Acetate consumption was monitored by HPLC to verify physiological activity.

Nitrate effect on dechlorination. The effect of nitrate (2 or 5 mM) on reductive dechlorination of 2-CPh (150 μM) was tested in anaerobic culture tubes with acetate (1 mM) as the electron donor. Duplicate cultures were inoculated with the isolates and monitored for the appearance of phenol and the disappearance of nitrate and nitrite.

Microscopy. Microscopic observations were done using a Leitz Orthoplan 2 microscope. Colonies on agar surfaces were illuminated with a focused beam of light and observed under the 5× objective. To determine if the 2CP strains were capable of gliding motility, cells were observed after suspending portions of colonies from young cultures or centrifuged broth cultures in 10 μl of sterile medium and spreading them on dried agarose-coated slides. Coverslips were sealed with a molten mixture of 50% paraffin and 50% Vaseline. Cells were observed under oil immersion with a 60× phase-contrast objective lens. Photographs were taken using TMAX 100 black-and-white film or Ektachrome color slide film. Transmission electron micrographs of strain 2CP-C were done with cells from colonies on plates using a JEOL 1200 EX-2 transmission electron microscope operated at 50 kV.

Hydrogen uptake and threshold determination. Hydrogen uptake was monitored in relation to dechlorination activity to test if the hydrogenase activity was closely linked to the terminal electron-accepting capacity of the 2CP strains. Hydrogen (5.0%) was added to the headspace of 100-ml cultures in 160-ml serum bottles. 2-CPh (100 μM) was added to these cultures, and the H_2 concentration was monitored by GC as dechlorination occurred. After the complete dechlorination of the 2-CPh, an additional 100 μM amendment was made. Threshold concentrations were measured with a GC equipped with a reduction gas detector (Trace Analytical, Menlo Park, Calif.) using the method described by Löffler et al. (17) with excess 2-CPh present.

Determination of f_e. As an indication of energetic efficiency and evidence of halorespiration, the fraction of electrons (f_e) from acetate used for the reductive dechlorination of 2-CPh was determined. Duplicate cultures of each strain were grown on acetate (1 mM) and 2-CPh (125 μM). Control cultures received acetate alone. Concentrations of 2-CPh were monitored daily by HPLC and amendments of new 2-CPh (125 μM) were made prior to the concentration reaching zero. Acetate was determined by HPLC. The f_e was calculated by plotting the acetate concentration consumed as 2H equivalents versus the micromoles of phenol produced (17). Hydrogen equivalents were used since each reductive dechlorination requires 1 mol of H_2 per mol of phenol formed. The hydrogen equivalents from acetate were calculated according to the following half-reaction: $CH_3COO^- + 4H_2O \rightarrow 2HCO_3^- + H^+ + 8H$. Thus, for every mole of acetate consumed 4 mol of 2H is generated.

16S rRNA gene isolation, sequencing, and analysis. Nearly complete sequences were obtained for the five isolates as described in the work of Cole et al. (5) with lengths (*Escherichia coli* positions) as follows: 2CP-1, 1,549 bp (8 to 1541); 2CP-2, 1,512 bp (8 to 1504); 2CP-3, 1,548 bp (8 to 1540); 2CP-C, 1,534 bp (8 to 1526); and 2CP-5, 1,548 bp (8 to 1540). Related sequences and a preliminary alignment were obtained using the Ribosomal Database Project (19, 20) programs Sequence Selection and Sequence Aligner. The alignment was completed using the Genetic Data Environment program, version 2.3 (33). A maximum-likelihood phylogenetic tree was created with the fastDNAml program (23), using a weighting mask to include only unambiguously aligned positions. This analysis was repeated on 100 bootstrap samples to obtain confidence estimates on branching order (12). The program CONSENSE from PHYLIP (Phylogeny Inference Package), version 3.5c (J. Felsenstein, Department of Genetics, University of Washington, Seattle, 1993), was used to determine the number of times that each group was monophyletic. The final tree was rooted by using *E. coli* and *Desulfovibrio desulfuricans* subsp. *desulfuricans* as the outgroup.

The following sequences were obtained in aligned format from the Ribosomal Database Project (GenBank accession number in parentheses): *E. coli* (J01695), *Cystobacter fuscus* ATCC 25194^T (M94276), *Stigmatella aurantiaca* ATCC 25190^T (M94281), *Archangium gephyra* ATCC 25201^T (M94273), *Angiococcus disciformis* ATCC 33172^T (M94374), *Myxococcus coralloides* M2 strain ATCC 25202^T (M94278), *Melittangium lichenicola* ATCC 25946 (M94277), *Myxococcus xanthus* strain DK1622 (M34114), environmental clone SJA-5 (AJ009449), *Chondromyces crocatus* strain Cm c6 (M94275), *Polyangium cellulosum* subsp. *ferrugineum* SMP 456 strain ATCC 25531 (M94282), strain NOSO-1 (AJ233948), *Nannocystis exedens* Na e1 strain ATCC 25963^T (M94279), *Polyangium vitellinum* strain Pl vt1 (AJ233944), and *D. desulfuricans* subsp. *desulfuricans* ATCC 27774 (M34113).

REP-PCR. To determine whether the dechlorinating isolates were different genotypes, repetitive extragenic palindromic sequence-PCR (REP-PCR) chromosome fingerprints were determined according to the protocol of Rademaker et al. (24) using REP primers and a small portion of the bacterial colony in the reaction mixture.

Analytical procedures. Samples (1.0 ml) were taken periodically for determination of nitrate, nitrite, and ammonia. Nitrate and nitrite were analyzed by HPLC using a Whatman Partisil 10 SAX column on a Shimadzu HPLC. The eluent was 50 mM phosphate buffer (pH 3.0) pumped at a rate of 1 ml/min. UV adsorption at 210 nm was used for detection. Ammonium was analyzed using the phenate method (4).

Chlorophenols, dichlorophenols, and aromatic products of dechlorination were analyzed on a Hewlett-Packard 1050 HPLC with a Chemstation analysis package. The eluent was phosphoric acid (0.1%)-buffered methanol pumped at 1.5 ml/min using a gradient from 48 to 55% methanol and a Hibar RP-18 (10-μm) column. Eluted peaks were detected at 218, 230, and 275 nm simultaneously on a UV multiwavelength detector. Samples (1 ml) from the enrichments were taken, made basic with 10 μl of 2 N NaOH, centrifuged for 6 min in a microcentrifuge, and filtered through Acrodisc LC13 polyvinylidene difluoride 0.45-μm-pore-size filters prior to HPLC analysis.

VFAs were analyzed using a Shimadzu HPLC with a Bio-Rad Aminex HPX-87H ion-exclusion column heated to 60°C and using 0.005 N H_2SO_4 as the eluent. Previously filtered samples were acidified to 0.25 N H_2SO_4 by adding 100 μl of 2.5 N H_2SO_4 to 900 μl of sample. Eluent was pumped at 0.6 ml/min, and detection of VFAs was performed at 210 nm with a UV detector.

The headspace of the incubation vessels was analyzed for N_2, H_2, and CO_2 using a Carle GC equipped with a 1.83-m Porapak Q column and a thermal conductivity detector. Headspace pressure was normalized to atmospheric pressure by venting with a needle prior to removing 0.3 ml of gas for injection into the GC. N_2O was quantified on a Perkin-Elmer 910 GC with a Porapak Q column and ^{63}Ni-electron capture detector. Potential denitrification products were measured in an Ar headspace.

Nucleotide sequence accession number. rRNA sequences have been submitted to GenBank (accession no. AF382396 [2CP-1], AF382397 [2CP-5], AF382398 [2CP-2], AF382399 [2CP-C], and AF382400 [2CP-3]).

RESULTS

Isolation of dechlorinating strains. Enrichment cultures that exhibited dechlorination of 2-CPh were derived from four different soil and sediment samples. Isolates were obtained by picking colonies from agar shakes and transferring them to broth cultures with 2-CPh and acetate as the electron donor. Of the colonies picked, only red-pigmented colonies were capable of dechlorinating 2-CPh. The purity of cultures that exhibited dechlorination activity was established by making several transfers of homogeneous colonies after anaerobic growth on fumarate-containing agar plates. After at least three transfers, colonies were transferred back into 2-CPh-containing medium and dechlorination activity was verified. Five strains, designated 2CP-1 (from Michigan stream sediment) (5), 2CP-2 (from Michigan pond sediment), 2CP-C (from

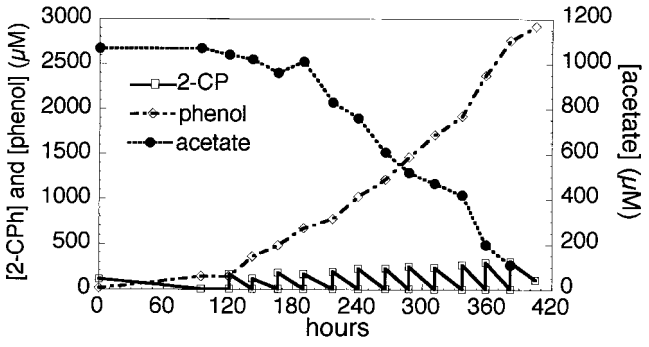

FIG. 1. Dechlorination of 2-CPh to phenol by strain 2CP-C and consumption of acetate.

Cameroon rain forest soil), 2CP-3 (from Michigan yard compost), and 2CP-5 (from Michigan yard compost), were isolated.

Acetate consumption followed the stoichiometric production of phenol from 2-CPh (Fig. 1). All strains exhibited similar behavior. 2CP colonies grown on R2A agar medium with fumarate as the electron acceptor developed a red pigmentation after several weeks of incubation (Fig. 2A). A similar red color was observed in broth cultures containing acetate and fumarate. This red pigment was not extractable with acetone. Colonies exhibited changes in morphology with time. A typical young colony is shown in Fig. 2B. As colonies aged, the pigmentation became denser, and cells appeared to concentrate in a mound at the center of the colony. These masses formed small raised colonies on the surface of the agar (Fig. 2A). Microscopic observations of cells from this concentrated colony mass showed the presence of refractile bodies, indicative of spores or cysts (Fig. 2C). Some vegetative cells from young cultures visualized by microscopy exhibited gliding motility. The terminal ends of cells formed hook-like structures and blebs (Fig. 2D). Transmission electron microscopy revealed pilus-like structures at the terminal end of the cells (Fig. 2E). All 2CP strains stained gram negative, and the cells were long slender rods with tapered ends (4 to 8 μm by 0.25 μm).

Phylogeny of 2CP strains. Nearly complete 16S ribosomal DNA (rDNA) sequences were obtained for all five isolates, and they are all very similar. Strain 2CP-1 and 2CP-5 sequences differ at six positions, 2CP-1 and 2CP-2 sequences differ at a single position, and 2CP-5 and 2CP-3 sequences also differ at a single position, while 2CP-3 and 2CP-C sequences are identical. The phylogenetic diversity of the myxobacteria has been previously examined in detail (32, 34). For comparison with the 2CP isolates, strains representing the range of myxobacterial diversity were chosen from these previous studies. Additionally, an unknown myxobacterium represented by a 16S rRNA clone isolated from an anaerobic, trichlorobenzene-transforming consortium was also included (39) (Fig. 3). Phylogenetic analysis indicated that these five strains form a coherent cluster deeply branching within the myxobacteria. The 2CP strain 16S rRNA sequence was 89 to 90% similar to that of *M. xanthus* (strain DK1622), and the maximum rRNA sequence similarity to a previously characterized myxobacterium was 91% to *C. fuscus* (ATCC 25194). In contrast, the rDNA sequence of *D. desulfuricans*, also a member of the delta-proteobacteria, was only 81% similar to those of the 2CP strains. Three genotypes were distinguished among the four strains examined by REP-PCR (Fig. 4). Strains 2CP-1 and 2CP-2, which were isolated from different Michigan sediments, appear to be very similar, as they were similar in 16S rDNA sequence. Further characterization of the 2CP strains was focused on the most robust strains, which also came from very different habitats: 2CP-1, 2CP-3, and 2CP-C.

Electron donors and acceptors. Acetate was the best electron donor for combined growth and dechlorination activity, although hydrogen was used in preference to acetate when both were present (data not shown). Succinate, pyruvate, and formate were also oxidized. Lactate appeared to be a poor electron donor, and butyrate and propionate were not utilized at all. The doubling time for exponentially growing cells with 2-CPh as an electron acceptor was 12 h. Up to 5 mM nitrate did not inhibit dechlorination. In general, strains 2CP-C, 2CP-3, and 2CP-1 used the same electron acceptors (Table 1) with one exception: 2CP-C dechlorinated 2,4-DCP and 2,4,6-TCP much faster and more completely than did 2CP-1. In addition to ortho-substituted chlorophenols, 2CP-C and 2CP-1 were able to grow with 2-bromophenol as an electron acceptor. Concentrations of chlorophenols above 250 μM were inhibitory to dechlorination activity. Iodo- and fluoro-substituted halophenols were not utilized. The fungal metabolite 3-chloro-4-anisaldehyde, found in soils (8), was not used by strain 2CP-C.

Nitrate, oxygen, and fumarate were also used as electron acceptors by the 2CP strains (Table 1). Plates that were incubated with 5% oxygen and 10% CO_2 added to the gas mixture exhibited colony formation within 1 to 2 weeks. The red pigment was not apparent under aerobic growth. Plates incubated in air exhibited poor or no growth. Oxygen supported microaerophilic growth of 2CP broth cultures when 3% (vol/vol) air (0.6% O_2) was added to serum bottle cultures with a N_2 headspace. To obtain visible growth, fresh air was repeatedly injected into the headspace to achieve 0.6% O_2 once the O_2 was depleted as determined by GC.

Fumarate and nitrate supported growth on plates and in broth cultures. The oxidized sulfur compounds sulfate and thiosulfate did not support growth in broth medium. Since nitrate supported the growth of the 2CP strains, the products of nitrate reduction in cultures grown with acetate were identified and quantified. After 21 days of incubation, strain 2CP-3 converted 52.6 and 60.3% of the nitrate added into ammonium at initial nitrate concentrations of 2.5 and 5.0 mM, respectively. Nitrite was also detected, although it appeared transiently when sufficient electron donor was available. Very small concentrations of N_2O and N_2 were also measured, accounting for less than 5% of total nitrate added in the 2.5 mM culture and insignificant levels in the 5 mM nitrate cultures. Strains 2CP-C and 2CP-1 exhibited similar ammonium formation from nitrate; however, both of these strains grew better if the initial nitrate concentration was lower (1 mM).

Dechlorination in the presence of nitrate. Since the original microcosms from which strains 2CP-3 and 2CP-C were enriched contained both 2-CPh and nitrate as electron acceptors, the preference exhibited by 2CP-1, 2CP-C, and 2CP-3 for one or the other was determined. For strain 2CP-C, dechlorination and nitrate reduction occurred in the same culture; however, 2-CPh was depleted completely (Fig. 5, arrows) on three oc-

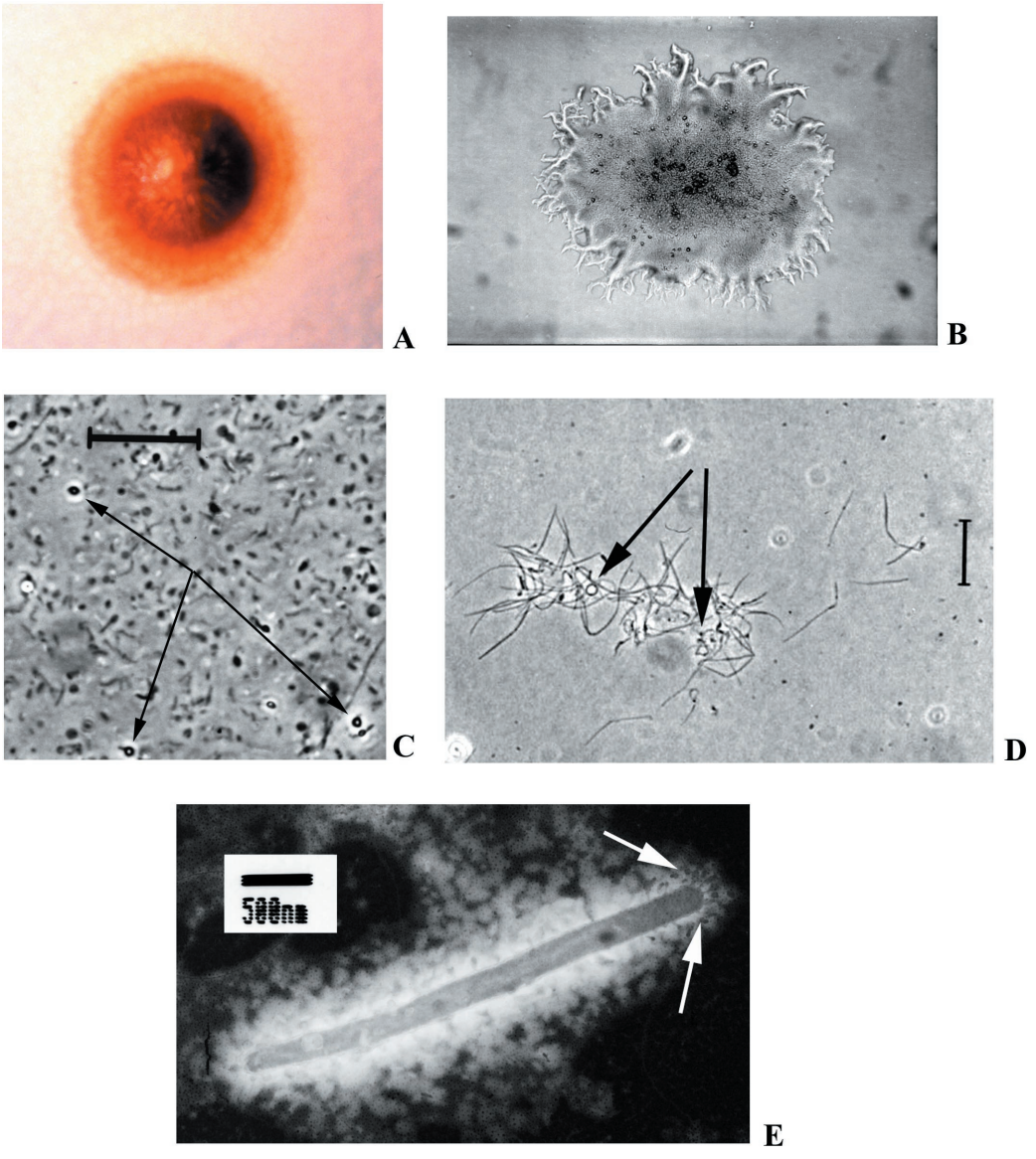

FIG. 2. (A) Red colony formed by 2CP-C on anaerobic agar, showing the formation of a central cell mass. (B) New 2CP-1 colony as it appears on agar surface. (C) Cells and cysts (refractile bodies indicated by arrows) from 2CP-C colony. Bar = 13 μm. (D) Vegetative cells of strain 2CP-C shown by phase-contrast microscopy. Hook-like ends of cells and blebs are shown by arrows. (E) Transmission electron microscopy of strain 2CP-C showing pilus-like structures (arrows) at the polar terminus of the cell.

casions. In a parallel experiment with 2-CPh maintained in the cultures at all times, nitrate reduction was considerably inhibited (data not shown). Similar results were obtained with strains 2CP-1 and 2CP-3. Reductive dechlorination in these same cultures was not affected by the presence of nitrate at concentrations of 5 mM, indicating a preferential use of 2-CPh as an electron acceptor. Control cultures without cells showed no loss of 2-CPh or nitrate in any of the experiments.

Indicators of halorespiration. The fraction of electrons from acetate used for reducing the electron acceptor, f_e, indicated that 64% (0.64) of the electrons from acetate were used for dechlorination by strain 2CP-C (Fig. 6A). Similar results were obtained with the other 2CP strains, with 2CP-1 having an f_e of 0.69 as calculated from the data of Cole et al. (5). With cell yield data from their paper, an f_s (fraction of electrons from the electron donor used for cell synthesis) of 0.34 was calculated for strain 2CP-1. By summing f_e and f_s for strain 2CP-1 (=1.03), there is good agreement with the theoretical sum of 1.0.

The threshold concentration for H_2 was measured as a confirmatory indicator of halorespiratory growth in the 2CP strains (Fig. 6B). Strain 2CP-C assimilated H_2 only in the presence of 2-CPh; a new addition of this substrate immediately stimulated H_2 uptake (Fig. 6B). Threshold concentrations of hydrogen were determined in the presence of excess 2,6-DCP since this substrate had twice the electron-accepting capacity of 2-CPh. Within 25 h the H_2 concentration was approximately 1 ppmv and still decreasing. Final threshold concentrations of less than 0.5 ppmv were measured after 48 h for all three 2CP strains (data not shown). The H_2 concentration

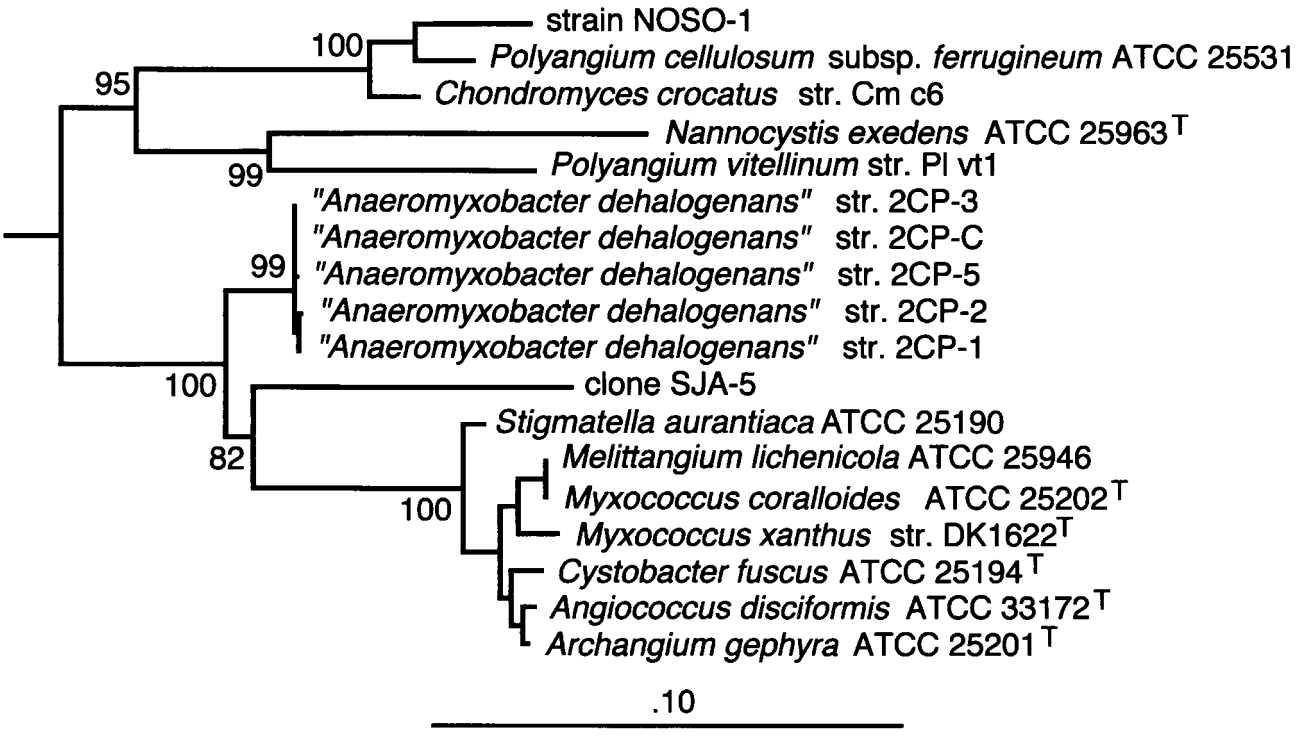

FIG. 3. Maximum-likelihood phylogenetic tree based on 16S rRNA sequences of 2CP strains and representative myxobacteria. Numbers indicate the number of times out of 100 bootstrap samples that the cluster defined by a given node was monophyletic.

was measured for the 2CP-C culture with a more sensitive H_2 detector and was found to be less than 0.02 ppmv of H_2.

DISCUSSION

The new group of isolates exhibits gliding motility, pigmentation, and cellular morphology which are consistent with *Myxococcus* species, but their anaerobic growth and a 9.0% difference in 16S rDNA sequence from those of other myxobacteria suggest that these bacteria should be a new genus and species. We propose *Anaeromyxobacter dehalogenans* to recognize their anaerobic growth and their halorespiratory ability. The previously described members of the order *Myxococcales* fall into three major phylogenetic branches (32, 34): the *Myxococcus* subgroup, containing the families *Myxococcaceae*, *Archangiaceae*, and *Cystobacteraceae*, and the *Chondromyces* and *Nannocystis* subgroups, both in the family *Polyangiaceae*. The *A. dehalogenans* sequences form a deep branch in the *Myxococcales* specifically related to the *Myxococcus* subgroup but outside any of the subgroup's three families. Several higher-order 16S rRNA structure features differentiating the three subgroups (32) also support the deep branching of *A. dehalogenans* in the *Myxococcales*. (i) The terminal structure between positions 1435 and 1466 (all positions based on *E. coli* sequence [2]) is severely truncated in the *Myxococcus* subgroup. This feature is not truncated in *A. dehalogenans*. Instead, *A. dehalogenans* matches the *Chondromyces* subgroup consensus in both primary sequence and secondary structure. The trichlorobenzene reactor clone SJA-5 shares a similar, but 1-bp-shorter, structure. (ii) The *Myxococcus* subgroup structure between positions 1128 and 1144 is missing a 2-base bulge and has the terminating loop size decreased to 4 bases. *A. dehalogenans* and clone SJA-5 retain the ancestral 2-base bulge, as do the *Chondromyces* and *Nannocystis* subgroups, and have a 6-base terminating loop, as does *Nannocystis*. (iii) The structure between positions 455 and 477 in *A. dehalogenans* contains an extra base pair not found in other members of the *Myxococcales*. (iv) Members of the *Myxococcus* subgroup, along with *A. dehalogenans*, retain the ancestral 5-base loop in the structure between positions 1161 and 1175. Most members of the *Chondromyces* and *Nannocystis* subgroups have loops of 6 and 4 bases, respectively.

Several physiological features of the 2CP strains are also shared with other myxobacteria. Bright red pigments were produced by all 2CP isolates and are common among the myxobacteria (27). A characteristic cell shape, bleb formation, and the ability to form spore-like structures are also commonly observed features (26). The colony morphology exhibited by 2CP-C, where cells appear to collect together in a raised mound, is very similar to the morphology shown by *M. xanthus* fruiting-body-deficient mutants (40). The loss of the ability to form fruiting bodies has been reported previously to be common in myxobacteria, particularly if they have been maintained in broth cultures (26). As all of the 2CP strains were enriched in liquid medium, it would be reasonable to expect that well-formed fruiting bodies would not occur. The gliding motility of the 2CP-C cells observed microscopically is also consistent with the behavior of myxobacteria (3). Possible reasons why this

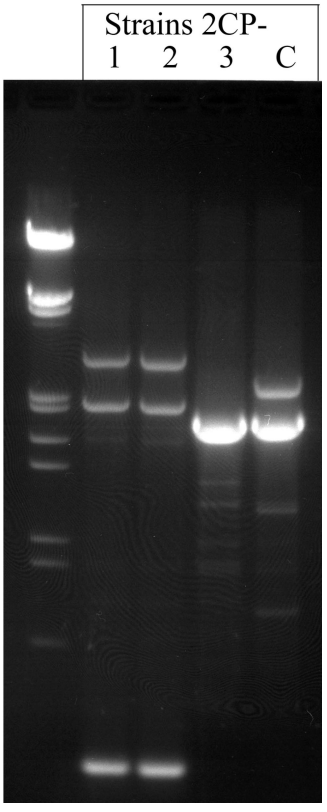

FIG. 4. REP-PCR fingerprints of strains 2CP-1, 2CP-2, 2CP-3, and 2CP-C.

motility was not always observed are the lack of a suitable surface and inappropriateness of environmental conditions such as pH, O_2 tension, or cell density.

The ability of the 2CP strains to grow anaerobically on a diverse group of electron acceptors implies that the description of the *Myxococcales* as having strictly aerobic lifestyles needs to be reevaluated. It is not unexpected to find anaerobic myxobacteria, since the delta subdivision of the proteobacteria is dominated by organisms with a diversity of anaerobic physiologies. An indication that some myxobacterial strains may have unrecognized anaerobic lifestyles is that rDNA sequences belonging to the myxobacterial group have been previously isolated from anaerobic marine sediments (25). Several myxobacterial 16S rDNA clones have also been found previously in other anaerobic environments, and recently one clone was extracted from an anaerobic enrichment culture that reductively dechlorinated trichlorobenzene (39). These results suggest that more anaerobic myxobacteria may exist.

Another unique physiological feature of the 2CP strains is the preferential utilization of 2-CPh as an electron acceptor over nitrate. On the surface this seems surprising, although from a thermodynamic perspective this is not the case. Energetically nitrate reduction to nitrite has a $\Delta G^{\circ\prime}$ of -81.6 kJ mol electron^{-1} (36), while 2-CPh reduction to phenol and chloride has a $\Delta G^{\circ\prime}$ of -78.5 kJ mol electron^{-1} (10), and the $\Delta G^{\circ\prime}$ of nitrate reduction to ammonia is only -75.0 kJ mol electron^{-1}. The energies available from these different reactions are comparable, and it is not likely that the preferences for substrate utilization are solely thermodynamically based. For example, one nonthermodynamic reason that the 2CP strains do not use nitrate before 2-CPh may be that nitrite accumulation is inhibitory to their growth. This is not unusual for nitrate-reducing bacteria (37). The apparent selective advantage of preferential reductive dechlorination also indirectly supports the case for halorespiratory metabolism for the 2CP strains. In order to gain an equal or greater benefit energetically from reductive dechlorination over the respiratory reduction of nitrate to ammonium, an electron transport chain is most likely involved. This would be consistent with the characteristics of all other previously isolated myxobacteria.

The ability to use acetate as the sole carbon and energy source is generally considered to be limited to the domain of respiring microbes, with the exception of aceticlastic methanogens. Aside from the 2CP strains, one such acetate-oxidizing 2-CPh-reducing bacterium, isolated from an estuarine environment, is *Desulfovibrio dechloracetivorans* SF3 (35). This unique sulfate-reducing organism can only use acetate as an electron donor for growth with 2-CPh as an electron acceptor and not with sulfate. Acetate utilization therefore provides evidence of halorespiration in bacteria, such as the 2CP strains. The fraction of electrons from the electron donor used to reduce the electron acceptor, f_e, has been shown elsewhere for halorespiring organisms to be between 0.6 and 0.7 (17). In the case of strain 2CP-C grown on acetate, the f_e of 0.64 is a little more than what is expected for denitrifiers but much less than expected for sulfate reducers (7, 21).

Since hydrogen oxidation also supports growth with 2-CPh

TABLE 1. Electron acceptors used by different 2CP strains[a]

Electron acceptor	Result for strain:		
	2CP-C	2CP-1	2CP-3
Halogenated			
2-CPh*	+	+	+
3-CPh	−	−	−
4-CPh	−	−	−
2,3-DCP	−	−	−
2,4-DCP	+	+/−	+
2,5-DCP	+	+	−
2,6-DCP*	+	+	+
3-Cl-4-OH-benzoate	−	ND	ND
2,4,6-TCP	+	+/−	ND
2,3,5-TCP	−	ND	ND
PCP	−	−	ND
2-Fluorophenol	−	−	ND
2-Bromophenol*	+	+	ND
2-Iodophenol	−	−	ND
3-Cl-anisaldehyde	−	ND	ND
Other			
$NO_3^- \rightarrow NH_4^+$*			
NO_3^- = 5 mM	+/−	+/−	+
NO_3^- = 1 mM	+	+	+
$SO_4^{2-} \rightarrow HS^-$	−	ND	−
$S_2O_3^{2-} \rightarrow HS^-$	−	ND	−
O_2*	+	+	+
Fumarate*	+	+	+

[a] +, reductive dechlorination and acetate consumption occurred over at least three additions of the halogenated substrate or growth was observed for the nonhalogenated electron acceptors; *, growth was confirmed by microscopic observation; +/−, activity was observed but was slow and/or complete degradation did not occur; ND, not determined; PCP, pentachlorophenol.

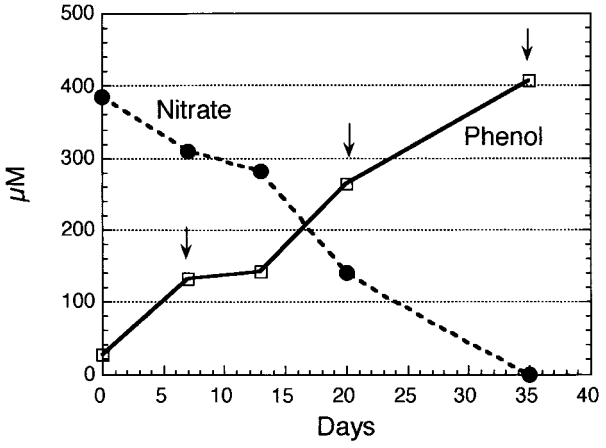

FIG. 5. Dechlorination as indicated by phenol accumulation from 2-CPh and nitrate utilization by 2CP-3. Arrows indicate when 2-CPh was completely dechlorinated and when new additions of 2-CPh were made.

and occurs only when 2-CPh is present (Fig. 6B), it too is an indicator of halorespiratory metabolism. This is particularly true when hydrogen threshold concentrations are less than 0.02 ppmv (17). This hydrogen concentration is related to the minimum amount of energy that an organism requires to grow (6, 18). Hydrogen threshold concentrations are therefore correlated with the energy available from the redox couples mediating their growth. For strain 2CP-C the hydrogen threshold of <0.02 ppmv is comparable to that observed previously for denitrification (6, 17) and is much less than that observed for sulfidogens, methanogens, and acetogens. Löffler et al. (17) suggest that hydrogen threshold measurements may be reliable indicators of halorespiratory metabolism in pure and mixed cultures where a halogenated substrate is the only electron acceptor provided other than CO_2.

One interesting feature of the microcosms from which strains 2CP-3, 2CP-5, and 2CP-C were enriched and isolated was the apparent sensitivity to electron acceptor concentrations greater than 1 mM. With the exception of fumarate, the 2CP strains grew well only when low concentrations (<1 mM) of nitrate, O_2, or 2-CPh were provided. It is possible that these anaerobic myxobacteria obligately require low concentrations of electron acceptor in order to grow. The fortuitous use of low chlorophenol concentrations, mainly for the purpose of toxicity reduction, may have led to the isolation of the 2CP microorganisms.

Description of the genus *Anaeromyxobacter* gen. nov. *Anaeromyxobacter* (An.aer.o.my.xo.bac′.ter. Gr. pref. *an*, not or without; Gr. n. *aer*, air; Gr. n. *myxa*, slime; N.L. masc. n. *bacter*, rod; N.L. masc. n. *Anaeromyxobacter*, slime rod [living] without air). This genus consists of myxobacterial species that are capable of facultative anaerobic growth using terminal electron acceptors such as nitrate, fumarate, and chlorophenolic compounds. Sulfur compounds are not reduced. Oxygen is used but only at low concentrations. The morphological description of this genus is the same as that of the type strain and only species in the genus, *Anaeromyxobacter dehalogenans*.

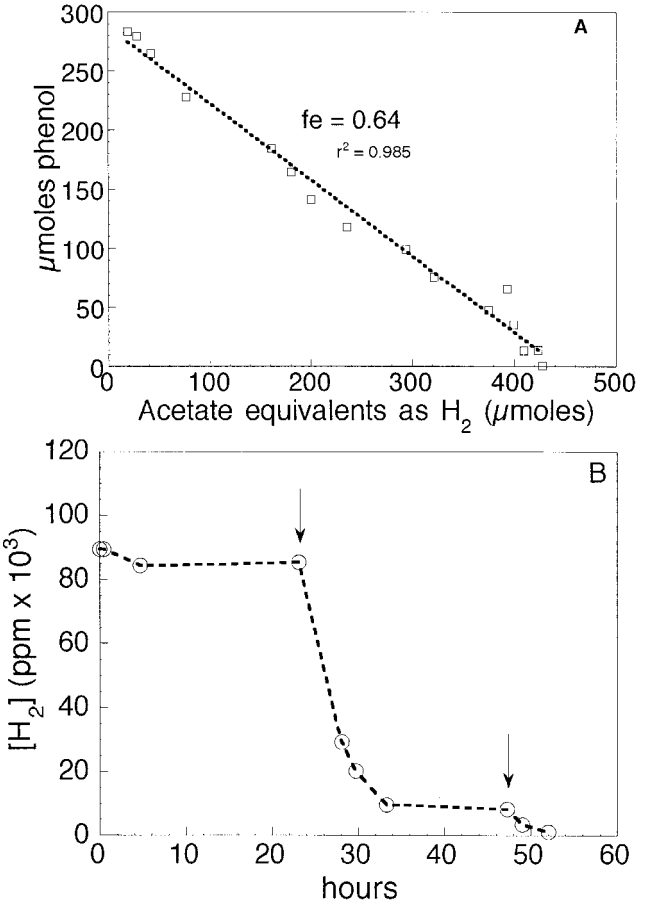

FIG. 6. (A) Fraction of electrons (f_e) from acetate used for reductive dechlorination of 2-CPh by strain 2CP-C. The f_e is derived from the slope of the regression line generated by plotting the acetate consumed as H_2 equivalents (4 H_2 equivalents/mole of acetate) versus the micromoles of phenol produced from the micromoles of 2-CPh dechlorinated. Electrons from acetate are partitioned to the electron acceptor, f_e, and to biomass; therefore, the slope of the regression line is less than 1.0 and is equal to the f_e. (B) Consumption of H_2 by strain 2CP-C under electron-acceptor-limited conditions. Arrows indicate when 2-CPh was added to the medium.

Description of *Anaeromyxobacter dehalogenans* sp. nov. *Anaeromyxobacter dehalogenans* (de.ha.lo′.ge.nans. N.L. adj. part. *dehalogenans*, dehalogenating). Cells are narrow rods 4 to 8 μm long and 0.25 μm wide that exhibit gliding motility. Terminal ends of cells have pilus structures and form blebs periodically. Refractile cysts are visible in older cultures. Cells stain gram negative. Growth is facultative and occurs only with low concentrations of the following electron acceptors: oxygen, nitrate (reduced to ammonia), fumarate, 2-CPh, 2-bromophenol, and 2,6-DCP. 2-CPh is the best substrate for halorespiration (doubling time = 12 h). Acetate, succinate, pyruvate, formate, and H_2 are used as electron donors. Cells grow by complete oxidation of organic electron donors to carbon dioxide. The best temperature and pH for growth are 30°C and 7.0, respectively. Colonies grown on R2A agar medium anaerobically with fumarate are red, raised, and round with a diameter of 1 to 2 mm after 2 weeks of incubation. A red pigmentation is produced in fumarate-grown cells. The type strain, 2CP-1,

was isolated from stream sediment near Lansing, Mich. Strains 2CP-1 and 2CP-C have been deposited in the American Type Culture Collection under accession no. ATCC BAA-258 and ATCC BAA-259, respectively.

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