Reductively debrominating strains of *Propionigenium maris* from burrows of bromophenol-producing marine infauna

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Two novel strains of *Propionigenium maris* able to reductively debrominate 2,4,6-tribromophenol (TBP) to monobromophenols were isolated from marine hemichordate and polychaete burrows. These two strains, DSL-1 and ML-1, were anaerobic, non-motile rods that stained Gram-negative and required 0.05% yeast extract for growth. Strain DSL-1 fermented pyruvate and succinate to predominantly butyrate and strain ML-1 fermented glucose and succinate primarily to propionate. No inorganic terminal electron acceptors were identified. The pH and temperature optima for growth were 7.6 and 30 °C for strain DSL-1 and 7.0 and 32 °C for strain ML-1, respectively; doubling times for strains DSL-1 and ML-1 were 0.32 h and 0.30 h, respectively. Both strains required 2–3% (w/v) NaCl for optimal growth. Morphological and physiological features, as well as the results of 16S rDNA sequence analysis, showed these to be new strains of *Propionigenium maris*. Because they differ from the *P. maris* type strain (DSM 9537^T) in a number of respects, including their ability to rapidly debrominate di- and tribromophenols, and in their specific habitats, the species description is amended to include these ecologically important properties.

Keywords: reductive dehalogenation, bromophenols, marine sediments, infaunal burrows, *Propionigenium*

INTRODUCTION

Biogenic bromoaromatic compounds are broadly distributed in intertidal marine sediments (King, 1986; D. E. Lincoln, K. T. Fielman, R. L. Marinelli and S. A. Woodin, unpublished data; Steward *et al.*, 1992; Woodin *et al.*, 1987). A variety of infaunal hemichordate and polychaete worms produce bromoaromatic compounds including, but not limited to, bromophenols, bromopyrroles, bromoindoles and bromobenzylalcohols (Fielman *et al.*, 1999; Woodin *et al.*, 1987). These bromometabolites are produced through the action of haloperoxidases (Chen *et al.*, 1991) and reach very high levels in the worm tissues (Fielman & Targett, 1995; Yoon *et al.*, 1994). The highest concentrations of bromometabolites external

to the animals are found in sediments lining the animals' burrows (King, 1986; D. E. Lincoln, K. T. Fielman, R. L. Marinelli and S. A. Woodin, unpublished data). Turnover of bromophenols in shallow marine sediments can be rapid (Steward & Lovell, 1997) and the burrow linings, due to the abundance of carbon co-substrates and potential electron acceptors and high levels of bacterial biomass (Steward *et al.*, 1996; Phillips & Lovell, 1999), are expected to be sites of enhanced debromination and degradation of bromoaromatic compounds. The microbiota inhabiting these burrow lining biofilms are thus of considerable interest for detailed studies of debromination processes. Presently little is known about the diversity of species carrying out reductive dehalogenation processes in marine systems.

The large extent of contaminated sediments and the diversity of microenvironments available in coastal marine systems implies the existence of a plethora of haloaromatic dehalogenating and/or degrading species there. However, although mixed culture and

Abbreviations: DBP, 2,4-dibromophenol; LPS, lipopolysaccharide; TBP, 2,4,6-tribromophenol.

The EMBL accession numbers for the 16S rDNA sequences of strains DSL-1 and ML-1 are Y16799 and Y16800, respectively.

sediment slurry studies of marine haloaromatic dehalogenation and degradation are abundantly documented in the literature (e.g. Abrahamsson & Klick, 1991; Allard *et al.*, 1991; Boothe *et al.*, 1997; Genthner *et al.*, 1989; Häggblom & Young, 1990, 1995; King, 1988; Monserrate & Häggblom, 1997; Remberger *et al.*, 1986) and dehalogenating bacteria from freshwater and terrestrial environments have been intensively studied (see El Fantroussi *et al.*, 1998; Fetzner & Lingens, 1994; Häggblom, 1992; Hale *et al.*, 1994; Janssen *et al.*, 1994; Mohn & Tiedje, 1992 for recent reviews), marine strains highly active against multiply substituted haloaromatics have proven difficult to enrich and isolate into pure culture. This is particularly true of reductively dehalogenating organisms, which can remove halogen atoms from the aromatic ring. This process is crucial in the consortial degradation of multiply substituted haloaromatics since compounds with few substituent halogen atoms, particularly monohalogenated aromatics, are mineralized much more readily than multiply substituted compounds with the same aromatic ring structure (Neilson, 1990). No pure culture able to dechlorinate multiply chlorinated chloroaromatic compounds has been reported from marine systems and only two marine strains able to debrominate multiply brominated aromatics have been described to date (Boyle *et al.*, 1999; Steward *et al.*, 1995). This limited roster of dehalogenators restricts studies of the physiology and biochemistry of reductive dehalogenation in marine micro-organisms, a reaction catalysed by several classes of enzymes differing in structure and, in some cases, in reaction mechanism (Y. P. Chen, J. Watson and C. R. Lovell, unpublished data; Holliger *et al.*, 1999; Löffler *et al.*, 1996; Ni *et al.*, 1995; Steward *et al.*, 1995). Here, the characterization and phylogenetic affiliation of two reductively debrominating marine anaerobic strains of *Propionigenium maris* are presented. The description of this species is amended since the two novel strains are capable of rapid reductive debromination of 2,4,6-tribromophenol (TBP) and 2,4-dibromophenol (DBP), producing monobromophenols as terminal products, a property not described for the type strain.

METHODS

Materials and strains. Bromophenol substrates and standards were obtained from Aldrich and used without further purification. *P. maris* DSM 9537^T was obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH. The isolation of strain DSL-1 has been described elsewhere (Steward *et al.*, 1995). Samples for enrichment of strain ML-1 were collected from the North Inlet salt marsh, Georgetown, South Carolina, USA (30°20'N, 79°10'W). These samples consisted of burrow linings of the capitellid polychaete *Notomastus lobatus*, a common inhabitant of clay-rich intertidal mud flats in this system and a well-studied producer of TBP, DBP and 4-bromophenol (Chen *et al.*, 1991; Lovell *et al.*, 1999; Steward *et al.*, 1992, 1996; Steward & Lovell, 1997; Yoon *et al.*, 1994). The burrow scrapings were transported on ice to

Columbia, South Carolina, where enrichment cultures were prepared following previously described methods (Steward *et al.*, 1995). Enrichment media had pH values of 5.5–7.5 and all contained 1 mM TBP, a concentration comparable to that of total bromophenols in *N. lobatus* burrow linings (D. E. Lincoln, K. T. Fielman, R. L. Marinelli and S. A. Woodin, unpublished data). These enrichment media also contained acetate, lactate, sodium benzoate or succinate (40 mM final concentration) as a supplemental carbon source. The cultures were incubated in the dark at 32 °C. Growth was seen within a few days in all cultures except those containing sodium benzoate. All growing cultures actively debrominated TBP as assessed by GC (Steward *et al.*, 1995). The pH 7.0, succinate-supplemented culture had few microscopically distinguishable cell types and was selected for pure culture isolation of debrominators using the agar shake method (Steward *et al.*, 1995). Colonies were visible within 1 week and were aseptically transferred into fresh succinate medium. This process was repeated four times, with each successive culture checked for homogeneity by phase-contrast microscopy. All cultures were homogeneous with respect to cell morphology and were apparently identical to each other. One pure culture from this group was selected arbitrarily and designated strain ML-1.

Growth media

DSL-1. The growth medium was a minor modification of the medium of Steward *et al.* (1995) and contained the following (g l⁻¹): KH₂PO₄, 0.2; NH₄Cl, 0.3; KCl, 0.5; CaCl₂·2H₂O, 0.15; NaCl, 20; MgCl₂·6H₂O, 3.0; Bacto yeast extract, 0.5; SL-9 trace metal solution (Tschech & Pfennig, 1984), 1 ml l⁻¹. The basal medium was adjusted to pH 5.5 and, after autoclaving, the medium was supplemented with sterile filtered 1 mM TBP, autoclaved cysteine sulfide (0.0028 % cysteine, 0.0028 % sodium sulfide, w/v, final concentration), and autoclaved NaHCO₃ (50 mM final concentration). In some experiments, a supplemental carbon source (acetate, benzoate, citrate, crotonate, ethanol, fumarate, glucose, lactate, malate, oxalate, propionate, pyruvate or succinate at 4–20 mM final concentration) and/or potential terminal electron acceptor (nitrate, fumarate and sodium thiosulfate at 5 mM final concentration) were added. Routine cultivation of strain DSL-1 was performed using 20 mM succinate as supplemental carbon source with no added terminal electron acceptor. The complete medium was adjusted to pH 7.5.

ML-1. The growth medium for strain ML-1 was derived from the DSL-1 medium recipe, substituting HEPES (4.8 g l⁻¹) for the carbonate buffer, and adding Bacto Casitone (1.0 g l⁻¹) as an additional supplement. Routine cultivation employed the ML-1 basal medium supplemented with 40 mM succinate. Culture growth was monitored by direct microscopic counting using a Petroff–Hauser counting chamber.

pH and temperature ranges and NaCl requirements. The pH and temperature ranges for growth of strains DSL-1 and ML-1 were determined in triplicate for cultures growing on succinate-supplemented basal media. Optimum pH and NaCl requirement determinations were performed at room temperature for strain DSL-1 and at 32 °C for strain ML-1. Basal medium pH values were adjusted to one unit below the desired pH of the complete medium and the final pH was checked after addition of TBP, cysteine sulfide and (for the DSL-1 medium) bicarbonate buffer. Addition of these supplements increased the pH of the media by roughly one unit. When necessary, additional minor adjustments were made using sterile degassed 1 M NaOH or 1 M HCl. The pH range tested for strain DSL-1 was 6.5–8.5 and that for strain

ML-1 was 5.5–7.5. The temperature range for growth of strain DSL-1 was determined at pH 7.5 and over 10–50 °C. The temperature range for strain ML-1 was determined at pH 7.0 and temperatures between 20 and 40 °C were used. Both strains were tested for growth within the ranges specified at 5 °C temperature intervals. Once the approximate temperature optima were determined, the strains were retested using 2 °C temperature intervals near the optima. The NaCl requirement for each organism was determined by varying the NaCl content of the succinate-supplemented basal medium recipes. The unmodified media both contained 2% NaCl (w/v). Inoculations from these 2% NaCl media were into media containing different NaCl levels (0–6%).

Determination of fermentation products. Fermentation products were determined using a Hewlett Packard 1100 series HPLC system equipped with a 25 cm Microsorb-MV C18 reversed-phase column (Rainin/Varian) and using a 0.005 M H₂SO₄ mobile phase at a flow rate of 1 ml min⁻¹. A₂₁₀ was detected. Retention times of products were compared to those of known standard compounds, including acetate, propionate and butyrate. Compound quantification was based on absorbances of known quantities of authentic compounds. Fermentation products were determined during early and late exponential phase, again during early stationary phase, and finally late in stationary phase.

Dehalogenation assays. Dehalogenation was determined at timed intervals throughout growth by measuring dehalogenation products by HPLC. Samples (1 ml) were taken and mixed with an equal volume of 100% acetonitrile. After centrifugation to remove precipitated proteins and polysaccharides, the samples were assayed for bromophenols using the Hewlett Packard 1100 series HPLC system with a 15 cm HP C18 reversed-phase column and using a methanol:H₂O:acetic acid (50:49:1) mobile phase at a flow rate of 1.5 ml min⁻¹. Detection was by A₂₈₀. Retention times of dehalogenation products were compared to those of authentic standard compounds, including 2-bromophenol, 4-bromophenol, DBP, 2,6-dibromophenol and TBP. Compound quantification was based on absorbances of known quantities of authentic compounds.

Determination of G + C content (mol %). DNA was isolated using a modification of the Marmur (1961) procedure, adding a cetyltrimethylammonium bromide extraction step for removal of contaminating polysaccharides and proteins (Ausubel *et al.*, 1987). The G + C contents (mol %) of each strain were determined after P1 nuclease digestion and HPLC separation of nucleosides (Mesbah *et al.*, 1989; Whitman *et al.*, 1986).

16S rDNA sequence determination and analysis. Genomic DNA was extracted and the gene encoding 16S rRNA (16S rDNA) was amplified as described previously (Rainey *et al.*, 1996). Purified PCR products were directly sequenced using the Taq DyeDeoxy Terminator Cycle Sequencing kit (Applied Biosystems). Sequence reactions were electrophoresed using Applied Biosystems models 373A and 310 DNA sequencers. BLAST analysis was performed on the sequence data to determine the phylogenetic grouping to which the strains were most closely related (Altschul *et al.*, 1990). The sequences determined in this study were aligned against those of *Propionigenium modestum* strain DSM 2376^T (accession number X54275) and *P. maris* strain 10succ1^T (accession number X84049) using the ae2 editor and sequence similarity values were calculated by pairwise sequence comparison (Maidak *et al.*, 1994, 1999).

SDS-PAGE. Cultures of the three strains were grown with and

without TBP to mid-exponential phase and harvested by centrifugation. Cell pellets were resuspended in 0.1 M NaOH and incubated in a boiling water bath for 10 min. Protein concentrations were determined by the Lowry assay (Lowry *et al.*, 1951). Approximately 0.5 µg sample was boiled in Laemmli sample buffer for 5 min, then electrophoresed on a 12.5% SDS-polyacrylamide slab gel (Laemmli, 1970). Proteins were visualized by Coomassie brilliant blue staining.

RESULTS AND DISCUSSION

Phylogenetic analyses

16S rDNA sequences comprising 1441 nt between positions 31 and 1493 (*Escherichia coli* numbering) were determined for strains DSL-1 and ML-1. Comparative analysis of the 16S rDNA sequences with those sequences available for representatives of the major bacterial groups using BLAST analyses indicated that both strains were members of the low-G + C Gram-positive subphylum. Alignment of the two sequences with those available for the members of the low-G + C Gram-positive subphylum and analysis of all nucleotide positions showed them to be most closely related to members of the genus *Propionigenium*. The 16S rDNA sequence of strain DSL-1 had 99.7% similarity to that of strain ML-1. The 16S rDNA sequence similarity between strain DSL-1 and *P. maris* was 99.6% whereas that to *P. modestum* was 96.0%. The 16S rDNA sequence of strain ML-1 had 99.9% similarity to *P. maris* and 95.7% to *P. modestum*. The high degree of phylogenetic relatedness among the *P. maris* strains was also supported by SDS-PAGE protein profiling (Fig. 1), which showed very similar profiles for all three strains grown with or without TBP. These results are consistent with the designation of DSL-1 and ML-1 as strains of *P. maris* and do not support identifying them as new species within the genus *Propionigenium*.

Fig. 1. Protein profiles of *Propionigenium maris* strains DSM 9537^T, DSL-1 and ML-1 on a 12.5% SDS-polyacrylamide gel. Molecular mass standards (with *M*, in thousands) are in the far left lane. Lanes: A, strain DSM 9537^T grown without TBP; B, strain DSM 9537^T grown with TBP; C, strain DSL-1 grown without TBP; D, strain DSL-1 grown with TBP; E, strain ML-1 grown without TBP; F, strain ML-1 grown with TBP.

Table 1. Selected properties of *Propionigenium maris* strains

P. modestum is included for comparison.

Property	<i>P. maris</i> DSL-1	<i>P. maris</i> ML-1	<i>P. maris</i> DSM 9537 ^{T*}	<i>P. modestum</i> [†]
Cell size (µm) [‡]	1.0 × 0.5	2.0 × 0.5	2.5 × 1.0	2.0 × 0.5
Inclusion	None	Present	None	None
Carbon substrate	Succinate, pyruvate	Succinate, glucose	Succinate, pyruvate, fumarate, citrate, 3-hydroxybutyrate, glucose, fructose, maltose	Succinate, pyruvate, fumarate, malate, oxaloacetate, L-aspartate
Fermentation products [§]	Butyrate (propionate)	Propionate (butyrate)	Propionate (butyrate, acetate, lactate, formate, ethanol)	Propionate (acetate)
Optimum pH	7.5–7.8	7.0	6.9–7.7	7.1–7.7
Optimum temperature (°C)	28	32	34–37	33
G + C (mol %)	39	41	40	33.9

* Data from reference Janssen & Liesack (1995).

† Data from reference Schink & Pfennig (1982).

‡ Cell dimensions (length × width) for exponentially growing cells. All *Propionigenium* cells are rods.

§ Products in parentheses for *P. maris* strains DSL-1 and ML-1 are minor products. Production of butyrate, acetate, lactate, formate and ethanol by *P. maris* DSM 9537^T and acetate by *P. modestum* is dependent on the carbon source.

Fig. 2. Phase-contrast photomicrograph of *Propionigenium maris* strain DSL-1 during stationary phase. Note asymmetric cell division (arrow). Scale bar, 10 µm.

Characterization of strains DSL-1 and ML-1

Cell morphology. Cells of strain DSL-1 in exponential growth phase were short rods averaging 1 µm in length and 0.5 µm in diameter (Table 1). Stationary phase cells were shorter, some were almost spherical (Fig. 1). This strain displayed asymmetric cell division, with one daughter cell noticeably larger than the other during stationary phase (Fig. 2). Strain ML-1 was also rod-shaped and 0.5 µm in diameter, but cell length during exponential growth was variable, ranging from

2 to 10 µm. Both strains became shorter and more uniform during stationary phase, with ML-1 length averaging 2 µm. Neither strain ML-1 nor the *P. maris* type strain displayed asymmetric cell division. Sporulation was not observed in either strain and is not a property of the genus *Propionigenium* (Schink & Pfennig, 1982). Strain ML-1 contained dense inclusions that were most numerous in stationary phase cells (Fig. 3). No such inclusions were found in DSL-1 or in the *P. maris* type strain in any growth phase.

Gram stain reaction. The Gram stain reaction was negative but electron microscopy of osmium-tetroxide-stained thin sections revealed a single dark-staining cell envelope layer (data not shown), consistent with a Gram-positive cell wall structure. No lipopolysaccharide (LPS), the characteristic compound of Gram type negative cell walls, was detected using the LPS-polymyxin B assay of Wiegel & Quandt (1982). Other *Propionigenium* strains also stain Gram-negative (Schink & Pfennig, 1982).

Growth properties. Neither strain could be grown in media that were not completely anoxic, indicating that both are obligate anaerobes. The pH range for growth of strain DSL-1 was 6.5–8.5, with optimal growth at pH 7.5–7.8. The pH range supporting growth of strain ML-1 was 5.5–7.5, and the optimum was pH 7.0. The lower pH optimum for strain ML-1 may reflect the stagnant conditions in *N. lobatus* burrows. *N. lobatus* burrow linings are compacted and heavily cemented with mucus (Steward *et al.*, 1996). This cohesive structure somewhat impedes exchange between burrow water and sediment porewater (Aller, 1983). In addition, *N. lobatus* does not irrigate its burrow very energetically, as demonstrated by the very thin oxidized layer lining the burrow wall (Steward *et al.*,

Fig. 3. Phase-contrast photomicrograph of *Propionigenium maris* strain ML-1 during stationary phase. Note inclusions (arrows). Scale bar, 10 μ m.

Fig. 4. (a) Growth of *Propionigenium maris* strain ML-1. (b) Debromination of TBP by *Propionigenium maris* strain ML-1: (○) TBP; (■) DBP; (△) 4-bromophenol; (▲) 2-bromophenol. Values are means of three cultures incubated in parallel. Error bars represent one standard deviation. For symbols with no error bars, error bars are smaller than the symbols.

1996). Burrow stagnation permits accumulation of volatile fatty acids (chiefly propionate, lactate and acetate) produced by the worm under conditions of anaerobiosis (Schöttler *et al.*, 1983; Surholt, 1977) and consequently, substantial decreases in burrow water pH (Scott, 1976). In contrast, the burrow linings of the hemichordate *Balanoglossus aurantiacus*, the source of DSL-1, are not very cohesive (Steward *et al.*, 1996) and have little effect on burrow water exchange. This animal moves very actively during feeding and burrow construction, forcing substantial exchange among burrow water, sediment porewater and overlying seawater. The result of this exchange should be burrow water pH values that are substantially higher than those of *N. lobatus* burrow water and this is consistent

with the higher pH optima of DSL-1. The temperature range for growth of strain DSL-1 was 10–35 °C and the optimum temperature was 30 °C. The optimum temperature for growth of strain ML-1 was similar, 32 °C. The shortest doubling times observed for strains DSL-1 and ML-1 at optimum pH and growth temperature were 0.30–0.32 h. Strain DSL-1 grew well within an NaCl concentration range of 1–5% with optimal growth at 2–3% NaCl and no growth at 0 or 6%. Strain ML-1 grew well from 1–4% NaCl with optimal growth at 3% and no growth at 0 or 5%. These results were consistent with the salinities observed in the euhaline saltmarsh environment from which these strains were isolated. The only defined carbon sources that were utilized in the presence of 0.05% yeast extract were pyruvate and succinate for strain DSL-1 and glucose and succinate for strain ML-1. The growth rate of strain DSL-1 was increased over that in the basal medium by addition of succinate or pyruvate at concentrations up to 20 mM, but not above, whereas the growth rate of strain ML-1 was increased by succinate at concentrations up to 40 mM. Substrates that did not measurably increase the growth rate of either strain in the presence of 0.05% yeast extract included acetate, benzoate, citrate, crotonate, ethanol, fumarate, lactate, malate, oxalate and propionate. In addition, strain DSL-1 did not utilize glucose and ML-1 did not utilize pyruvate. Strain DSL-1 growing on either pyruvate or succinate showed exponential decline in cell numbers in stationary phase. In the absence of these supplemental carbon sources the decline in stationary phase cell numbers was less marked. A similar effect was observed for glucose-grown cultures of strain ML-1, which goes into exponential decline after stationary phase. These stationary phase cultures could not always be successfully subcultured. Strain ML-1 cultures grown with succinate could be successfully subcultured after as long as 4 months if they were kept at room temperature. No appreciable change in growth rate or yield of either strain resulted from addition of nitrate, fumarate or thiosulfate as potential terminal electron acceptors. The predominant fermentation product of strain DSL-1 at all growth stages was butyrate. Small amounts of propionate were also observed. In the absence of enzymology data the pathway used for

butyrate production from succinate could not be determined. Strain ML-1 consistently produced propionate as its predominant fermentation product. Small amounts of butyrate were also observed. Similar to strain ML-1, the *P. maris* type strain (DSM 9537^T) utilizes succinate and glucose (as well as several other substrates), requires yeast extract and consistently produces propionate as its predominant fermentation product (Janssen & Liesack, 1995).

Debromination. TBP was rapidly debrominated by both strains (Steward *et al.*, 1995; Fig. 3). Other tri-bromophenol isomers were not tested. DBP was also debrominated by both strains. DSL-1 produced primarily 2-bromophenol as the terminal debromination product from both TBP and DBP; ML-1 produced a mixture of 2- and 4-bromophenol. The basis for the discrepancy between the previous report of 4-bromophenol production by strain DSL-1 (Steward *et al.*, 1995) and the current observation of 2-bromophenol is not completely clear, but may be due to a change in the culture between the initial determinations and the present work. Neither strain could utilize 2- or 4-bromophenol as carbon substrates. 3-Bromophenol was not tested. Neither strain dechlorinated chlorophenols. A newly isolated *Desulfovibrio* strain that debrominated bromophenols also did not dechlorinate chlorophenols (Boyle *et al.*, 1999).

Bromophenols supplied at millimolar concentrations did not affect yield or doubling time of strain DSL-1 or ML-1. The absence of growth stimulation or change in growth yields indicates that debromination by these strains does not lead to energy production. This result is in contrast to a newly isolated *Desulfovibrio* strain, which can use bromophenols as terminal electron acceptors (Boyle *et al.*, 1999). *P. maris*, the closest related organism to the two novel isolates based on 16S rDNA sequence analysis, was also tested for dehalogenation activity. Growth of unadapted *P. maris* type strain DSM 9537^T was strongly inhibited by 1 mM TBP. After adaptation through successive subculturing using media containing 100–500 µM TBP, type strain DSM 9537^T could be grown in the presence of up to 500 µM TBP and trace amounts of the debromination terminal product, 2-bromophenol, were detected. No indication of growth stimulation or evidence that debromination is coupled to energy production were obtained for any *P. maris* strains. *Propionigenium* species are regarded as purely fermentative anaerobes (Schink & Pfennig, 1982; Janssen & Liesack, 1995). No increases in cell yields by *P. modestum* (Schink & Pfennig, 1982) or any strain of *P. maris* in response to added nitrate, fumarate or thiosulfate have been observed. This indicates that no energy yielding electron transport phosphorylation system is functioning in these species.

G+C content. The G+C content of genomic DNA from DSL-1 is 39±0.3 mol% (SD, *n* = 6), 41±0.1 mol% (SD, *n* = 4) for ML-1 DNA. These values are in good agreement with G+C contents of other *Propionigenium* strains (Table 1).

In conclusion, the high 16S rDNA sequence similarity found between strains DSL-1 and ML-1 and the two described species of the genus *Propionigenium* clearly indicate their membership in the genus *Propionigenium*. Based on several similarities to the *P. maris* type strain and phylogenetic analysis, the new isolates can be placed as novel strains in the species *Propionigenium maris*. The new strains have a high degree of 16S rDNA sequence similarity to that of the *P. maris* type strain (99.6–99.9%), but differ from the latter organism in several physiological features including cell size, substrate preferences, temperature and pH optima and, most importantly, ability to rapidly debrominate TBP. The infaunal burrow, a dynamic microenvironment that supports high bacterial biomass and activities, may promote rapid evolution of these *Propionigenium* strains, resulting in substantial physiological variability without concurrent changes in 16S rDNA sequence. Our identification of these isolates as strains of *P. maris* is conservative and based on the high degree of 16S rDNA sequence homology and the strong similarity of protein profiles among the new strains and the *P. maris* type strain.

The new *P. maris* strains have been isolated from infaunal burrows differing in key microenvironmental features. Such burrows can differ very substantially in size, shape and placement in the sediments, the texture and organic matter content of sediment in the burrow linings, and the porosity of the surrounding sediments (which affects solute transport rates) (see Steward *et al.*, 1996 for further discussion). In addition, the behaviours of the host invertebrates can also differ substantially. Given this range of structures and microbiologically significant activities of the host animals, it is probable that many distinct strains of *P. maris*, and likely new *Propionigenium* species, occur in marine infaunal burrows.

Emendation

The species description for *P. maris* (Janssen & Liesack, 1995) is emended for the property of reductive dehalogenation of bromophenols found in the linings of some marine infaunal burrows. In contrast to strains isolated from the burrows, which are regarded as a special habitat for some *P. maris* strains, the type strain DSM 9537^T exhibits only minor debromination activity and only after prolonged adaptation. Furthermore, some strains may not utilize sugars and butyrate can be the major fermentation product from pyruvate and succinate, although the type strain forms propionate as the major product from glucose and succinate. Depending on the isolate, the cell size varies between 0.5 and 1 µm in diameter and 1 and 10 µm in length.

ACKNOWLEDGEMENTS

We are grateful to W. B. Whitman for help with determination of the G+C contents of DSL-1 and ML-1. This research was supported by the Office of Naval Research, grant number N00014-96-0403 to C. R. L.

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