Cryptanaerobacter phenolicus gen. nov., sp. nov., an anaerobe that transforms phenol into benzoate via 4-hydroxybenzoate

Pierre Juteau, Valérie Côté, Marie-France Duckett, Réjean Beaudet, François Lépine, Richard Villemur and Jean-Guy Bisailon

Correspondence

Pierre Juteau

pierre.juteau@inrs-iaf.quebec.ca

INRS – Institut Armand-Frappier, Université du Québec, 531 boulevard des Prairies, Laval, Quebec, Canada H7V 1B7

An anaerobic bacterium that transforms phenol and 4-hydroxybenzoate (4-OHB) into benzoate, strain LR7.2^T, was isolated from a culture originating from a mixture of swamp water, sewage sludge, swine waste and soil. Cells of strain LR7.2^T are Gram-positive short rods (1 × 2 µm) that are electron-dense when observed by electron microscopy. The optimum pH and temperature for growth and transformation activity of 4-OHB are 7.5–8.0 and 30–37 °C, respectively. The bacterium does not use sulphate, thiosulphate, nitrate, nitrite, FeCl₃, fumarate or arsenate as an electron acceptor. It does not normally use sulphite, although stimulation of growth and 4-OHB transformation activity at a low concentration (up to 2 mM) has been reported previously under different culture conditions. The presence of 4-OHB or phenol is essential for growth; transformation of 4-OHB or phenol into benzoate is used to produce energy for growth. Using [⁶D]-phenol, 4-OHB was shown to be an intermediate in the transformation of phenol into benzoate. No spore was observed. The bacterium has a DNA G + C content of 51 mol% and its major membrane fatty acid is anteiso-C_{15:0}. The 16S rRNA gene sequence of strain LR7.2^T shows only 90% similarity to its closest relative (*Pelotomaculum thermopropionicum*). From these results, a new taxon is proposed: *Cryptanaerobacter phenolicus* gen. nov., sp. nov. The type strain is LR7.2^T (=ATCC BAA-820^T = DSM 15808^T).

Phenol-transforming consortia enriched from methanogenic environments metabolize phenol via 4-hydroxybenzoate (4-OHB) and benzoate (Bisaillon *et al.*, 1991a, 1993; Gallert *et al.*, 1991; Sharak Genthner *et al.*, 1990; Zhang *et al.*, 1990). Most attempts to isolate the strain responsible for the transformation of phenol from these consortia have failed. *Sedimentibacter hydroxybenzoicus* (previously '*Clostridium hydroxybenzoicum*'), isolated from such a consortium, decarboxylates 4-OHB into phenol (Breitenstein *et al.*, 2002; Zhang *et al.*, 1994). Phenol carboxylation was observed with that bacterium but only with whole-cell suspensions and cell extracts (Zhang & Wiegel, 1994). In addition, our group reported the isolation of a phenol-transforming bacterium from a methanogenic consortium originating from a mixture of swamp water, sewage sludge, swine waste and soil (Li *et al.*, 1996). This bacterium,

designated strain 6, was related to the genus *Clostridium*. However, we later determined that the culture also contained a low concentration of another bacterium, which we designated strain 7 (Letowski *et al.*, 2001). Each organism was enriched by centrifugation on a Percoll gradient, and strain 6 was purified by dilution and plating. Because strain 7 did not grow on solid medium, strain 6 was removed by subculturing in the presence of ampicillin and by means of dilution. Strain 7 was shown to be responsible for the transformation of phenol and 4-OHB into benzoate, and strain 6 provides an unknown factor that has a positive effect on metabolism of strain 7. Duckett (2000) observed that the supernatant from autoclaved cultures of other anaerobic bacteria, such as *Clostridium sporogenes* M55, also has a beneficial effect on transformation activity. The component of the spent medium responsible for the stimulatory effect was not identified but it was shown to be thermotolerant and oxygen resistant, with a molecular mass under 1000 Da. Because the purity of the strain 7 culture was questionable, in the present work we isolated strain LR7.2^T from that culture using more conventional methods. This strain was characterized and we propose that it should be included in a novel genus, *Cryptanaerobacter* gen. nov., as *Cryptanaerobacter phenolicus* sp. nov.

Published online ahead of print on 6 August 2004 as DOI 10.1099/ij.s.0.02914-0.

Abbreviations: FAME, fatty acid methyl ester; 4-OHB, 4-hydroxybenzoate.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain LR7.2^T is AY327251.

Clostridium sporogenes M55 (= ATCC 13732 = DSM 754) was taken from our collection. Strains 6 and 7 are those described by Letowski *et al.* (2001). *Clostridium sporogenes* M55 and strain 6 were cultured at 37 °C in SBM1 medium, which consisted of the supplemented Boyd's medium (SBM) used by Letowski *et al.* (2001) but without phenol or 4-OHB. Strain 7 was grown at 37 °C in SBM2 or SBM3 media. SBM2 medium contained 1.5 mM 4-OHB, 45 ml fresh SBM1 and 25 ml of the supernatant from an autoclaved culture (5–8 days old) of strain 6. SBM3 was similar to SBM2 except that an autoclaved culture of *Clostridium sporogenes* M55 was used instead of strain 6. Strain LR7.2^T was isolated in semi-solid (0.3 % w/v agar) SBM2 medium using Veillon tubes (8 × 200 mm). After 10–15 days of incubation, small colonies were recovered with a syringe and then diluted and inoculated in semi-solid SBM2 medium. This procedure was repeated three times. Purity of the resulting culture was confirmed by microscopic observation and the strain was designated LR7.2^T. This strain was cultivated in a modified SBM3 medium (named SBM4): yeast extract was used instead of proteose peptone and the concentrations of Na₂S and iron were reduced by a factor of 10 in order to minimize precipitation of FeS. In addition, after verifying the effect of different concentrations, 4-OHB was increased to 3.0 mM.

Phenol, 4-OHB, benzoate, 3-phenylpropionate, phenylalanine and cinnamate were analysed using a gas chromatograph coupled to a flame-ionization detector (GC-FID) (Bisaillon *et al.*, 1991b). Fatty acids present in the spent medium of *Clostridium sporogenes* were also quantified by GC-FID using an SPB-1000 column (30 m × 0.32 mm; Supelco). GC-MS was used to analyse [⁶D]-phenol and [⁴D]-4-OHB and to identify the 3-phenylpropionate found in the spent medium of *Clostridium sporogenes* (Bisaillon *et al.*, 1991b). Various compounds related to 4-OHB and phenol were analysed by HPLC (Dennie *et al.*, 1998). Growth was normally monitored by optical density measurements at 400 nm with a spectrophotometer (Spectronic 1001 plus; Milton Roy). Equivalence to cell concentration and biomass weight was determined by acridine orange direct count and dry weight measurement (Gerhardt *et al.*, 1994). Observations by light and electron microscopy were performed as described by Letowski *et al.* (2001).

Different growth conditions were tested: addition of a second reducing agent (Na₂S₂O₄ 1.5 % w/v) to SBM4; use of different gas mixtures (H₂/CO₂/N₂ at 10:10:80, 10:0:90, 20:0:80, 80:0:20 and 0:0:100); variation of the concentration of 4-OHB from 0 to 12 mM and phenol from 0 to 14 mM; addition of 2 ml yeast extract 20 % (w/v), 25 ml supernatant from an autoclaved culture of *Clostridium sporogenes* M55 or 10 ml fresh SBM4 after 6 days of incubation; variation of pH from 6.0 to 9.0; and incubation at temperatures varying from 4 to 55 °C. Potential inorganic acceptors were tested in SBM4 in which Na₂S was replaced by cysteine, with and without 4-OHB. Compounds related to phenol or 4-OHB were tested in SBM4 without 4-OHB.

The DNA G + C content was determined by HPLC (Mesbah & Whitman, 1989). The 16S rRNA gene sequence was PCR-amplified with pA and pH primers (Edwards *et al.*, 1989) and cloned in pGEM-T Easy vector (Promega) using the manufacturer's protocol. The recombinant plasmids were used to transform competent *Escherichia coli* DH5α. Plasmids were purified by PEG precipitation (Sambrook *et al.*, 1989). The insert was sequenced with a 4200 DNA analysis system (LI-COR) using the following primers: pA and pH, 530f, 907r and 926f (Gerhardt *et al.*, 1994) and 533r [5'-TTACCGCGGC(T/G)GCTG-3'].

Cultures inoculated with strain LR7.2^T alone and together with strain 6 or *Clostridium sporogenes* M55 were verified for spore formation by heating them at 70, 75 or 80 °C for 10 min after 6–10 days of incubation at 37 °C. Viability after treatment was checked by inoculating samples in fresh SBM4. Other samples were stained with malachite green and observed by light microscopy. For fatty acid methyl ester (FAME) analysis, cells grown in SBM4 for 6 days were used. FAMES were separated and analysed with an MIS system version 2.11 according to the manufacturer's protocol (MIDI).

Colonies of strain LR7.2^T in semi-solid medium were 1 mm in diameter with diffuse margins and were brownish after 10 days of incubation at 37 °C. Light and electron microscopic observations revealed that this strain was similar to strain 7 (1 × 2 µm, Gram-positive, electron-dense) described by Letowski *et al.* (2001). Its activity was also similar to strain 7 in that it transformed phenol and 4-OHB into benzoate. The 16S rRNA gene sequence of strain LR7.2^T is identical to that of strain 7 (accession no. AF072863) except for two nucleotides, at positions 12 and 383. This shows that strains 7 and LR7.2^T represent the same organism.

Based on a phylogenetic analysis (Fig. 1), the closest relative to strain LR7.2^T among other type strains is *Pelotomaculum thermopropionicum* DSM 13744^T (90 % sequence similarity), a thermophilic, syntrophic propionate-oxidizing bacterium (Imachi *et al.*, 2002). Other species present on the tree are clearly not in the same lineage. Some sequences available in public databases show a closer relationship to strain LR7.2^T but they are from uncultured or non-isolated bacteria (Letowski *et al.*, 2001). The DNA G + C content of strain LR7.2^T was 51 mol%.

The addition of a second reducing agent (Na₂S₂O₄) to SBM4 had no effect on strain LR7.2^T. Growth and 4-OHB transformation activity were observed in cultures made under all the different tested atmosphere compositions, but the best result was obtained with the gas mixture that is normally used (H₂/CO₂/N₂ at 10:10:80). The strongest negative effects were correlated with an increase in the proportion of hydrogen. When tested under the same conditions, growth and 4-OHB transformation activity were faster (completed after 6 days instead of 10) in the presence of 4-OHB compared with phenol. Different supplements added after 6 days of incubation (yeast extract,

Fig. 1. Phylogenetic tree showing *Cryptanaerobacter phenolicus* gen. nov., sp. nov. LR7.2^T and its closest relatives. 16S rRNA gene sequences were aligned with CLUSTAL W 1.8. Distance-matrix analysis and tree construction were conducted as described by Bouchard *et al.* (1996). Numbers at nodes are bootstrap percentages obtained from 1000 resamplings. Bar, 0.02 nucleotide substitutions per position.

supernatant from an autoclaved culture of *Clostridium sporogenes*, fresh SBM4) did not stimulate growth or 4-OHB transformation activity. The optimal pH and temperature for growth and 4-OHB transformation activity were 7.5–8 and 30–37 °C, respectively. Even under optimal conditions, growth of strain LR7.2^T was weak [maximum final OD = 0.1, which corresponds to 4×10^6 cells ml⁻¹ or 10 (mg dry weight) l⁻¹].

The major fatty acid of strain LR7.2^T is anteiso-C_{15:0}, which is different from other related bacteria (Table 1). No spore was observed in heat-treated cultures of this strain alone or in co-culture with strain 6 or *Clostridium sporogenes* M55. In addition, there was no growth or 4-OHB transformation 30 days after inoculation of a sample of the heat-treated cultures in fresh SBM4. This is surprising given that pasteurization was used during the enrichment process that led to the isolation of LR7.2^T (Létourneau *et al.*, 1995). We hypothesize that strain LR7.2^T can sporulate under the specific conditions that prevailed in the original consortium but that we did not reproduce here.

The transient presence of [⁴D]-4-OHB (1 µM maximum) was observed in a culture to which 3 mM [⁶D]-phenol had been added, confirming that 4-OHB is an intermediate in the transformation of phenol into benzoate (phenol → 4-OHB → benzoate). This metabolic pathway has been reported with phenol-transforming methanogenic consortia (Bisaillon *et al.*, 1991b; Gallert *et al.*, 1991; Sharak Genthner *et al.*, 1991) but never with a pure culture. Observation of 4-OHB as an intermediate in phenol transformation is difficult because phenol carboxylation is a reversible reaction in which the decarboxylation is thermodynamically favoured at equilibrium. When 4-OHB was added to the strain LR7.2^T culture, most of the 4-OHB was first transformed into phenol until the 4-OHB concentration was low enough to permit transformation of phenol (4-OHB → phenol → 4-OHB → benzoate), a phenomenon that has been observed with many phenol-transforming consortia derived from methanogenic environments (Knoll & Winter, 1989; Létourneau *et al.*, 1995; Sharak Genthner *et al.*, 1990; Zhang & Wiegand, 1990). Phenol and 4-OHB are not a source of carbon for strain LR7.2^T because they are stoichiometrically transformed into benzoate, which is not

metabolized (Fig. 2). Nevertheless, no growth was observed in the absence of phenol or 4-OHB, indicating that the strain cannot grow fermentatively. Cell yield was proportional to the concentration of these compounds up to optimal values (6.5 mM for 4-OHB, 3.5 mM for phenol). This observation invalidates the initial hypothesis made for

Table 1. Fatty acid composition of strain LR7.2^T and related species

Percentages of total acids are shown. Taxa: 1, *Cryptanaerobacter phenolicus* gen. nov., sp. nov. LR7.2^T; 2, *Pelotomaculum thermopropionicum*; 3, *Desulfotomaculum thermosapovorans*; 4, *Desulfotomaculum thermocisternum*; 5, *Desulfotomaculum thermobenzoicum*. Data for reference taxa are from Imachi *et al.* (2002).

Fatty acid	1	2	3	4	5
C _{11:0}			0.5		
iso-C _{14:0}		0.8	1.4		
C _{14:0}	5.8	3.2	2.1	6.2	2.0
iso-C _{15:1}			1.0		
iso-C _{15:0}	13.7	76.4	23.1	63.3	79.4
anteiso-C _{15:0}	18.9			3.9	
C _{15:0}	3.5		1.4	3.9	
C _{16:0} aldehyde			0.6		
iso-C _{16:0}	7.5		2.6	2.4	
C _{16:1} ω9c*	13.7				
C _{16:1}			5.4		
C _{16:0}	12.5	10.7	15.7	10.4	7.4
iso-C _{17:1}			5.0		
iso-C _{17:0}	5.7	2.9	8.9		11.3
Branched C _{17:0}				2.4	
anteiso-C _{17:0}	7.3				
C _{17:0}			8.9		
iso-C _{18:0}					
C _{18:1} ω9c	4.6				
C _{18:1} ω11			0.9		
C _{18:0}	4.2	6.7	3.2	2.4	
Summed feature 4†	2.6				

*Present as methyl ester (7.8%) and dimethyl aldehyde (5.9%) in the analysis.

†Unresolved peak composed of C_{15:2} and an unknown compound.

Fig. 2. Transformation of 4-OHB into benzoate by strain LR7.2^T. Symbols: ■, 4-OHB; ●, phenol; ▲, benzoate; □, sum of 4-OHB, phenol and benzoate; ×, growth. The experiment was conducted in duplicate. Error bars represent standard deviations.

the consortium from which strain LR7.2^T was isolated, namely that phenol and 4-OHB could be transformed into benzoate by co-metabolism (Bécharde *et al.*, 1990). Instead, strain LR7.2^T seems to use these transformations as an energy source for growth. This could represent an unusual anaerobic respiration in which 4-OHB would be the external electron acceptor via its reduction into benzoate. It would be necessary to elucidate completely the energetic metabolism of strain LR7.2^T in order to confirm this hypothesis.

The electron donor and the carbon source have yet to be identified. The complex supplements that are required to ensure growth of strain LR7.2^T (proteose peptone or yeast extract plus a spent medium from *Clostridium sporogenes* or strain 6) are probably the source of more than one essential element. This makes it difficult to design a basal medium to test pure compounds. Instead, the medium was analysed before and after growth of strain LR7.2^T. 3-Phenylpropionate was found in the spent medium of *Clostridium sporogenes* and it was consumed by strain LR7.2^T (its concentration decreasing from 0.5 to 0.2 mM). This compound has already been identified as an intermediate in the synthesis of phenylalanine from phenol in the methanogenic consortium from which strain LR7.2^T was isolated (Lépine *et al.*, 1996). However, 3-phenylpropionate as well as phenylalanine and cinnamate (another compound that was used to form phenylalanine in the originating consortium) had no effect on growth of strain LR7.2^T when given as pure compounds in the absence of *Clostridium sporogenes* spent medium. Acetate, propionate, butyrate and isovalerate were also found in the spent medium of *Clostridium sporogenes* but they were not consumed by strain LR7.2^T. Growth under a 100% N₂ atmosphere suggests that H₂ is not used as an electron donor. By contrast, growth under an atmosphere of up to 80% H₂ confirmed that strain LR7.2^T does not require a syntrophic association with H₂-consuming bacteria.

The following potential inorganic electron acceptors did not enable growth in the absence of 4-OHB (concentrations

in mM are given in parentheses): sulphite (0.25, 0.5, 2, 5, 10), sulphate (0.25, 0.5, 2, 5, 10), sulphur (62), thiosulphate (10), nitrate (10), nitrite (10), FeCl₃ (1), fumarate (10) and arsenate (10). When tested in the presence of 4-OHB, growth and 4-OHB transformation rates were lower than or equal to those observed in the non-amended media. Letowski *et al.* (2001) reported that growth of strain 7 and 4-OHB transformation activity were stimulated by low concentrations of sulphite (up to 2 mM). However, they did not confirm that strain 7 used sulphite as an electron acceptor. We did not observe such stimulation here. The difference seems to be due to the medium we used: contrary to Letowski *et al.* (2001), our medium was supplemented with spent medium from a *Clostridium sporogenes* M55 culture. We hypothesize that strain LR7.2^T could be stimulated by sulphite only under certain less favourable conditions.

Strain LR7.2^T could not use for growth or transform any of the following compounds related to 4-OHB and phenol (concentrations in mM are given in parentheses): 4-hydroxybenzamide (0.7), methyl 4-OHB (3), 4-hydroxysulphonic acid (3), 4-hydroxyacetophenone (0.7), 4-hydroxybenzoic alcohol (3), hydroquinone (3), 4-chlorophenol (0.8), 4-hydroxycinnamic acid (3), 4-OHB hydrazide (0.6), 4-hydroxybenzaldehyde (3), 4-hydroxyphenyl pyruvic acid (3), 3-(4-hydroxyphenyl) propionic acid (3), *p*-cresol (3), 3-OHB (3), 4-hydroxypyridine (1), catechol (3), 2-bromophenol (3), 2-chlorophenol (3), 2-fluorophenol (3) and 2-aminophenol (3).

Strain LR7.2^T can be clearly differentiated from its closest known relative, *P. thermopropionicum*, which normally grows in syntrophic association with hydrogen-scavenging methanogens (Imachi *et al.*, 2002). *P. thermopropionicum* grows in pure culture only on pyruvate and fumarate under a hydrogen-free atmosphere. In contrast, strain LR7.2^T grows in pure culture in the presence of hydrogen. Moreover, *P. thermopropionicum* is a thermophilic organism (optimal growth temperature 55 °C) whereas strain LR7.2^T is mesophilic. Finally, the fatty acid compositions of cells of *P. thermopropionicum* and strain LR7.2^T are different. In addition, strain LR7.2^T can be easily differentiated from other related genera (*Desulfotomaculum*, *Moorella*, *Sporotomaculum*) based on morphological, physiological and phylogenetic criteria, as stated by Letowski *et al.* (2001). It also differs from *Desulfotomaculum* species that use sulphite as an electron acceptor. The ability of strain LR7.2^T to use the transformation of phenol and 4-OHB to generate energy for growth is unique. Consequently, based on these phenotypic differences and the phylogenetic analysis, we propose that strain LR7.2^T should be included in a new genus, *Cryptanaerobacter* gen. nov., as *Cryptanaerobacter phenolicus* sp. nov.

Description of *Cryptanaerobacter* gen. nov.

Cryptanaerobacter [Crypt.an.ae'ro.bac.ter. Gr. adj. *kryptos* hidden; Gr. pref. *an* not; Gr. n. *aer* air; *anaero* not (living)

in air; N.L. masc. n. *bacter* rod; N.L. masc. n. *Cryptanaerobacter* an anaerobic rod that is hidden within the consortium].

Gram-positive, anaerobic bacteria in the form of short rods; electron-dense. Sulphate, thiosulphate, nitrate, nitrite, FeCl₃, fumarate and arsenate are not used as electron acceptors. Sulphite is not normally used even though stimulation of growth and 4-OHB transformation activity at low concentrations (up to 2 mM) has been noted under certain culture conditions. The type species is *Cryptanaerobacter phenolicus*.

Description of *Cryptanaerobacter phenolicus* sp. nov.

Cryptanaerobacter phenolicus (phen.o'li.cus. N.L. n. *phenol*-*olis* phenol; N.L. masc. adj. *phenolicus* relating to phenol).

Cells are 1 µm in diameter and 2 µm long. Metabolism of the bacteria is stimulated by supplementing the culture medium with spent medium from *Clostridium sporogenes* M55 (=ATCC 13732=DSM 754). Colonies in soft agar are 1 mm in diameter with diffuse margins and are brownish after 10 days of incubation. Optimum growth occurs under an atmosphere consisting of 10% H₂, 10% CO₂ and 80% N₂ at 30–37 °C and pH 7.5–8.0 but remains low (4 × 10⁶ cells ml⁻¹). Phenol is carboxylated into 4-OHB, 4-OHB is decarboxylated into phenol and 4-OHB is dehydroxylated into benzoic acid. Phenol or 4-OHB is essential for growth; the transformation of phenol or 4-OHB into benzoate produces energy that is conserved for growth. None of the following compounds related to 4-OHB and phenol is transformed or used for growth: 4-hydroxybenzamide, methyl 4-OHB, 4-hydroxysulphonic acid, 4-hydroxyacetophenone, 4-hydroxybenzoic alcohol, hydroquinone, 4-chlorophenol, 4-hydroxycinnamic acid, 4-OHB hydrazide, 4-hydroxybenzaldehyde, 4-hydroxyphenyl pyruvic acid, 3-(4-hydroxyphenyl) propionic acid, *p*-cresol, 3-hydroxybenzoate, 4-hydroxypyridine, catechol, 2-bromophenol, 2-chlorophenol, 2-fluorophenol and 2-aminophenol. Sporulation has not been observed in pure culture or in defined co-culture but the capacity to form spores probably exists, since pasteurization was used during the enrichment process that led to the isolation. The major membrane fatty acid is anteiso-C_{15:0}. The DNA G+C content is 51 mol%.

The type strain, LR7.2^T (=ATCC BAA-820^T=DSM 15808^T), was isolated from a methanogenic consortium that resulted from the mixture of swamp water, sewage sludge, swine waste and soil.

Acknowledgements

This work was supported by the Natural Sciences and Engineering Research Council of Canada (NSERC). We thank L. Racine for providing outstanding technical assistance.

References

- Béchar, G., Bisaillon, J. G., Beaudet, R. & Sylvestre, M. (1990). Degradation of phenol by a bacterial consortium under methanogenic conditions. *Can J Microbiol* **36**, 573–578.
- Bisaillon, J.-G., Lépine, F. & Beaudet, R. (1991a). Study of the methanogenic degradation of phenol via carboxylation to benzoate. *Can J Microbiol* **37**, 573–576.
- Bisaillon, J.-G., Lépine, F., Beaudet, R. & Sylvestre, M. (1991b). Carboxylation of *o*-cresol by an anaerobic consortium under methanogenic conditions. *Appl Environ Microbiol* **57**, 2131–2134.
- Bisaillon, J.-G., Lépine, F., Beaudet, R. & Sylvestre, M. (1993). Potential for carboxylation-dehydroxylation of phenolic compounds by a methanogenic consortium. *Can J Microbiol* **39**, 642–648.
- Bouchard, B., Beaudet, R., Villemur, R., McSween, G., Lépine, F. & Bisaillon, J.-G. (1996). Isolation and characterization of *Desulfotobacterium frappieri* sp. nov., an anaerobic bacterium which reductively dechlorinates pentachlorophenol to 3-chlorophenol. *Int J Syst Bacteriol* **46**, 1010–1015.
- Breitenstein, A., Wiegel, J., Haertig, C., Weiss, N., Andreesen, J. R. & Lechner, U. (2002). Reclassification of *Clostridium hydroxybenzoicum* as *Sedimentibacter hydroxybenzoicus* gen. nov., comb. nov., and description of *Sedimentibacter saalensis* sp. nov. *Int J Syst Evol Microbiol* **52**, 801–807.
- Dennie, D., Gladu, I., Lépine, F., Villemur, R., Bisaillon, J. & Beaudet, R. (1998). Spectrum of the reductive dehalogenation activity of *Desulfotobacterium frappieri* PCP-1. *Appl Environ Microbiol* **64**, 4603–4606.
- Duckett, M.-F. (2000). *Étude d'une souche bactérienne anaérobie capable de transformer le phénol et l'acide 4-hydroxybenzoïque en acide benzoïque*. Masters thesis, INRS – Institut Armand-Frappier, Université du Québec.
- Edwards, U., Rogall, T., Blöcker, H., Emde, M. & Böttger, E. C. (1989). Isolation and direct complete nucleotide determination of entire genes. Characterization of a gene coding for 16S ribosomal RNA. *Nucleic Acids Res* **17**, 7843–7853.
- Gallert, C., Knoll, G. & Winter, J. (1991). Anaerobic carboxylation of phenol to benzoate: use of deuterated phenols revealed carboxylation exclusively in the C4-position. *Appl Microbiol Biotechnol* **36**, 124–129.
- Gerhardt, P., Murray, R. G. E., Wood, W. A. & Krieg, N. R. (editors) (1994). *Methods for General and Molecular Bacteriology*. Washington, DC: American Society for Microbiology.
- Imachi, H., Sekiguchi, Y., Kamagata, Y., Hanada, S., Ohashi, A. & Harada, H. (2002). *Pelotomaculum thermopropionicum* gen. nov., sp. nov., an anaerobic, thermophilic, syntrophic propionate-oxidizing bacterium. *Int J Syst Evol Microbiol* **52**, 1729–1735.
- Knoll, G. & Winter, J. (1989). Degradation of phenol via carboxylation to benzoate by a defined, obligate syntrophic consortium of anaerobic bacteria. *Appl Environ Microbiol* **55**, 318–324.
- Lépine, F., Bisaillon, J.-G., Milot, S., Tawfik, H. K., Beaudet, R. & Villemur, R. (1996). Transformation of phenol into phenylalanine by a methanogenic consortium. *Appl Environ Microbiol* **62**, 809–814.
- Létourneau, L., Bisaillon, J.-G., Lépine, F. & Beaudet, R. (1995). Spore-forming bacteria that carboxylate phenol to benzoic acid under anaerobic conditions. *Can J Microbiol* **41**, 266–272.
- Letowski, J., Juteau, P., Villemur, R., Beaudet, R., Lépine, F. & Bisaillon, J.-G. (2001). Separation of a phenol carboxylating organism from a two-member, strict anaerobic coculture. *Can J Microbiol* **47**, 373–381.

Li, T., Bisailon, J. G., Villemur, R., Létourneau, L., Bernard, K., Lépine, F. & Beaudet, R. (1996). Isolation and characterization of a new bacterium carboxylating phenol to benzoic acid under anaerobic conditions. *J Bacteriol* **178**, 2551–2558.

Mesbah, M. & Whitman, W. B. (1989). Measurement of deoxyguanosine/thymidine ratios in complex mixtures by high-performance liquid chromatography for determination of the mole percentage guanine + cytosine of DNA. *J Chromatogr* **479**, 297–306.

Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989). *Molecular Cloning: a Laboratory Manual*, 2nd edn. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.

Sharak Genthner, B. R., Townsend, G. T. & Chapman, P. J. (1990). Effect of fluorinated analogues of phenol and hydroxybenzoates on the anaerobic transformation of phenol to benzoate. *Biodegradation* **1**, 65–74.

Sharak Genthner, B. R., Townsend, G. T. & Chapman, P. J. (1991). *para*-Hydroxybenzoate as an intermediate in the anaerobic

transformation of phenol to benzoate. *FEMS Microbiol Lett* **78**, 265–269.

Zhang, X. & Wiegel, J. (1990). Isolation and partial characterization of a *Clostridium* species transforming *para*-hydroxybenzoate and 3,4-dihydroxybenzoate and producing phenols as the final transformation products. *Microb Ecol* **20**, 103–121.

Zhang, X. & Wiegel, J. (1994). Reversible conversion of 4-hydroxybenzoate and phenol by *Clostridium hydroxybenzoicum*. *Appl Environ Microbiol* **60**, 4182–4185.

Zhang, X., Morgan, T. V. & Wiegel, J. (1990). Conversion of ¹³C-1 phenol to ¹³C-4 benzoate, an intermediate step in the anaerobic degradation of chlorophenols. *FEMS Microbiol Lett* **67**, 63–66.

Zhang, X., Mandelco, L. & Wiegel, J. (1994). *Clostridium hydroxybenzoicum* sp. nov., an amino acid-utilizing, hydroxybenzoate-decarboxylating bacterium isolated from methanogenic freshwater pond sediment. *Int J Syst Bacteriol* **44**, 214–222.