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NOTE

Reclassification of the only species of the genus *Desulfomonas*, *Desulfomonas pigra*, as *Desulfovibrio piger* comb. nov.

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The growth characteristics, DNA G+C content and sequences of 16S rDNA and the transcribed 16S–23S rDNA internal spacer were determined for *Desulfomonas pigra* ATCC 29098^T^, *Desulfovibrio desulfuricans* subsp. *desulfuricans* strains Essex 6^T^ (= ATCC 29577^T^) and MB (= ATCC 27774) and '*Desulfovibrio fairfieldensis*' ATCC 700045. Despite phenotypic differences (shape and motility) between *Desulfomonas pigra* and *Desulfovibrio* strains, the molecular analysis suggests that *Desulfomonas pigra* should be reclassified within the genus *Desulfovibrio*. Thus, the reclassification is proposed of *Desulfomonas pigra*, the type and only species of the genus, as *Desulfovibrio piger* comb. nov., which implies the emendation of the description of the genus *Desulfovibrio*.

Keywords: *Desulfomonas pigra*, *Desulfovibrio*, sulfate-reducing bacteria

Sulfate-reducing bacteria (SRB) are anaerobic micro-organisms that conduct dissimilatory sulfate reduction to obtain energy. This process leads to the release of hydrogen sulfide, a corrosive and cytotoxic compound. SRB have been isolated mostly from environmental sources, but are also present in the digestive tract (mouth and gut) of animals and humans (Gibson, 1990; Postgate, 1984a; Van der Hoeven *et al*., 1995). Human isolates belong mostly to the genera *Desulfomonas* and *Desulfovibrio* (Gibson *et al*., 1988, 1991; Moore *et al*., 1976; Willis *et al*., 1997). They are anaerobic, Gram-negative rods that contain desulfoviridin (Postgate, 1984a). Both genera belong to the family *Desulfovibrionaceae*, within the *δ-Proteobacteria* (Castro *et al*., 2000). They are phylogenetically closely related to several pathogens, such as *Bilophila wadsworthia* (Baron *et al*., 1989) and *Lawsonia intracellularis* (McOrist *et al*., 1995). Recent findings suggest that SRB may be involved in human disease. They have been proposed to play a role in the pathogenesis of inflammatory bowel diseases (Gibson *et al*., 1988, 1991; Pitcher & Cummings, 1996; Willis *et al*., 1997) and periodontitis (Langendijk *et al*., 2000). *Desulfovibrio* species have also been isolated from profound abscesses (abdominal or brain), blood and urine (La Scola & Raoult, 1999; Loubinoux *et al*., 2000; McDougall *et al*., 1997; Tee *et al*., 1996). In these settings, most strains have been identified as '*Desulfovibrio fairfieldensis*', a recently proposed novel species (Tee *et al*., 1996). *Desulfomonas pigra*, the only species of the genus *Desulfomonas*, and '*Desulfovibrio fairfieldensis*' have been described exclusively in humans to date, whilst *Desulfovibrio desulfuricans*, the type species of the genus *Desulfovibrio*, is also present in the environment. *Desulfomonas pigra* and *Desulfovibrio desulfuricans* differ in phenotypic traits such as cell shape and motility (Moore *et al*., 1976; Postgate, 1984a). However, the inclusion of the former within the genus *Desulfovibrio* has been suggested (Devereux *et al*., 1989; Widdel & Bak, 1992). To clarify the taxonomic status of *Desulfomonas pigra*, we performed a phenotypic and molecular comparison of *Desulfomonas pigra* ATCC 29098^T (= DSM 749^T), *Desulfovibrio desulfuricans* subsp. *desulfuricans* strains Essex 6^T (= ATCC 29577^T = DSM 642^T) and MB (= ATCC 27774) and '*Desulfovibrio fairfieldensis*' ATCC 700045.

Abbreviations: ITS, internal transcribed spacer; SRB, sulfate-reducing bacteria.

The GenBank accession numbers for the 16S rDNA sequences of *Desulfomonas pigra* ATCC 29098^T and *Desulfovibrio desulfuricans* subsp. *desulfuricans* strains Essex 6^T and MB are respectively AF192152–AF192154 and the accession numbers for the ITS sequences of *Desulfomonas pigra* ATCC 29098^T, *Desulfovibrio desulfuricans* subsp. *desulfuricans* strains Essex 6^T and MB and '*Desulfovibrio fairfieldensis*' ATCC 700045 are respectively AY033878–AY033881.

Table 1. Differential utilization of growth substrates by *Desulfomonas pigra* and the most-closely related strains

All strains use lactate, pyruvate, ethanol and hydrogen (growth with hydrogen requires acetate and CO_2 for cell synthesis) and do not use acetate as electron donors. All strains use sulfate, sulfite and thiosulfate and do not use nitrite as electron acceptors.

Substrate	*Desulfomonas pigra* ATCC 29098^T	*Desulfovibrio desulfuricans* subsp. *desulfuricans* strains Essex 6^T and MB	'*Desulfovibrio fairfieldensis*' ATCC 700045
Electron donors (growth on sulfate):			
Formate	−	+	+
Malate	−	+	+
Fumarate	−	+	+
Propanol	−	+	+
Butanol	−	+	+
Use of nitrate as an electron acceptor (growth on lactate)	−	+	+

The strains were grown in Postgate's medium B at 37 °C under anaerobic conditions for molecular analysis (Postgate, 1984a). The electron donors utilized by the strains were determined in a basal medium supplemented with sterile stock solutions (10 mM) and with 10 mM sulfate as the terminal electron acceptor, as described previously (Devereux *et al*., 1990). Hydrogen was added in a mixture with CO_2 (4:1, v/v) by the Hungate technique into the gas phase of half-filled tubes sealed with black rubber stoppers (Widdel & Bak, 1992). The use of electron acceptors was determined with 10 mM sodium lactate as the electron donor. After bacterial inoculation (1 %, v/v), cell growth was checked by measurement of the OD_{600} and regarded as positive if it exceeded 0·1 within 14 days.

DNA extraction for molecular analysis was performed using the phenol/chloroform method (Brenner *et al*., 1982). The G+C content of DNA was determined by reverse-phase HPLC using a Spectra Physics chromatograph with a Supelcosil LC-18S column (Supelco) and a forward Spectra Focus scanning detector (Spectra Physics). Enzymic hydrolysis of DNA samples was carried out using a procedure adapted from methods described previously for tRNAs (Desgres *et al*., 1989). The enzymic hydrolysates were submitted to boronate chromatography to eliminate ribonucleosides (Kuo *et al*., 1990). The remaining deoxyribonucleosides were quantified by HPLC with synthetic N^6-methyldeoxyadenosine as internal standard (Gehrke *et al*., 1990). The 16S rRNA gene was amplified using the consensus primers 27f and 1525r and sequenced as described previously (Loubinoux *et al*., 2000; Tee *et al*., 1996). 16S rDNA sequences from members of *Desulfovibrio* and phylogenetically related strains were obtained from the GenBank database. All 16S rDNA sequences were aligned with CLUSTAL X and a phylogenetic tree was constructed using DENDROGRAF, a program of the Taxotron package (Taxolab Institut Pasteur, Paris, France). The internal transcribed spacer (ITS) region between the 16S and 23S rDNAs was amplified using primers 1525f (5′-GGCTGGATCACCTCCTT-3′) and 23Sr52 (5′-TGCCAAGGCATCCACC-3′) and sequenced as described previously (Gürtler & Stanisich, 1996).

Desulfomonas pigra ATCC 29098^T utilized fewer substrates than did *Desulfovibrio desulfuricans* subsp. *desulfuricans* strains Essex 6^T and MB or '*Desulfovibrio fairfieldensis*' ATCC 700045 (Table 1). The G+C contents of DNA from *Desulfomonas pigra* ATCC 29098^T, *Desulfovibrio desulfuricans* subsp. *desulfuricans* strains Essex 6^T and MB and '*Desulfovibrio fairfieldensis*' ATCC 700045 were respectively 64, 59, 59 and 62 mol%. The phylogenetic tree of *Desulfomonas pigra* ATCC 29098^T and phylogenetically related strains based on comparative analysis of the 16S rDNA sequences showed that *Desulfomonas pigra* is closely related to *Desulfovibrio* species (Fig. 1). The closest relatives were '*Desulfovibrio fairfieldensis*' (96% similarity), *Desulfovibrio desulfuricans* Essex 6^T (96%), *Desulfovibrio desulfuricans* MB (95·5%) and *Desulfovibrio intestinalis* (95%). The lengths of the ITS sequences of *Desulfomonas pigra* ATCC 29098^T, *Desulfovibrio desulfuricans* subsp. *desulfuricans* strains Essex 6^T and MB and '*Desulfovibrio fairfieldensis*' ATCC 700045 were respectively 274, 427, 396 and 529 bp. The ITS sequence of *Desulfomonas pigra* ATCC 29098^T contained one tRNA gene (Ile), whereas the sequences of *Desulfovibrio desulfuricans* subsp. *desulfuricans* strains Essex 6^T and MB and '*Desulfovibrio fairfieldensis*' ATCC 700045 contained two tRNA genes (Ile, Ala).

Cells of *Desulfomonas pigra* are non-motile, straight rods whereas cells of *Desulfovibrio* strains are usually curved, typically comma-shaped, motile rods. The creation of the genus *Desulfomonas* in 1976 relied on this phenotypic difference (Moore *et al*., 1976). A non-motile species, *Desulfovibrio carbinolicus*, has already been included within the genus *Desulfovibrio* (Nanninga & Gottschal, 1987). *Desulfomonas pigra* is usually considered as a commensal bacterium in humans, which may explain the limited interest in this species suggested by only two publications (Moore *et al*., 1976; Sperry & Wilkins, 1977). More recently,

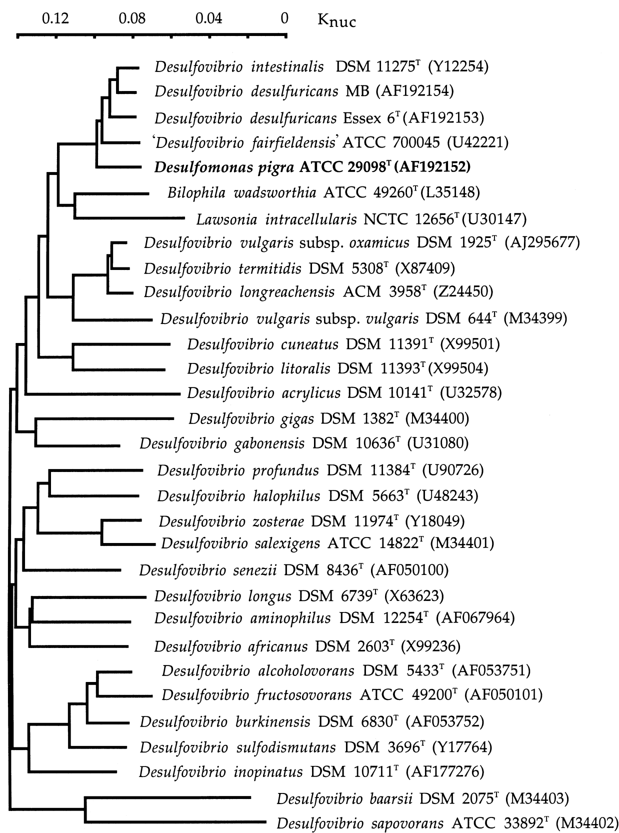

Fig. 1. Phylogenetic tree of *Desulfomonas pigra* ATCC 29098^T (= DSM 749^T) and phylogenetically related species based on comparative analysis of 16S rRNA gene sequences. The dendrogram was generated by using the neighbour-joining algorithm.

however, *Desulfomonas pigra* has attracted more interest as it was found to be the most prevalent species of SRB in faeces of patients with inflammatory bowel disease (Loubinoux *et al.*, 2002). Despite its shape and the absence of motility, *Desulfomonas pigra* shares several important phenotypic features with strains of *Desulfovibrio*, such as the presence of desulfoviridin, cytochrome c_3 and menaquinone MK-6 (Moore *et al.*, 1976; Postgate, 1984a; Sperry & Wilkins, 1977). These biochemical characteristics are usually considered as diagnostic characters for the genus *Desulfovibrio*. Moreover, members of *Desulfomonas* and *Desulfovibrio* oxidize organic compounds incompletely to acetate. Thus, they are not able to grow with acetate as electron donor (Table 1).

The G+C content of the DNA of *Desulfomonas pigra* ATCC 29098^T, *Desulfovibrio desulfuricans* subsp. *desulfuricans* strains Essex 6^T and MB and '*Desulfovibrio fairfieldensis*' ATCC 700045 varies from 59 to 64 mol%. This variation is below 10 mol%, which is currently accepted within a genus (Vandamme *et al.*, 1996). The value of 64 mol% obtained for *Desulfomonas pigra* ATCC 29098^T differs from the value of 66 mol% reported previously for the same strain (Moore *et al.*, 1976). This could be explained by differences in the methods used, because HPLC is a more precise method than thermal denaturation. The major argument for proposing the reclassification of *Desulfomonas pigra* within the genus *Desulfovibrio* relies on the 16S rDNA sequence analysis.

Thus, on the basis of previous work (Devereux *et al.*, 1989; Widdel & Bak, 1992) and our findings, it is proposed that *Desulfomonas pigra*, the type and only species of the genus, be assigned to the genus *Desulfovibrio* as *Desulfovibrio piger* comb. nov.

Emended description of the genus *Desulfovibrio*

Desulfovibrio (De.sul.fo.vi′bri.o. L. pref. *de* from; L. n. *sulfur* sulfur; N.L. masc. n. *Vibrio* a genus name; N.L. masc. n. *Desulfovibrio* a vibrio that reduces sulfur compounds).

The description of the genus *Desulfovibrio* is identical to that given by Postgate (1984b) except for the shape and motility of rods. We propose the genus *Desulfovibrio* to include curved or straight rods, non-motile or motile by means of a single or lophotrichous polar flagellum.

Description of *Desulfovibrio piger* comb. nov.

Desulfovibrio piger (pi′ger. L. adj. *piger* lazy, referring to the limited substrate utilization of the species).

Basonym: *Desulfomonas pigra* Moore *et al.* 1976.

The description is identical to that of Moore *et al.* (1976) except for the G+C content of the DNA, which is 64 mol%. Obligately anaerobic, sulfate-reducing, non-saccharolytic, non-proteolytic, non-spore-forming, non-motile Gram-negative rods that are straight and have rounded ends (0·8–1·0 × 2·5–10·0 µm). Uses lactate, pyruvate, ethanol and hydrogen as electron donors for sulfate reduction, but not acetate. Oxidizes lactate and pyruvate incompletely to acetate. The optimum temperature for growth is 37 °C. Growth is not affected by 20% bile. Colonies on anaerobic blood agar are translucent, 1–2 mm in diameter, circular and non-haemolytic. Cells contain desulfoviridin and cytochrome c_3. Isolated from human specimens (faeces, peritoneal fluids and intra-abdominal collections). The type strain, isolated from human faeces, is ATCC 29098^T (= DSM 749^T).

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