Reclassification of the only species of the genus Desulfononas, Desulfononas 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 Desulfononas pigra ATCC 29098T, Desulfovibrio desulfuricans subsp. desulfuricans strains Essex 6T (= ATCC 29577T) and MB (= ATCC 27774) and ‘Desulfovibrio fairfieldensis’ ATCC 700045. Despite phenotypic differences (shape and motility) between Desulfononas pigra and Desulfovibrio strains, the molecular analysis suggests that Desulfononas pigra should be reclassified within the genus Desulfovibrio. Thus, the reclassification is proposed of Desulfononas 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: Desulfononas pigra, Desulfovibrio, sulfate-reducing bacteria

Sulfate-reducing bacteria (SRB) are anaerobic microorganisms that conduct dissipative 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 Desulfononas 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 β-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). Desulfononas pigra, the only species of the genus Desulfononas, 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. Desulfononas 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 Desulfononas pigra, we performed a phenotypic and molecular comparison of Desulfononas pigra ATCC 29098T (= DSM 749T), Desulfovibrio desulfuricans subsp. desulfuricans strains Essex 6T (= ATCC 29577T = DSM 642T) 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 Desulfononas pigra ATCC 29098T and Desulfovibrio desulfuricans subsp. desulfuricans strains Essex 6T and MB are respectively AF192152–AF192154 and the accession numbers for the ITS sequences of Desulfononas pigra ATCC 29098T, Desulfovibrio desulfuricans subsp. desulfuricans strains Essex 6T 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

<table>
<thead>
<tr>
<th>Substrate</th>
<th><em>Desulfomonas pigra</em> ATCC 29098&lt;sup&gt;T&lt;/sup&gt;</th>
<th><em>Desulfovibrio desulfuricans</em> subs. <em>desulfuricans</em> strains Essex 6&lt;sup&gt;T&lt;/sup&gt; and MB</th>
<th>‘<em>Desulfovibrio fairfieldensis’</em> ATCC 700045</th>
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<tbody>
<tr>
<td>Electron donors (growth on sulfate):</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Formate</td>
<td>−</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Malate</td>
<td>−</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Fumarate</td>
<td>−</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Propanol</td>
<td>−</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Butanol</td>
<td>−</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Use of nitrate as an electron acceptor (growth on lactate)</td>
<td>−</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

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<sub>2</sub> (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<sub>600</sub> 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<sup>9</sup>-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 (Gürtler & Stanisch, 1996).

*Desulfomonas pigra* ATCC 29098<sup>T</sup> utilized fewer substrates than did *Desulfovibrio desulfuricans* subs. *desulfuricans* strains Essex 6<sup>T</sup> and MB or ‘*Desulfovibrio fairfieldensis’* ATCC 700045 (Table 1). The G + C contents of DNA from *Desulfomonas pigra* ATCC 29098<sup>T</sup>, *Desulfovibrio desulfuricans* subs. *desulfuricans* strains Essex 6<sup>T</sup> and MB and ‘*Desulfovibrio fairfieldensis’* ATCC 700045 were respectively 64, 59, 59 and 62 mol%. The phylogenetic tree of *Desulfomonas pigra* ATCC 29098<sup>T</sup> 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<sup>T</sup> (96%), *Desulfovibrio desulfuricans* MB (95.5%) and *Desulfovibrio intestinalis* (95%). The lengths of the ITS sequences of *Desulfomonas pigra* ATCC 29098<sup>T</sup>, *Desulfovibrio desulfuricans* subs. *desulfuricans* strains Essex 6<sup>T</sup> and MB and ‘*Desulfovibrio fairfieldensis’* ATCC 700045 were respectively 274, 427, 396 and 529 bp. The ITS sequence of *Desulfomonas pigra* ATCC 29098<sup>T</sup> contained one tRNA gene (Ile), whereas the sequences of *Desulfovibrio desulfuricans* subs. *desulfuricans* strains Essex 6<sup>T</sup> 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,
Desulfovibrio piger 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, Desulfovibrio piger shares several important phenotypic features with strains of Desulfovibrio, such as the presence of desulfoviridin, cytochrome c₅₅ 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 Desulfovibrio 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 Desulfovibrio piger ATCC 29098ᵀ, Desulfovibrio desulfuricans subsp. desulfuricans strains Essex 6ᵀ 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 Desulfovibrio piger ATCC 29098ᵀ 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 Desulfovibrio piger 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 Desulfovibrio piger, 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.brio. 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.**


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-phaemolytic. Cells contain desulfoviridin and cytochrome c₅₅. Isolated from human specimens (faeces, peritoneal fluids and intra-abdominal collections). The type strain, isolated from human faeces, is ATCC 29098ᵀ (= DSM 749ᵀ).

**Acknowledgements**

Part of this work was supported by an FCT grant POCTI 36562/ESP/2000 to I.A.C.P. We are indebted to the late Dr Wee Tee (University of Melbourne, Australia) for kindly providing the strain ‘Desulfovibrio fairfieldensis’ ATCC 700045. We are grateful to Professor J. Desgres (Laboratoire de Biochimie Spécialisée, Hôpital d’Enfants, CHU de Dijon) for DNA G + C content determinations. We thank D. Meng and A.M. Carpentier (Laboratoire de Bactériologie-Virologie UMR CNRS 7565) for their excellent technical assistance.

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