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 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 *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

Abbreviations: ITS, internal transcribed spacer; SRB, 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 *Desulfovibrio* and *Desulfovibrio* (Gibson et al., 1988, 1991; Moore et al., 1976; Willis et al., 1997). They are anaerobic, Gram-negative rods that contain desulfoviriadin (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). *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 29098T (= DSM 749T), *Desulfovibrio desulfuricans* subsp. *desulfuricans* strains Essex 6T (= ATCC 29577T = DSM 642T) and MB (= ATCC 27774) and ‘Desulfovibrio fairfieldensis’ ATCC 700045.

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The GenBank accession numbers for the 16S rDNA sequences of *Desulfo*-

References


All strains use lactate, pyruvate, ethanol and hydrogen (growth with hydrogen requires acetate and CO₂ 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.

### 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> subsp. <em>desulfuricans</em> strains Essex 6&lt;sup&gt;T&lt;/sup&gt; and MB</th>
<th><em>Desulfovibrio fairfieldensis</em> ATCC 700045</th>
</tr>
</thead>
<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 CO₂ (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) by the 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°-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 & Stansch, 1996).

*Desulfomonas pigra* ATCC 29098<sup>T</sup> utilized fewer substrates than did *Desulfovibrio desulfuricans* subsp. *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* subsp. *desulfuricans* strains Essex 6<sup>T</sup> and MB and *Desulfovibrio fairfieldensis* ATCC 700045 were respectively 64, 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* subsp. *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* subsp. *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 carinolicus*, has already been included within the genus *Desulfovibrio* (Narringer & Gottschall, 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,
however, *Desulfovomonas 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, *Desulfovomonas piger* shares several important phenotypic features with strains of *Desulfovibrio*, such as the presence of desulfoviridin, cytochrome c₃ and menaquinone MK-6 (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 *Desulfovomonas 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 *Desulfovibrio 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).


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 c₃. Isolated from human specimens (faeces, peritoneal fluids and intra-abdominal collections). The type strain, isolated from human faeces, is ATCC 29098T (= DSM 7497).


