Isolation and characterization of *Desulfovibrio burkinensis* sp. nov. from an African ricefield, and phylogeny of *Desulfovibrio alcoholivorans*

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A sulfate-reducing bacterium, strain HDvT (T = type strain), was isolated from an anoxic ricefield soil. Cells were Gram-negative, non-sporulating curved rods motile by means of a single polar flagellum. Cytochrome c₃ and desulfoviridin were present. In the presence of sulfate, glycerol, 1,2- and 1,3-propanediol, dihydroxyacetone, pyruvate, lactate, fumarate, maleate, malate and succinate were incompletely oxidized mainly to acetate. Sulfite, thiosulfate, elemental sulfur, fumarate, maleate and malate were utilized as alternative electron acceptors. In the absence of added electron acceptors, pyruvate, fumarate, maleate, malate and dihydroxyacetone were fermented. The DNA base composition was 67 mol% G+C. The phylogenetic, phenotypic and physiological characteristics of strain HDvT indicate that it is a new species of the genus *Desulfovibrio*, for which the name *Desulfovibrio burkinensis* sp. nov. is proposed; the type strain is HDvT (= DSM 6830). Phylogenetic analysis confirmed that *Desulfovibrio alcoholivorans* was a distinct species supporting the previously published phenotypic data.

**Keywords:** sulfate reduction, ricefields, sulfate-reducing bacteria, *Desulfovibrio burkinensis*, *Desulfovibrio alcoholivorans*

*Desulfovibrio* species are a phylogenetically coherent group of sulfate-reducing bacteria (SRB) which shows great versatility in its ability to oxidize organic compounds including sugars (Ollivier et al., 1988; Trinkerl et al., 1990), amino acids (Stams et al., 1985), and polyols such as glycerol, 1,2- and 1,3-propanediol (Kremer & Hansen, 1987; Nanninga & Gottschal, 1987; Oppenberg & Schink, 1990; Qatibi et al., 1991; Tanaka, 1990). We have investigated the role of SRB in sulfide toxicity observed in many ricefields of the Kou Valley (Burkina Faso, West Africa) and this has led us to isolate a number of strains of SRB capable of using a variety of substrates including acetate, propionate, ethanol and lactate. We report in this paper the characterization of strain HDvT which was previously isolated from this ecosystem (Ouattara et al., 1992) and, based on its phenotypic and phylogenetic characteristics, designate it a new species of *Desulfo-

Strain HDvT was isolated from an anoxic layer (10–25 cm) of ricefield soil of the Kou Valley in Burkina Faso. The in situ temperature averaged 33 °C and the pH was near 7. A medium containing lactate and sulfate was used for enrichment cultures (Ouattara et al., 1992). The techniques for isolation, cultivation and chemical analysis used in this paper have been described previously (Ouattara et al., 1992). DNA was extracted from strain HDvT and *Desulfovibrio alcoholivorans* (DSM 5433) (Redburn & Patel, 1993). The 16S rRNA gene was amplified and purified as described previously (Redburn & Patel, 1993; Andrews & Patel, 1996). Sequencing reactions were prepared by

The GenBank accession numbers for the 16S rDNA sequences of strain HDvT and *Desulfovibrio alcoholivorans* are AF053752 and AF053751, respectively.
A vibrio-shaped isolate, highly motile by means of a single polar flagellum was isolated from enrichment cultures and designated HDvT. Cells stained Gram-negative, occurred singly or in pairs and were 0.8–1.2 \times 2.2–3.1 \, \mu m in size. In stationary-phase cultures, the cells became spirilloid and lost their motility. Spores were not observed. Strain HDvT was strictly anaerobic. The optimum growth temperature was 37 °C and no growth was observed above 42 °C or below 13 °C. The strain grew optimally at pH 6.8 within a pH range of 5.8–8.0. NaCl was not required for growth, and complete inhibition of growth was observed in a medium containing 1% NaCl. No growth occurred in the absence of vitamins. Vitamins could be replaced by yeast extract. Sulfate, sulfite, thiosulfate, elemental sulfur, fumarate, maleate and malate served as electron acceptors; nitrate or ferric iron were not reduced. Various compounds excluding sugars, amino acids and fatty acids were used as electron donors in the presence or the absence of sulfate as an electron acceptor. Growth with molecular hydrogen or formate required acetate as a carbon source. Ethanol, 1,2-propanediol, glycerol, dihydroxyacetone (DHA), pyruvate, lactate and succinate were incompletely oxidized to acetate and \( \text{CO}_2 \) (presumably) in the presence of sulfate. Fumarate, maleate and malate were converted to acetate and traces of succinate. Malate was produced as an intermediate during fumarate and maleate oxidation. 1,3-Propanediol was converted to 3-hydroxypropionate and a trace of...
FICHE DESCRIPTIVE


Titre original : Isolation and characterization of Desulfovibrio burkinensis sp. nov. from an African ricefield, and phylogeny of Desulfovibrio alcoholivorans.


Titre en Français : Isolément et caractérisation de Desulfovibrio burkinensis sp. nov. d’une rizière africaine, et phylogénie de Desulfovibrio alcoholivorans.

Mots-clés matières : Desulfovibrio burkinensis, Desulfovibrio alcoholivorans, Desulfovibrionaceae, sulfato-réduction, anaérobiose, phylogénie, taxonomie, rizières
(10 au plus)

Résumé en Français :
(150 mots maximum)

Plan de classement : Monde végétal et Animal - Fermentations
Desulfovibrio burkinensis sp. nov.

**Table 1.** Comparison of some physiological characteristics between strain HDv\(^T\), *Desulfovibrio alcoholivorans* and *Desulfovibrio carbinolicus*

<table>
<thead>
<tr>
<th>Metabolism</th>
<th>Strain HDv(^T)</th>
<th><em>Desulfovibrio alcoholivorans</em></th>
<th><em>Desulfovibrio carbinolicus</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Electron donors (with sulfate)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1,2-Propanediol</td>
<td>+ Acetate</td>
<td>+ Acetate + propionate</td>
<td>- * ND</td>
</tr>
<tr>
<td>1,3-Propanediol</td>
<td>w 3-HP + acetate</td>
<td>+ Acetate</td>
<td>+ 3-HP</td>
</tr>
<tr>
<td>Ethylene glycol</td>
<td>(+) Glycolaldehyde</td>
<td>(+) Acetate</td>
<td>+ Acetate</td>
</tr>
<tr>
<td>Succinate</td>
<td>w Acetate</td>
<td>+ Acetate</td>
<td>+ Acetate</td>
</tr>
<tr>
<td>Dihydroxyacetone</td>
<td>+ Acetate</td>
<td>-</td>
<td>+ Acetate</td>
</tr>
<tr>
<td>+ Electron acceptors (with lactate as electron donor and carbon source)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fumarate</td>
<td>+ Succinate</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Maleate</td>
<td>+ Succinate</td>
<td>- ND</td>
<td>- ND</td>
</tr>
<tr>
<td>Malate</td>
<td>+ Succinate</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Fermentation (without sulfate)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glycerol</td>
<td></td>
<td>+ 1,3-Propanediol</td>
<td>1,3-Propanediol</td>
</tr>
<tr>
<td>Dihydroxyacetone</td>
<td>+ Acetate</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

† From Nanninga & Gottschal (1987).

acetate (Ouattara *et al.*, 1992). Propanol-1, butanol-1, pentanol-1 were oxidized to propionate, butyrate and valerate, respectively. Formate was presumably oxidized to \(\text{CO}_2\). Ethylene glycol was slightly oxidized to glycolaldehyde without growth. A slight increase in turbidity was observed on 1,3-propanediol, succinate and formate (\(\Delta OD \leq 0.07\)). Strain HDv\(^T\) was able to ferment DHA, pyruvate, fumarate, maleate and malate in the absence of sulfate. DHA and pyruvate were fermented to acetate. Fumarate, maleate and malate were fermented to succinate and acetate. The soluble fraction of strain HDv\(^T\) exhibited the characteristic absorption bands of cytochrome \(c_3\) with maxima at 419, 523 and 553 nm. The oxidized extract showed the cytochrome Soret peak at 408 nm. The cytochrome was not reduced by sodium ascorbate, which indicated that it had a low midpoint redox potential. The spectrum showed a strong absorption band at 628 nm and a weaker one at 582 nm, characteristic of desulfoviridin (Postgate, 1956; Lee & Peck, 1971).

The G+C content of the DNA, determined at the DSMZ as described previously (Magot *et al.*, 1997), was 67 mol\% (mean of three determinations). Using 12 primers, we determined an almost complete sequence consisting of 1540 nucleotides for strain HDv\(^T\) and 1524 nucleotides for *Desulfovibrio alcoholivorans* corresponding to positions 8-1540 and 8-1524, respectively (*Escherichia coli* numbering, according to Winker & Woese, 1991). Phylogenetic analysis revealed that strain HDv\(^T\) and *Desulfovibrio alcoholivorans* were related to species of the genus *Desulfovibrio* and in particular to *Desulfovibrio fructosivorans* with which they shared a similarity of 95%. Strain HDv\(^T\) and *Desulfovibrio alcoholivorans* were also more similar to each other (similarity of 95%) than to other *Desulfovibrio* species (mean similarity of 88%). A dendrogram generated by the neighbour-joining method depicting these relationships is shown in Fig. 1.

Based on 16S rRNA gene sequences and other characteristics, strain HDv\(^T\) is related to the genera *Desulfovibrio* and *Desulfomicrobium* (Pfenning *et al.*, 1981; Widdel, 1988; Rozanova *et al.*, 1988). Since desulfoviridin and cytochrome \(c_3\) are present in the soluble extract of the isolate, it can be assigned to the genus *Desulfovibrio* (Widdel, 1988). The morphological and physiological characteristics of strain HDv\(^T\) are similar to those of *Desulfovibrio alcoholivorans* (Qatibi *et al.*, 1991), *Desulfovibrio carbinolicus* (Nanninga & Gottschal, 1987) (Table 1) and *Desulfovibrio fructosivorans* (Ollivier *et al.*, 1988). However, strain HDv\(^T\) is phylogenetically distinct from *Desulfovibrio fructosivorans* and *Desulfovibrio alcoholivorans* and cannot be regarded as a strain of these species. *Desulfovibrio carbinolicus* is non-motile, is unable to oxidize 1,2-propanediol, and converts glycerol and 1,3-propanediol to 3-hydroxypropionate in the presence of sulfate. Strain HDv\(^T\) oxidizes glycerol to acetate and
1,3-propanediol to a mixture of 3-hydroxypropionate and acetate in the presence of sulfate. In addition, strain HDvT cannot ferment glycerol but both Desulfovibrio fructosovorans and Desulfovibrio carboxidicus can. On the account of the differences mentioned above, we propose that strain HDvT be classified as the type strain of a new species of genus Desulfovibrio, Desulfovibrio burkinensis sp. nov. We also confirm that Desulfovibrio alcoholivorans described by Qatibi et al. (1991) is a distinct species based on the phylogenetic evidence presented in this report.

Description of Desulfovibrio burkinensis sp. nov.

Desulfovibrio burkinensis (bur.ki.nen’sis. N.L. adj. burkinensis pertaining to Burkina Faso, West Africa).

Cells are curved rods, motile by a single polar flagellum, 0.8–1.2 μm wide and 2.2–3.1 μm long. They occur singly or in pairs and become non-motile and spiraloid in stationary phase cultures. Cells do not form spores and stain Gram-negative. Optimum growth occurs at 37 °C and at pH 6.8. NaCl is not required for growth. Strictly anaerobic. Elemental sulfur, sulfite, thiosulfate, sulfate, fumarate, maleate and malate serve as electron acceptors; hydrogen sulfide and succinate are the end products of inorganic and organic electron acceptors, respectively. Nitrate or ferric iron are not reduced. Molecular hydrogen, formate, lactate, pyruvate, fumarate, maleate, malate, succinate, dihydroxyacetone, glycerol, 1,3-propanediol, 1,3-propanediol, ethanol, propanol-1, butanol-1, pentanol-1 serve as electron donors. Growth with hydrogen and formate requires acetate as carbon source. Ethylene glycol is oxidized without growth. Formate, succinate and 1,3-propanediol yield very slight growth. Pyruvate, dihydroxyacetone, fumarate, maleate, malate are fermented. Not used: acetate, propionate, butyrate, fructose, citrate, oxalate, oxamate, choline, benzoate. Vitamins are required for growth but could be replaced by yeast extract. Sodium chloride is not required and inhibits growth above 1% (w/v). Desulfoviridin and cytochrome c₅₇ are present. DNA base composition: 67 mol% G+C (HPLC). Isolated from an anoxic layer of a ricefield soil in Burkina Faso. Type strain: strain HDvT (= DSM 6830T), deposited in the German Collection of Microorganisms (DSMZ), Braunschweig, Germany.

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References


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