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NOTE

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 HDv^T (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 c_3 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 HDv^T 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 HDv^T (= DSM 6830^T). 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 HDv^T 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 *Desulfovibrio*, *Desulfovibrio burkinensis* sp. nov. We have also included a phylogenetic analysis of *Desulfovibrio alcoholivorans*, a species that was studied earlier in the laboratory of some of the authors (Qatibi *et al.*, 1991). In addition, we have corrected the epithets in the names *Desulfovibrio alcoholovorans* and *Desulfovibrio fructosovorans* to *Desulfovibrio alcoholivorans* and *Desulfovibrio fructosivorans*, respectively.

Strain HDv^T 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 HDv^T 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 HDv^T and *Desulfovibrio alcoholivorans* are AF053752 and AF053751, respectively.

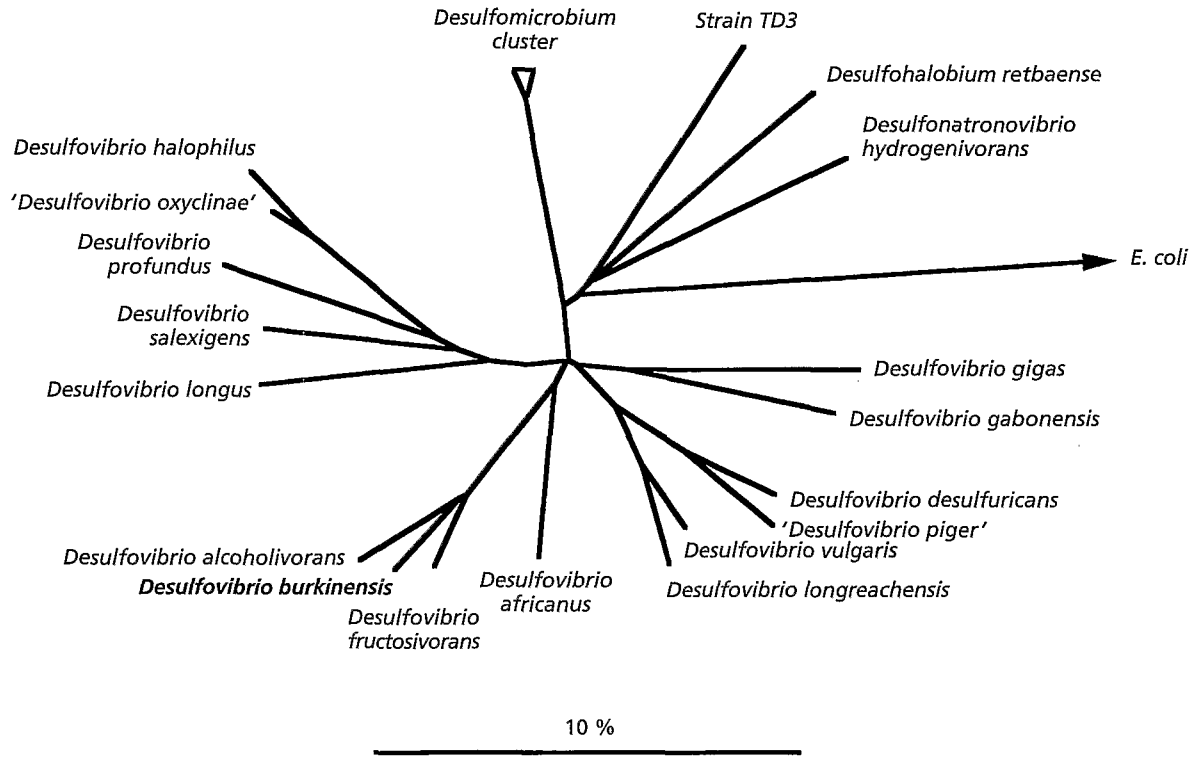

Fig. 1. Phylogenetic relationships of strain HDv^T and *Desulfovibrio alcoholivorans* with members of the family *Desulfovibrionaceae*, and other related genera. Sequences were extracted from the RDP (Maidak *et al.*, 1996), with the exception of the sequences for *'Desulfovibrio oxyclinae'*, *Desulfovibrio profundus*, *Desulfovibrio fructosivorans* and *Desulfonatronovibrio hydrogenivorans* which were extracted from GenBank (accession nos U33316, U90726 and AF050101) and EMBL (accession no. X99234), respectively. Bar, 10 nucleotide changes per 100 nucleotides.

using a Prism dideoxy terminator cycle sequencing kit and 12 primers as recommended by the manufacturer and the products were separated using an ABI 373 automated DNA sequencer (both from Applied Biosystems). The determined 16S rDNA sequences were manually aligned with reference sequences of various members of the domain *Bacteria* by using the alignment editor ae2 (Maidak *et al.*, 1996). All sequences were obtained from the Ribosomal Database Project (RDP) (Maidak *et al.*, 1996) with the exception of sequences from *'Desulfovibrio oxyclinae'*, *Desulfovibrio profundus* and *Desulfovibrio fructosivorans* which were extracted from the GenBank (accession nos U33316, U90726 and AF050101, respectively). Positions of sequence and alignment uncertainty were omitted from the analysis. Pairwise evolutionary distances based on 895 unambiguous nucleotides were computed by using the method of Jukes & Cantor (1969) and dendrograms were constructed from these distances by using the neighbour-joining method. Both programs form part of the PHYLIP package (Felsenstein, 1993). Bootstrap analysis was also performed using the PHYLIP package.

A vibrio-shaped isolate, highly motile by means of a single polar flagellum was isolated from enrichment cultures and designated HDv^T. Cells stained Gram-negative, occurred singly or in pairs and were 0·8–1·2 × 2·2–3·1 μm in size. In stationary-phase cultures, the cells became spirilloid and lost their motility. Spores were not observed. Strain HDv^T 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 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

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Titre en Français : Isolement 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
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Table 1. Comparison of some physiological characteristics between strain HDv^T, *Desulfovibrio alcoholivorans* and *Desulfovibrio carbinolicus*

Abbreviations: 3-HP, 3-hydroxypropionate; ND, not determined; +, growth; −, no growth; (+), utilization without growth; W, weak growth.

Metabolism	Strain HDv^T		*Desulfovibrio alcoholivorans**		*Desulfovibrio carbinolicus*†	
	Growth	Product(s)	Growth	Product(s)	Growth	Product(s)
Electron donors (with sulfate)						
1,2-Propanediol	+	Acetate	+	Acetate + propionate	−*	ND
1,3-Propanediol	W	3-HP + acetate	+	Acetate	+	3-HP
Ethylene glycol	(+)	Glycolaldehyde	(+)	Acetate	+	Acetate
Succinate	W	Acetate	+	Acetate	+	Acetate
Dihydroxyacetone	+	Acetate	−	−	+	Acetate
Electron acceptors (with lactate as electron donor and carbon source)						
Fumarate	+	Succinate	−	−	−	−
Maleate	+	Succinate	−	ND	−	ND
Malate	+	Succinate	−	−	−	−
Fermentation (without sulfate)						
Glycerol	−		−		+	1,3-Propanediol + 3-HP
Dihydroxyacetone	+	Acetate	ND		ND	

* From Qatibi *et al.* (1991).

† 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 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 ($\Delta OD \leqslant 0{\cdot}07$). 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* (Pfennig *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 HDv^T cannot ferment glycerol but both *Desulfovibrio fructosivorans* and *Desulfovibrio carbinolicus* can. On the account of the differences mentioned above, we propose that strain HDv^T 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 spirilloid 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, 1,2-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 c_3 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 HDv^T (= DSM 6830^T), deposited in the German Collection of Microorganisms (DSMZ), Braunschweig, Germany.

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