Desulfovibrio marrakechensis sp. nov., a 1,4-tyrosol-oxidizing, sulfate-reducing bacterium isolated from olive mill wastewater

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A novel mesophilic sulfate-reducing bacterium, EMSSDQ$_4$^{T}, was isolated from olive mill wastewater in the semi-arid region of Morocco (Marrakech). Cells were Gram-negative, catalase-positive, straight rods that were non-motile and non-spore-forming and contained cytochrome c_3 and desulfoviridin. The DNA G+C content was 65.1 mol%. Phylogenetic analysis based on 16S rRNA gene sequences revealed that the isolate was a member of the genus *Desulfovibrio* with *Desulfovibrio carbinoliphilus* D41^T, *Desulfovibrio alcoholivorans* SPSN^T, *Desulfovibrio fructosivorans* JJ^T and *Desulfovibrio carbinolicus* EDK82^T as the most closely related strains with validly published names. In addition to the classical substrates used by *Desulfovibrio* species, the isolate oxidized 1,4-tyrosol, one of the most abundant phenolic compounds occurring in olive mill wastewater, to 4-hydroxyphenylacetate without ring cleavage. *D. alcoholivorans* SPSN^T was also found to carry out this reaction. Under air, strain EMSSDQ$_4$^{T} exhibited limited growth on lactate and yeast extract in the absence of sulfate. On the basis of genotypic and phenotypic characteristics, it is proposed that the isolate represents a novel species, *Desulfovibrio marrakechensis* sp. nov. The type strain is EMSSDQ$_4$^{T} (=DSM 19337^T =ATCC BAA-1562^T).

Olive oil manufacturing results annually in the production of more than 3×10^7 m^3 black olive mill wastewater (OMW), which has a high polluting organic load due to the high content of organic substances, including sugars, tannins, polyphenols, polyalcohols, pectins, lipids and a wide variety of simple aromatic compounds, resulting from olive cell-wall degradation during the oil extraction process (Labat *et al.*, 2000; Lesage-Meessen *et al.*, 2001). OMW is frequently discharged into evaporation ponds, dumped directly in rivers or spread on soil, resulting in a major environmental problem in the main olive-producing countries of the Mediterranean region, mainly those in the south, such as Morocco. Microbiological studies on OMW and digesters treating these effluents have revealed the presence of metabolically diverse anaerobic bacteria (Mechichi *et al.*, 2000; Chamkha *et al.*, 2001; Thabet *et al.*, 2004), all of which have been identified as anaerobic species belonging to the genus *Clostridium*.

In the course of a study on microbiological biodiversity in an aeration basin (a very large pond used formerly as an

The authors dedicate this paper to the memory of Professor Norbert Pfennig (1925–2008).

Abbreviation: OMW, olive mill wastewater.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain EMSSDQ$_4$^{T} is AM947130.

Photomicrographs of cells of strain EMSSDQ$_4$^{T} and growth curves of strain EMSSDQ$_4$^{T} and related strains cultivated in air in the absence of sulfate are available as supplementary material with the online version of this paper.

underground gallery for water for irrigation of the desert) located 10 km north-east of Marrakech city in Morocco (31° 38′ 52″ N 7° 54′ 7.3″ W) and used to treat OMW by natural evaporation, several aerobic and anaerobic bacteria that are resistant to the simple aromatic compounds present in these effluents have been isolated and characterized. One of them has been identified recently as belonging to a new genus (Liebgott *et al.*, 2008) and is a Gram-positive, anaerobic, rod-shaped, spore-forming bacterium that is unable to carry out dissimilatory sulfate reduction. In the present paper, strain EMSSDQ$_4^T$, a novel Gram-negative, anaerobic, straight-rod-shaped, non-spore-forming, sulfate-reducing bacterium that oxidizes 1,4-tyrosol to 4-hydroxyphenylacetate, which was isolated from the same OMW evaporation pond, is described. To our knowledge, this is the first bacterium to be described that is capable of performing this metabolic process. 1,4-Tyrosol is one of the major simple aromatic compounds present in OMW (Fernandez-Bolanos *et al.*, 1998; Mulinacci *et al.*, 2001) and exhibits toxicity towards several micro-organisms (Capasso *et al.*, 1995; Sayadi *et al.*, 2000). It has been reported that only aerobic bacteria under oxic conditions are able to oxidize 1,4-tyrosol (Allouche *et al.*, 2004; Abdelkafi *et al.*, 2005; Liebgott *et al.*, 2007).

The basal medium used consisted of (l^{-1} distilled water): 0.5 g K_2HPO_4, 0.5 g KH_2PO_4, 0.5 g NH_4Cl, 0.4 g NaCl, 0.05 g $CaCl_2 . 2H_2O$, 0.3 g $MgCl_2 . 6H_2O$, 0.1 g KCl, 0.25 g cysteine hydrochloride, 0.1 g yeast extract (Difco), 1 ml trace element mineral solution (Widdel & Pfennig, 1981) modified by Imhoff-Stuckle & Pfennig (1983) and 1 ml 0.1 % (w/v) resazurin. The pH was adjusted to 7.2 with 10 M KOH. Unless otherwise indicated, aliquots of 9 ml were dispensed into Hungate tubes under a stream of O_2-free N_2/CO_2 (80 : 20, v/v) gas, closed with butyl rubber stoppers and subsequently autoclaved. Prior to inoculation, $NaHCO_3$ (30 mM), $Na_2S . 9H_2O$ (1.7 mM) and 1 ml filter-sterilized vitamin solution l^{-1} (Balch *et al.*, 1979) were added. For solid roll-tube media, 2 % (w/v) agar was added.

Samples of OMW (total content of phenols 2770–2820 mg l^{-1}), collected as described by Liebgott *et al.*, (2008) during the 2004–2005 harvest season at a depth of 60 cm, were inoculated (10 %, v/v) into basal medium containing 10 mM lactate and 20 mM sulfate and then incubated for 2–3 weeks at 37 °C. After several transfers under the same conditions and using repeated agar serial dilutions, brown colonies, approximately 1–2 mm in diameter, were observed within 1 week of incubation in agar roll-tubes (Hungate, 1969). Well-separated colonies were picked from the highest dilutions (10^{-10}) and transferred to liquid basal medium containing 10 mM lactate and 20 mM sulfate. The purity of the culture was checked routinely by phase-contrast microscopy and by performing growth tests in basal medium containing 1 % (w/v) glucose, 1 % (w/v) yeast extract and 1 % (w/v) biotrypticase. Seven pure strains were obtained and subsequently shown to be similar by amplified rRNA gene restriction analysis profiles (data not shown). One strain, designated EMSSDQ$_4^T$, was studied in detail. All physiological and metabolic tests were performed in triplicate at 37 °C (except for temperature experiments) using cells growing on lactate and sulfate. Unless otherwise indicated, positive growth was determined by monitoring changes in OD_{580} and by production of sulfides (Cord-Ruwisch, 1985), compared with negative controls.

Cells of strain EMSSDQ$_4^T$ were non-motile, straight rods with rounded ends, approximately 1.3–1.6 μm in diameter and 3–4 μm in length, occurring either singly or in pairs. Motility was not observed by optical microscopy, even in young cells during their first step of exponential growth. The isolate did not possess a flagellum, as clearly shown by electron microscopy (Supplementary Fig. S1, available in IJSEM Online). Cells were Gram-negative, oxidase-negative and catalase-positive. Spores were not observed either by microscopic examination or in heat-resistance experiments (Liebgott *et al.*, 2007).

Optimal growth conditions were determined in basal medium containing 10 mM lactate and 20 mM sulfate as described by Qatibi *et al.* (1991). Optimum growth temperature was estimated to be 37 °C. No growth was observed at temperatures above 50 °C or below 20 °C. Growth was optimal at pH 7, with growth occurring between pH 6.5 and 8.5. When tested at various NaCl concentrations, growth was observed between 0 and 35 g l^{-1}, with optimum growth in the absence of NaCl.

Reduction of different electron acceptors was performed in basal medium with 10 mM lactate and supplied with distinct electron acceptors: 20 mM sulfate, 10 mM sulfite, 10 mM thiosulfate, 1 % (w/v) elemental sulfur, 5 mM nitrate, 5 mM nitrite and 10 mM fumarate. Positive growth with the latter three electron acceptors was estimated by monitoring changes in optical density. In addition to sulfate, sulfite, thiosulfate, elemental sulfur and fumarate also served as electron acceptors. The isolate did not reduce nitrate or nitrite.

Because several aerobic bacteria were also isolated (see for example Liebgott *et al.*, 2007) from the same site as strain EMSSDQ$_4^T$, the capacity of the isolate to tolerate oxygen (air) was assessed; cysteine hydrochloride and sulfides were omitted from the basal medium for these experiments. Cultivation was carried out in tubes containing 4.5 ml basal medium with 10 mM lactate under an air atmosphere corresponding to two-thirds of the culture volume (the calculated amount of oxygen in the headspace was 80 μmol) and incubated horizontally using 10 % (v/v) inoculum growing in basal medium containing 10 mM sulfate and 20 mM lactate (to ensure that sulfate derived from the inoculum was completely consumed); after inoculation, the sulfide concentration was approximately 0.5 mM. Positive growth was determined by monitoring changes in optical density. In the presence of air, some limited growth ($OD_{580} \leqslant 0.3$) was observed in the absence of sulfate (Supplementary Fig. S2); the maximum growth

rate and the doubling time were approximately 0.008 h^{-1} and 90 h, respectively. Increased growth ($OD_{580} \leqslant 0.47$, 4.65 mM sulfide) of the isolate on 10 mM lactate in the presence of 20 mM sulfate was observed as it shifted back to anaerobic conditions; the maximum growth rate and the doubling time were approximately 0.16 h^{-1} and 5 h, respectively. Under aerobic conditions, cells became elongated (results not shown) and growth was poor or absent after a second transfer. From these preliminary tests, it can be assumed that the isolate was at least able to survive oxygen exposure. It would be interesting to investigate more fully whether oxygen served as a direct or indirect electron acceptor by the isolate.

Aromatic compounds (filter-sterilized) were tested at a final concentration of 5 mM in modified basal medium with increased yeast extract (0.05 %, w/v) and 20 nM sodium selenite; non-inoculated tubes were observed under the same conditions to verify that aromatic compounds were not partially transformed abiotically. In addition to sulfide production and optical density, positive growth was also determined by analysis of degradation products. Aromatic compounds were analysed by HPLC using a Waters apparatus composed of a 1525 binary pump, a 2996 diode array detector, a Rheodyne injector (model 7725i) fitted with a 20 µl loop, a temperature control system and an in-line degasser. Separations were carried out on a Symmetry C_{18} reversed-phase column (4.6 × 150 mm, ODS2, 5 µm particle size). Elution was performed at 30 °C with a flow rate of 0.8 ml min^{-1} using a linear gradient of acetonitrile (A) in water acidified with 1 % (v/v) acetic acid (B) in two steps: the first step was from 5 to 20 % A for 25 min and the second step was from 20 to 100 % A for 5 min (total 30 min). Aromatic compounds were visualized at 280 nm and total spectra from each peak were analysed from 200 to 400 nm. Peaks were determined by comparing their retention times and respective UV spectra online (diode array detection) with commercial standards, except for *o*-methylgallate, which is not available commercially and was synthesized according to Scheline (1966).

Utilization of 21 simple aromatic compounds present in OMW in the presence of sulfate as electron acceptor was tested. Strain $EMSSDQ_4^T$, *Desulfovibrio alcoholivorans* $SPSN^T$, *Desulfovibrio fructosivorans* JJ^T and *Desulfovibrio carbinolicus* $EDK82^T$ were negative for utilization of phenol, catechol, resorcinol, 4-hydroxyphenylacetate, benzoate, 4-hydroxybenzoate, 3-hydroxybenzoate, protocatechuate, syringate, gallate, *o*-methylgallate, veratrate, *p*-anisate, cinnamate, *p*-, *o*- and *m*-coumarate, vanillate, ferulate and caffeate; the phenolic compound 1,4-tyrosol was utilized by strain $EMSSDQ_4^T$ and *D. alcoholivorans* $SPSN^T$ only (Table 1). 1,4-Tyrosol was oxidized by both these strains to 4-hydroxyphenylacetate at 100 % recovery leaving the aromatic ring intact (5 mmol 1,4-tyrosol was oxidized by strain $EMSSDQ_4^T$ and *D. alcoholivorans* $SPSN^T$ resulting in production of approximately 3.1 mmol and 2.47 mmol sulfide, respectively). These observations strongly suggest the following oxidation equation:

$$C_8O_2H_{10} + 0.5SO_4^{2-} \rightarrow C_8O_3H_8 + 0.5S^{2-} + H_2O$$

4-Hydroxyphenylacetate was not metabolized further by either strain even after more than 1 month of incubation. Under fermentation conditions, both strains were unable to utilize 1,4-tyrosol. It is interesting to note that, in the presence of sulfate, both strains were unable to metabolize other phenolic compounds such as, for example, unsubstituted phenol, 2-hydroxyphenol (catechol), 3-hydroxyphenol (resorcinol), 4-hydroxycinnamate, 4-hydroxybenzoate and 4-hydroxyphenylacetate, indicating that the composition and the position of the side chain were determinants for substrate specificity. A primary alcohol side chain in the *para* position to the hydroxyl group was required for the transformation of aromatic substrates.

When phylogenetic analysis revealed that strain $EMSSDQ_4^T$ was related to some *Desulfovibrio* species (see below), other simple substrates commonly used by these bacteria were tested. Substrate utilization was tested in basal medium in the presence of 20 mM sulfate (except for fermentation tests) containing different electron donors at a final concentration of 10 mM. Formate, methanol and hydrogen (H_2/CO_2 at 80 : 20 %, v/v) were tested in the absence or presence of 5 mM acetate as carbon source. Yeast extract, peptone, Casamino acids and biotrypticase were tested at a final concentration of 0.5 g l^{-1}. Lactate and acetate were analysed by HPLC using a Bio-Rad Aminex HPX-87H column, with 0.005 M sulfuric acid as eluent (elution rate of 0.6 ml min^{-1}) and an operating temperature of 50 °C. A UV detector (Merck) set at 210 nm was used. Growth of the isolate occurred in the absence of vitamins or yeast extract after three transfers. Several non-aromatic substrates were used in the presence of sulfate including ethanol, 2-methoxyethanol, 1-propanol, 2-propanol, 1-butanol, 1,2-propanediol, 1,3-propanediol, 1,4-butanediol, hydrogen, formate, lactate, pyruvate, fumarate and malate. With the latter three substrates, small amounts of sulfide were produced ($\leqslant$1.5 mM). When sulfate was replaced by sulfite (10 mM), which is the energetically more favourable electron acceptor, two to three times more sulfide was produced. Lactate was oxidized to acetate and presumably carbon dioxide. Growth with molecular hydrogen and formate required acetate as carbon source. Growth with formate, 1,4-butanediol and 2-propanol was weak. In the absence of sulfate, fumarate, malate and pyruvate could be fermented. No growth was observed on acetate, propionate, butyrate, methanol, 2-butanol, glycerol, ethylene glycol, 2,3-butanediol, crotonate, alanine, glycine, arginine, lysine, choline, lactose, sucrose, glucose, fructose, maltose, mannose, ribose, xylose, yeast extract, biotrypticase, Casamino acids or peptone.

Pigments were assayed as described previously (Qatibi *et al.*, 1991), except that cells were disrupted by ultrasound cycles and the resulting cell-free extract was concentrated in an Amicon ultrafiltration cell (10 kDa pore size) before

Table 1. Differential characteristics of strain EMSSDQ$_4$^{T} and type strains of related *Desulfovibrio* species

Strains: 1, *D. marrakechensis* sp. nov. EMSSDQ$_4$^{T} (data from this study); 2, *D. carbinoliphilus* D41^T (Allen *et al.*, 2008) 3, *D. alcoholivorans* SPSN^T (Qatibi *et al.*, 1991); 4, *D. fructosivorans* JJ^T (Ollivier *et al.*, 1988; Qatibi *et al.*, 1991); 5, *D. carbinolicus* EDK82^T (Nanninga & Gottschal, 1987; Qatibi *et al.*, 1991). +, Positive; −, negative; (+), weakly positive; ND, no data available.

Character	1	2	3	4	5
Morphology	Straight rods	Curved rods	Curved rods	Vibrioid rods	Rod-shaped
Motility	−	+	+	+	−
Cell length (μm)	3–4	2.4–3.0	2.8–3.2	2–4	1.5–5.0
Cell diameter (μm)	1.3–1.6	0.6–0.7	0.7–0.9	0.5–0.7	0.6–1.1
DNA G+C content (mol%)	65.1	63	64.5	64.1	65
Growth temperature (°C)					
Range	20–50	22–36	20–42	20–45	5–44
Optimum	37	30	35–37	35	37–38
pH for growth					
Range	6.5–8.5	5.8–8.4	5.5–8.5	ND	5.3–8.7
Optimum	7	7–8	7	6.5–7	7–7.3
NaCl concentration for growth (g l^{-1})					
Range	0–35	ND	0–20	0 to <40	ND
Optimum	0	ND	5–10	0	0
Electron donors					
Formate plus acetate	(+)	+	+	+	(+)
Succinate	−	−	+	−	(+)
Methanol	−	(+)	(+)	−	+
2-Propanol	(+)	(+)	−	−*	−
2-Butanol	−	+	−	−	−
Glycerol	−	−	+	+	+
1,2-Propanediol	(+)	+	+	−†	−†
Ethylene glycol	−	+	(+)	+	+
1,4-Tyrosol	+	ND	+*	−*	−*
Fructose	−	−	−	+	−
Fermentation of:					
Fumarate	+	−	+	+	+
Malate	+	−	+	+	+
Glycerol	−	−	−	+	+
Fructose	−	−	−	+	−
Electron acceptors					
Fumarate	+	−	−	+	−
Elemental sulfur	+	−	+	+	(+)

*Tested in the present study.
†Degraded with glycerol as co-substrate in the absence of sulfate (Qatibi *et al.*, 1998).

examination for cytochrome c_3 and desulfoviridin. The spectrum of the oxidized extract showed a characteristic absorption band at 629 nm, indicating the presence of desulfoviridin. When reduced by sodium dithionite, the spectrum of cell-free extracts exhibited the characteristic absorption bands of cytochrome c_3, with maxima at 415.0, 522.5 and 553.0 nm.

The DNA G+C content of strain EMSSDQ$_4$^{T}, determined by HPLC (Mesbah *et al.*, 1989) at the Identification Service of the DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany), was 65.1 mol%, which is within the range reported for *Desulfovibrio* species (48–65 mol%).

Phylogenetic analysis was carried out as follows. DNA from strain EMSSDQ$_4$^{T} was extracted using the DNeasy tissue kit according to the manufacturer's protocol (Qiagen). Purified PCR product was sequenced using the CEQTMDTCS-Quick Start kit (Beckmann Coulter) as directed in the manufacturer's protocol. Sequence reactions were electrophoresed using the CEQTM 8000 genetic analysis system. Primers F (5′-GAGTTTGATCCTGGCTCA-3′) and R (5′-AGAAAGGAGGTGATCCAGCC-3′) were used to amplify the 16S rRNA gene. The nucleotide sequence was aligned manually using the sequence alignment editor ae2 (Maidak *et al.*, 2001). Sequences used in the phylogenetic analysis were obtained from the RDP (Maidak *et al.*, 2001) and GenBank (Benson *et al.*, 1999).

Pairwise evolutionary distances were calculated using the method of Jukes & Cantor (1969). A dendrogram was constructed using the neighbour-joining method (Saitou & Nei, 1987). Confidence in the tree topology was determined using 1000 bootstrapped trees (Felsenstein, 1985). Analysis of the complete sequence (1539 bp) of the 16S rRNA gene of strain EMSSDQ$_4^T$ revealed that the isolate was related to species of the genus *Desulfovibrio* in the family *Desulfovibrionaceae* in the *Deltaproteobacteria*. It represents a new sublineage (supported by a bootstrap value of 100 %) within the genus *Desulfovibrio* (Fig. 1) and, in particular, forms a subline with a subcluster of species consisting of *Desulfovibrio carbinoliphilus* D41^T (97.5 % sequence similarity with the type strain; Allen *et al.*, 2008), *D. alcoholivorans* SPSN^T (97.4 %; Qatibi *et al.*, 1991), *D. fructosivorans* JJ^T (97.2 %; Ollivier *et al.*, 1988), *D. carbinolicus* EDK82^T (96.7 %; Nanninga & Gottschal, 1987), *Desulfovibrio burkinensis* HDv^T (96.0 %; Ouattara *et al.*, 1999), *Desulfovibrio magneticus* RS-1^T (95.6 %; Sakaguchi *et al.*, 2002) and *Desulfovibrio sulfodismutans* ThAc01^T (94.2 %; Bak & Pfennig., 1987) Strain EMSSDQ$_4^T$ shared a branching node with *D. fructosivorans* JJ^T, although this relationship was not supported by a statistically significant bootstrap value (67 %), demonstrating that this is not a particularly specific relationship. A 16S rRNA gene sequence similarity of 97 % is commonly considered as the upper limit for the definition of separate species (Stackebrandt & Goebel, 1994). Although more than 97 % similarity indicates that strains may belong to the same species, it is now generally acknowledged that this rule does not always apply for several *Desulfovibrio* species. Furthermore, Table 1 shows that strain EMSSDQ$_4^T$ exhibited several characteristics that clearly distinguish it from closely related species with validly published names.

Strain EMSSDQ$_4^T$ differed from *D. carbinoliphilus* D41^T (isolated from a gas condensate-contaminated aquifer), *D. alcoholivorans* SPSN^T (isolated from a bioethanol production plant) and *D. fructosivorans* JJ^T (isolated from estuarine sediment) in its cell shape and motility; unlike these three motile *Desulfovibrio* species, cells of strain EMSSDQ$_4^T$ were non-motile, straight rods. Two species of non-motile, straight rods, *D. carbinolicus* and *Desulfovibrio piger* (Loubinoux *et al.*, 2002), isolated from an anaerobic purification plant and human faeces, respectively, have already been included in the genus *Desulfovibrio*. Moreover, in contrast to the three motile *Desulfovibrio* species, the isolate did not possess a flagellum. It is improbable that the isolate was motile with a corkscrew-like motion as has been observed in some spirilloid *Desulfovibrio* species. Strain EMSSDQ$_4^T$ also differed from *D. carbinoliphilus* D41^T in its DNA G+C content, its temperature range and optimum temperature for growth, its inability to grow with methanol and 2-butanol and its ability to use fumarate and elemental sulfur as electron acceptors. Additionally, in contrast to *D. carbinoliphilus* D41^T, the isolate fermented fumarate and malate. Strain EMSSDQ$_4^T$ differed from *D. alcoholivorans* SPSN^T by its temperature range and optimum salinity for growth, its inability to grow with succinate, methanol and glycerol and its ability to grow on 2-propanol and to use fumarate as electron acceptor. The isolate also differed from *D. fructosivorans* JJ^T by its inability to use fructose and glycerol (in the presence or absence of sulfate) and its ability to use 1, 2-propanediol, 1,4-tyrosol and 2-propanol. Unlike the non-motile *D. carbinolicus* EDK82^T, strain EMSSDQ$_4^T$ was unable to use methanol, glycerol (in the presence or absence of sulfate) and succinate, was able to use 1,2-propanediol, 1,4-tyrosol and 2-propanol as substrates and was able to use fumarate as electron acceptor. The temperature growth range also differed for the two strains. On the other hand, it is important to note that, even though *D. alcoholivorans* SPSN^T, *D. carbinolicus* EDK82^T, *D. fructosivorans* JJ^T (present study) and *Desulfovibrio magneticus* RS-1^T (Sakaguchi *et al.*, 2002) were also catalase-positive, they could not be cultivated in the presence of oxygen (air). This enzyme was supposed to be one of the two enzymes responsible for hydrogen peroxide elimination in *Desulfovibrio* species (for reviews see Dolla *et al.*, 2006 and references therein).

Our data strongly indicate that strain EMSSDQ$_4^T$ represents a novel species belonging to the genus *Desulfovibrio* and classification of this isolate as a representative of a novel species, *Desulfovibrio marrakechensis* sp. nov., is therefore proposed.

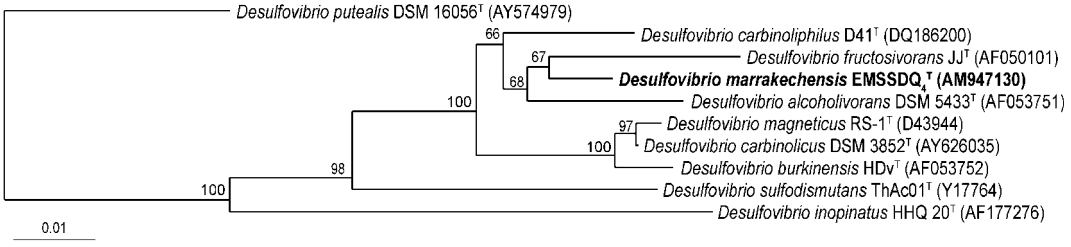

Fig. 1. Unrooted phylogenetic dendrogram, based on 1445 unambiguous base pairs of 16S rRNA gene sequence data, indicating the position of *Desulfovibrio marrakechensis* strain EMSSDQ$_4^T$ amongst the most closely related strains of the genus *Desulfovibrio*. GenBank accession numbers are given in parentheses. Bootstrap values, expressed as percentages of 1000 replications, are shown at branching points. Bar, 1 difference per 100 nucleotide positions.

Description of *Desulfovibrio marrakechensis* sp. nov.

Desulfovibrio marrakechensis [mar.ra.kech.en′sis. N.L. masc. adj. *marrakechensis* pertaining to Marrakech, in south-west Morocco, the source of isolation of the type strain; also referring to the first sulfate-reducing bacterium isolated from Morocco (Marrakech was the old name of Morocco)].

Cells are straight rods with rounded ends, 1.3–1.6 × 3.0–4.0 μm, occurring either singly or in pairs, non-motile, Gram-negative, oxidase-negative and catalase-positive. Optimum growth occurs at 37 °C and pH 7. Grows in 0–35 g NaCl l^{-1}, with optimum growth in the absence of NaCl. Able to use sulfate, sulfite, thiosulfate and elemental sulfur, with production of sulfide. Fumarate also serves as electron acceptor, whereas nitrate and nitrite do not. Strictly anaerobic, but exhibits limited growth in the absence of sulfate under air in basal medium containing lactate and yeast extract. Substrates that are oxidized by anaerobic respiration of sulfate are hydrogen, formate, lactate, pyruvate, fumarate, malate, ethanol, 2-methoxyethanol, 1-propanol, 2-propanol, 1-butanol, 1,2-propanediol, 1,3-propanediol, 1,4-butanediol and 1,4-tyrosol. Hydrogen and formate are only utilized in the presence of acetate. Lactate is oxidized to acetate. 1,4-Tyrosol is transformed to 4-hydroxyphenylacetate at 100 % recovery, leaving the aromatic ring intact. Fumarate, malate and pyruvate are fermented. Acetate, propionate, butyrate, methanol, 2-butanol, glycerol, ethylene glycol, 2,3-butanediol, crotonate, alanine, glycine, arginine, lysine, choline, lactose, sucrose, glucose, fructose, maltose, mannose, ribose, xylose, yeast extract, biotrypticase, Casamino acids, peptone, phenol, catechol, resorcinol, 4-hydroxyphenylacetate, benzoate, 4-hydroxybenzoate, 3-hydroxybenzoate, protocatechuate, syringate, gallate, *o*-methylgallate, veratrate, *p*-anisate, cinnamate, *p*-coumarate, *o*-coumarate, *m*-coumarate, vanillate, ferulate and caffeate are not utilized. Desulfoviridin and cytochrome c_3 are present.

The type strain is EMSSDQ$_4^T$ (=DSM 19337^T =ATCC BAA-1562^T), isolated from an aeration basin in Marrakech, Morocco, used for the elimination of OMW by natural evaporation. The DNA G+C content of the type strain is 65.1 mol%.

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