Salinivibrio costicola* subsp. *vallismortis* subsp. nov., a halotolerant facultative anaerobe from Death Valley, and emended description of *Salinivibrio costicola

Chi-Yu Huang,^{1†} Jean-Louis Garcia,² Bharat K. C. Patel,³ Jean-Luc Cayol,² Larry Baresi⁴ and Robert A. Mah¹

Author for correspondence: Jean-Louis Garcia. Tel: +33 4 91 82 85 72. Fax: +33 4 91 82 85 70.
e-mail: garcia@esil.univ-mrs.fr

¹ University of California Los Angeles, School of Public Health, Department of Environmental Health Sciences, Los Angeles, CA 90024-1772, USA

² IRD, Laboratoire de Microbiologie, Université de Provence, ESIL case 925, 163 Av. de Luminy, 13288 Marseille Cedex 9, France

³ School of Biomolecular and Biomedical Sciences, Faculty of Science, Griffith University, Brisbane, Queensland 4111, Australia

⁴ California State University Northridge, 18111 Nordhoff St, Northridge, CA 91330-8303, USA

Strain DV^T, a halotolerant, Gram-negative, facultatively anaerobic bacterium, was isolated from a hypersaline pond located in Death Valley, California. The cells were non-spore-forming, motile, curved rods (1.0–1.8 × 0.5–0.6 µm) and occurred singly, in pairs or rarely in chains. Strain DV^T was oxidase-, catalase-, Voges–Proskauer-, amylase-, gelatinase- and lipase-positive and indole-negative. Nitrate, sulfate and fumarate were not used as electron acceptors. Carbohydrates served as energy sources both aerobically and anaerobically. Strain DV^T grew optimally at 37 °C (temperature range 20–50 °C) with 2.5 % NaCl (NaCl range 0–12.5 %) and pH 7.3 (pH range of 5.5–8.5) in a glucose/yeast extract medium with a doubling time of 20 min (aerobically) or 41 min (anaerobically). The end products of glucose fermentation were ethanol, isobutyrate, propionate, lactate, formate and CO₂. Strain DV^T was resistant to penicillin, D-cycloserine, streptomycin and tetracycline (200 µg ml⁻¹). The G+C content was 50 mol %. 16S rRNA gene sequence analysis indicated that it was closely related to *Salinivibrio costicola* (97.7 %) and this was confirmed by DNA–DNA hybridization (93 % relatedness). However, phenotypic characteristics such as halotolerance, gas production, growth at 50 °C, antibiotic resistance, sugar-utilization spectrum and phylogenetic signatures are sufficiently different from *Salinivibrio costicola* to warrant designating strain DV^T as a new subspecies of *Salinivibrio costicola*, *Salinivibrio costicola* subsp. *vallismortis* subsp. nov. (= DSM 8285^T).

Keywords: *Salinivibrio costicola* subsp. *vallismortis* subsp. nov., halotolerance, facultative anaerobe, taxonomy

INTRODUCTION

Hypersaline ecosystems are extreme environments and are generally inhabited by a limited variety of halophilic bacterial species, even though a wide range of substrates are available for growth. The dead cell bodies or metabolic end-products of invertebrates, algae and prokaryotes are the major sources of

oxidizable compounds in these environments (Ollivier *et al.*, 1994). Brine shrimps or brine flies produce proteins and chitin. Cyanobacteria and *Halobacteriaceae* at high salt concentrations provide significant quantities of organic matter from decomposition of their cell walls, which are composed of sugars, proteins and lipids. Organic osmolytes, which maintain cell turgor pressure under high salt concentrations, also contribute to the overall carbon cycle in hypersaline ecosystems. In addition to potassium, halophilic bacteria accumulate low-molecular-mass organic compounds (such as betaine) to adapt to osmotic stress (Galinski & Trüper, 1982; Reed *et al.*, 1986; Robertson *et al.*, 1990, 1992; Lai *et al.*, 1991; Lai & Gunsalus, 1992).

[†] **Present address:** Department of Environmental Science, Tunghai University, Taichung 40704, Taiwan.

Abbreviation: VFA, volatile fatty acid.

The GenBank accession number for the 16S rRNA gene sequence of strain DV^T is AF057016.

Relatively little work has been reported on the diversity of anaerobic halophilic microbes or the breakdown of organic matter under anaerobic saline conditions. Increasing salt concentration results in an abnormal accumulation of H_2 (Zeikus, 1983) and a diversity of volatile fatty acids (VFA) in sediments (Klug *et al.*, 1985; Oren, 1988), suggesting that oxidation of organic matter is incomplete at high levels of NaCl, as compared with other ecosystems (digestors, marine ecosystems, etc.), where methane is the end product. Accumulation of H_2 , acetate and other VFAs indicates that interspecies hydrogen transfer metabolism is limited in hypersaline environments. Even if VFAs are oxidized, the process is very slow as compared with the fermentative metabolism of carbohydrates and other substrates used by most anaerobes. Consequently, VFAs, H_2 and other end-products accumulate (Oren, 1988).

The ability to grow at varying concentrations of salt is used to classify halophilic microbes into three groups (Larsen, 1962): (i) the slight halophiles (optimum growth at 2–5% NaCl), (ii) the moderate halophiles (optimum growth at 5–20% NaCl) and (iii) the extreme halophiles (optimum growth at 20–30% NaCl). The three types have been reported to occur in anaerobic saline habitats (Vreeland & Hochstein, 1993). Most bacteria isolated from sea water are also found in hypersaline waters and, in particular, members of the genus *Vibrio* (Ramos-Cormenzana, 1993), which are slightly halophilic, tend to dominate. We have isolated a new strain from sediment samples collected from a hypersaline pond located in Death Valley, California. The site, located 86 m below sea level, is the lowest land point in the United States. A huge surface of this area is covered by a large amount of salt. About 10000 years ago, the increasingly arid climate transformed Lake Manly, which used to occupy the valley, into the current salt flats.

METHODS

Origin of sample. Sediment and water samples were collected from a site at Badwater, Death Valley, California. Sediment samples were collected from underneath a salt-encrusted layer immersed under several centimetres of water. The samples were kept under anaerobic conditions in the dark, transported at ambient temperature to our laboratory and processed within 48 h. The chemical composition of the water from Badwater was marginally different from that of sea water (low in magnesium and chloride but high in calcium, sulfate and carbonate) and contained (as percentages): sodium, 3.4; calcium, 0.28; phosphate, 0.19; magnesium, 0.37; chloride, 4.78; sulfate, 1.06; and carbonate, 0.23.

Isolation and culture techniques. A modified HS-1 medium (Liaw & Mah, 1992), prepared aerobically or anaerobically, was used for enrichment and axenic cultures. The anaerobic technique of Hungate (1969), modified for use with syringes (Macy *et al.*, 1972), was used. The medium contained (l^{-1}): 1 g NH_4Cl , 7 g $MgCl_2 \cdot 6H_2O$, 9.6 g $MgSO_4 \cdot 7H_2O$, 0.5 g $CaCl_2 \cdot 2H_2O$, 3.8 g KCl, 0.4 g $K_2HPO_4 \cdot 3H_2O$, 3 g Na_2CO_3 ,

120 g (for enrichment) or 25 g (for axenic culture) NaCl, 10 ml trace mineral solution (Balch *et al.*, 1979) and, for anaerobic cultures, 0.5 g L-cysteine and 0.001 g resazurin. Anaerobic medium was prepared by boiling the medium under a stream of N_2/CO_2 (70:30, v/v) gas, cooling it to room temperature and dispensing it, while flushing with N_2/CO_2 gas, into culture tubes (9 ml) or into serum bottles (25 ml). Both types of vessel were then sealed with rubber septa and aluminium crimp cap seals (Bellco Glass). Aerobic medium was prepared by dispensing 9 ml medium into 16 × 150 mm culture tubes and capping the tubes loosely with plastic caps. All media were then autoclaved at 121 °C for 20 min. Prior to inoculation, sterile stock solutions were added to the pre-dispensed autoclaved medium to obtain a final concentration of 0.5% glucose, 0.1% yeast extract (for both aerobic and anaerobic medium) and 0.3% $NaHCO_3$ and 0.05% $Na_2S \cdot 9H_2O$ (for anaerobic medium). For preparation of roll tubes, 1.8% Bacto-agar (Difco) was added to the medium.

Enrichments were initiated by inoculating a few grams of the sediment sample to a serum bottle containing 25 ml anaerobic enrichment medium (12% NaCl and 0.5% glucose) followed by incubation at 37 °C without shaking. Cultures were transferred five times at intervals of 3 d with a 10% inoculum prior to culture purification. Cultures were purified by the repeated use of the roll-tube method of Hungate (1969). Culture purity was assessed from the uniformity of colony morphology and by examination of single cells in phase-contrast microscopy.

Physiological and biochemical tests. Unless indicated, all experiments were conducted in triplicate. The effect of NaCl on growth was determined in anaerobic culture medium. A culture resulting from four successive growths in anaerobic medium lacking NaCl was used as inoculum to avoid carry-over of NaCl. The effect of temperature was determined by using aerobic growth medium containing the optimum salt concentration. The effect of salt concentrations and of various temperatures on growth were also determined by using the aerobic growth medium. The effect of pH was tested aerobically by using two organic buffers, MES and 1,3-bis[tris(hydroxymethyl)methylamino]propane (Bis-Tris propane), at a final concentration of 500 mM. The heat resistance of cells was determined in growth medium. After 1 and 2 d incubation at 37 °C, duplicate cultures were heated at 90 or 100 °C for 20 min and subcultured into fresh growth medium [inoculum, 20% (v/v)]. The resulting cultures were incubated at 37 °C for 2 d. In addition, cultures grown (i) in the basal medium or (ii) in the basal medium enriched with 5 g yeast extract and bio-Trypticase l^{-1} were examined for the presence of spores at different growth phases.

The ability to oxidize or ferment a variety of substrates was determined by using glucose-free aerobic and anaerobic media containing the test substrate (final concentration of 0.5%). The growth rate for each test compound was compared with that of a control tube containing 0.1% yeast extract only. The criterion for positive substrate utilization was based on achieving a maximum growth rate of at least 0.2 h^{-1} . Prototrophic growth was tested in the aerobic medium containing glucose but no yeast extract.

Biochemical tests were conducted as described by Smibert & Krieg (1981) and by using API Rapid NFT strips (API Analytab Products). Gram reaction was determined by using heat-fixed liquid cultures stained with a Difco Gram stain kit. Microscopic examination of cultures grown under

different conditions was used to determine sporulation. The effect of antibiotics on growth was determined aerobically by using D-cycloserine, penicillin-G, streptomycin and tetracycline at concentrations ranging from 100 to 600 µg ml⁻¹. Oxygen was tested as an electron acceptor in liquid and solid media. Fumarate (10 mM), nitrate (10 mM) and sulfate (10 mM) with lactate as carbon source were tested as electron acceptors in anaerobic liquid medium. Growth on a H₂/CO₂ gas mixture (80:20) was examined in anaerobic liquid medium in order to test for homoacetogenesis.

Analytical techniques. Growth rates were determined by measuring the OD₅₆₀ of cultures using a Perkin-Elmer Junior model 35 spectrophotometer. VFAs, carboxylic acids and gases from the fermentation of glucose were analysed from culture supernatants by gas chromatography (Cord-Ruwisch *et al.*, 1986) and by HPLC (Shimadzu) with a reverse-phase mC18 column (4 mm × 30 cm Spherisorb C18; Interchim) and a UV SPD-6A spectrophotometric detector (Shimadzu) at a wavelength of 225 nm. The mobile phase consisted of 20% acetonitrile in 0.01 M chlorhydric acid. H₂S was determined photometrically as colloidal CuS after reaction with a mixture containing 50 mM HCl and 5 mM CuSO₄ (Cord-Ruwisch, 1985).

Scanning microscopy. Cells were fixed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer and were attached to polylysine-coated glass coverslips by using 0.1% aqueous polylysine. They were then rinsed in cacodylate buffer followed by dehydrating in a 30–100% acetone series, and dried in liquid CO₂. Samples were sputter coated with about 10 nM gold and viewed under a Philips model SEM 501 scanning electron microscope. Photographs were taken at 10000 × magnification using a Polaroid type 55-P/N film.

DNA base composition and DNA relatedness. DNA was extracted by the technique of Pitcher *et al.* (1989). The mol% G+C content was determined at the DSMZ according to Mesbah *et al.* (1989) and Tamaoka & Komagata (1984). DNA–DNA hybridization studies were performed at the DSMZ and the procedures have been reported previously (Mechichi *et al.*, 1998).

Isolation of nucleic acids and sequencing. The methods for the extraction and purification of genomic DNA have been described previously (Redburn & Patel, 1993). Twelve primers, as described previously for the *Bacteria* domain (Andrews & Patel, 1996), were used to amplify the 16S rRNA gene from genomic DNA. The amplified product was purified (Redburn & Patel, 1993; Andrew & Patel, 1996) and the sequence was determined with an ABI automated DNA sequencer by using a Prism dideoxy terminator cycle sequencing kit and the protocol recommended by the manufacturer (Applied Biosystems).

The 16S rRNA gene sequence that we determined was aligned manually with reference sequences of various members of the domain *Bacteria* by using the ae2 alignment editor (Maidak *et al.*, 1996). All reference sequences were obtained from the Ribosomal Database Project (Maidak *et al.*, 1996) with the exception of the sequences of *Salinivibrio costicola* NCIMB 701^T, *S. costicola* AV3, *S. costicola* E-367, *S. costicola* V-15, *S. costicola* 6 and *S. costicola* H-178, which were extracted from the EMBL database under accession numbers X95527 to X95532, respectively. Positions of sequence and alignment uncertainty were omitted from the analysis. Pairwise evolutionary distances based on 1319 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).

RESULTS

Isolation

A short vibrio with a tendency to form chains was isolated from the environmental sample and designated strain DV^T. Axenic cultures of strain DV^T were obtained by inoculating Hungate roll tubes after several successive transfers of the glucose enrichment. Strain DV^T was the predominant organism in the enrichment at this time and was the only colony-forming organism at the highest dilutions.

Morphology

Colonies grown in anaerobic roll tubes and on aerobic agar plates were similar in morphology. They were round with entire edges, smooth, convex, opaque and white. Colony diameter reached 3 mm in 1 week on aerobic agar plates. The cells of strain DV^T were small, curved rods ranging from 1.0 to 1.8 by 0.5 to 0.6 µm in size (Fig. 1), very motile in young cultures by means of one or rarely two subpolar flagella (Fig. 2), but non-motile in old cultures. Cells occurred singly, in pairs or in chains, as observed normally for other vibrios. Strain DV^T stained Gram-negative. Spores were not observed.

Growth and physiology

Strain DV^T was a chemoorganotrophic facultative anaerobe and, hence, the conditions for optimum growth were determined under anaerobic and aerobic conditions. The isolate grew in NaCl concentrations ranging from 0 to 12.5%, with the optimal concentration being 2.5% (Fig. 3a). The optimum temperature for growth was 37 °C, and no growth was observed above 50 °C or below 20 °C (Fig. 3b). The optimum pH for growth was 7.3 and the pH growth range was 5.5–8.5 (Fig. 3c). The doubling time for strain DV^T in the presence of glucose and yeast extract under optimal conditions was 20 min aerobically and 41 min anaerobically. Growth occurred in mineral medium with glucose as sole carbon and energy source.

Under aerobic conditions, strain DV^T used the following substrates: D-glucose, D-mannose, sucrose, trehalose, D-gluconate, pyruvate, adipate, caprate, L-glutamic acid and yeast extract. Slight growth was observed on D-xylose, L-histidine and L-proline. The following compounds were not used: L-arabinose, cellobiose, D-galactose, D-galacturonate, lactose, maltose, L-rhamnose, salicin, citrate, 2-oxoglutarate, DL-lactate, L-malate, malonate, phenylacetate, propionate, valerate, ethanol, D-mannitol, *m*-inositol, D-sorbitol, DL-α-alanine, β-alanine, L-leucine, L-serine, L-tyrosine, benzoate, *p*-hydroxybenzoate, betaine, sarcosine and spermine.

Fig. 1. Phase-contrast micrograph of strain DV^T. Bar, 5 µm.

Fig. 2. Scanning electron micrograph of strain DV^T. Bar, 2 µm.

The following substrates were fermented anaerobically: cellobiose, fructose, glucose, maltose, mannose, trehalose, *N*-acetylglucosamine, glycogen, starch and pyruvate. The following compounds were not fermented: arabinose, galactose, lactose, melibiose, raffinose, rhamnose, ribose, sucrose, xylose, adonitol, dulcitol, erythritol, glycerol, inositol, mannitol, methanol, sorbitol, formate, acetate, butyrate, propionate, ascorbate, citrate, fumarate, lactate, oxalate, succinate, tartrate, betaine, methylamine, trimethylamine, sarcosine, Casamino acids, cellulose, chitin and pectin.

The only end-products detected from glucose (28 mM) fermentation by strain DV^T after 24 h at 37 °C were: 15 mM ethanol, 8 mM isobutyric acid, 8 mM propionic acid, 3 mM lactic acid, 1 mM formic acid and

only CO₂ in the gas phase. Fumarate, nitrate and sulfate did not serve as electron acceptors. No growth was observed on H₂/CO₂.

The following biochemical tests were positive for strain DV^T: oxidase, catalase, amylase, gelatinase, lipase, arginine dihydrolase and Voges–Proskauer. Negative results were shown for aesculin and urea hydrolysis, β-galactosidase, lysine and ornithine decarboxylase activities and indole production. Strain DV^T was insensitive to antibiotics and growth was only inhibited by streptomycin at a concentration of 200 µg ml⁻¹, by penicillin-G at 250 µg ml⁻¹ and by both D-cycloserine and tetracycline at 600 µg ml⁻¹. Protoplasts were observed when the isolate was grown on penicillin-G or D-cycloserine.

DNA studies

The G + C content of DNA from strain DV^T determined in triplicate by HPLC was 50 mol %. DNA–DNA hybridization studies indicated that strain DV^T and *S. costicola* were closely related (93 % similarity).

16S rRNA sequence analysis

By using 12 primers, an almost complete sequence was determined, consisting of 1548 nucleotides corresponding to positions 8–1542 (*Escherichia coli* numbering according to Winker & Woese, 1991). The comparison of 1319 unambiguous bases of the 16S rRNA gene of strain DV^T with sequences from other members of the domain *Bacteria* indicated its close relationship to *S. costicola*. Phylogenetic analysis revealed that strain DV^T was a member of the family *Vibrionaceae* and the closest relative was *S. costicola* (mean similarity 97.7 %). A dendrogram generated by the neighbour-joining method showing this relationship is shown in Fig. 4.

DISCUSSION

The salt content of the pond known as Badwater in Death Valley, California, from which strain DV^T was isolated, is close to that of sea water, although periodically, and between rains, the salinity must approach saturation. At the time of sampling, a salt crust had formed, floating below the water surface, close to the bottom sediment. The sample was collected from the sediment layer below the brine, after breaking through the crust. Although the sample contained only 3.76 % NaCl (determined by drying and weighing triplicate samples), the presence of a salt crust indicated considerable periodic concentration of the brine, sufficient to elicit precipitation of salt, which would consequently create a localized high-salt environment. Strain DV^T is unusual among vibrios as it is halo-tolerant (does not need NaCl) and yet requires 2.5 % NaCl for optimal growth. However, it is not surprising to have isolated such a strain from such an environment, which fluctuates in salinity.

Fig. 3. Effects of NaCl concentration (a), temperature (b) and pH (c) on growth of strain DV^T. The experiments were conducted with anaerobic culture medium adjusted to the optimum conditions required for growth with the exception of the parameter to be determined. Sp., Specific.

Cells of three genera of the family *Vibrionaceae*, namely *Photobacterium*, *Aeromonas* and *Plesiomonas*, are straight rods (Baumann & Schubert, 1984) and hence, on morphology alone, strain DV^T can be excluded from these genera. The genera *Vibrio* and *Salinivibrio* are the only genera that contain curved cells resembling strain DV^T. However, 16S rRNA gene sequence analysis revealed that the closest relative of

strain DV^T is *S. costicola* (mean similarity 97.7%). Therefore, characterization of strain DV^T is based on a comparison with *S. costicola*, which is the only species so far described for this genus (Mellado *et al.*, 1996).

The strains of *S. costicola* can be differentiated from members of the genus *Vibrio* on the basis of two unique helical sequences and secondary structures between positions 178 to 197 and 197 to 219 of the rRNA (*E. coli* 16S rRNA gene sequence numbering according to Winker & Woese, 1991) (Mellado *et al.*, 1996). Strain DV^T contains a genus signature in its 16S rRNA that is the same as that of *S. costicola* strains between positions 197 and 219, but a secondary structure that is similar to that of *Vibrio* species rather than that of *S. costicola* strains between positions 178 and 197 (Fig. 5). It has been suggested that, if the level of 16S rDNA similarity is greater than 97%, other additional phenotypic and/or genotypic characteristics should be used for taxonomic purposes (Stackebrandt & Goebel, 1994).

From the phenotypic characteristics exhibited by the new isolate, some notable traits appear very important for distinguishing strain DV^T from previously described strains of *S. costicola* (Table 1). *S. costicola* is a moderate halophile, optimum growth of which occurs at 10% NaCl, but it is unable to grow at NaCl concentrations lower than 1.2–1.5% or at 50 °C. It cannot produce gas from glucose and ferments galactose, mannose, mannitol and sucrose. L-Alanine, lactate and mannitol are used aerobically as sole carbon and energy sources but mannose is not, and amylase is negative (Garcia *et al.*, 1987; Farmer & Hickman-Brenner, 1992). As strain DV^T and *S. costicola* differ in many phenotypic characteristics, this reflects heterogeneity of the genomes of the two isolates. However, DNA relatedness studies indicate a very close relationship (similarity of 93%) and, according to current taxonomic norms, strain DV^T should be accorded the status of a strain of *S. costicola* rather than a new species. We therefore propose that strain DV^T should be designated *Salinivibrio costicola* subsp. *vallismortis* subsp. nov., which automatically creates *Salinivibrio costicola* subsp. *costicola* subsp. nov. The different nucleotide signatures for the two strains will enable their distribution in saline and hypersaline environments to be studied more readily.

Fig. 4. Unrooted dendrogram showing the phylogenetic position of strain DV^T within the radiation of the members of the genera *Vibrio* and *Salinivibrio*. The tree was determined by using 1319 unambiguous nucleotides of the 16S rDNA using the Jukes and Cantor and neighbour-joining methods. All six sequences of *S. costicola* used in the analysis were identical and hence only the sequence of the type strain NCIMB 701^T is represented in the dendrogram. Bar, evolutionary distance of 0.01.

Fig. 5. Comparison of the secondary 16S rRNA structures from strain DV^T (a) and *S. costicola* (b). The numbers represent the two helices from positions 178 to 197 and from 197 to 219 (*E. coli* numbering according to Winker & Woese, 1991).

Emended description of *Salinivibrio costicola* Mellado et al. 1996

Salinivibrio costicola (cos.ti'co.la. L. n. *costa* rib; L. subst. *cola* dweller; M.L. n. *costicola* rib-dweller).

Cells are Gram-negative, non-spore-forming, curved rods, 0.5–0.6 × 1.0–3.2 µm, highly motile by means of one or rarely two subpolar flagella. Occur singly, in pairs or rarely in chains. Circular, convex, opaque, smooth, cream-coloured pigmented colonies (2–3 mm). The optimum NaCl concentration for growth is between 2.5 and 10% (w/v) at 37 °C; grows at NaCl concentrations between 0 and 20% (w/v). Growth at 5–50 °C and pH 5–10 (optimal growth at 37 °C and pH 7.3–7.5). No growth factors are required for growth, but yeast extract enhances growth.

Facultative anaerobe, chemoorganotrophic. Catalase and oxidase are produced. Gelatin and casein are hydrolysed; Voges–Proskauer and arginine decarboxylase tests are positive. Indole, β-galactosidase,

lysine and ornithine decarboxylase tests are negative. Nitrates usually not reduced to nitrites; no reduction of nitrite. Acid is produced aerobically from D-glucose, sucrose, D-trehalose and xylose. D-Glucose, D-trehalose, N-acetylglucosamine and pyruvate are fermented.

Isolated from hypersaline habitats and from salted food. G + C content of the DNA is between 49.4 and 50.5 mol %. The type strain is NCIMB 701^T.

Description of *Salinivibrio costicola* subsp. *costicola* subsp. nov.

Salinivibrio costicola subsp. *costicola* (cos.ti'co.la. L. n. *costa* rib; L. subst. *cola* dweller; M.L. n. *costicola* rib-dweller).

Cells are Gram-negative, non-spore-forming, curved rods, 0.5 × 1.5–3.2 µm, motile by means of one polar flagellum. Circular, convex, opaque, cream-coloured pigmented colonies (2–3 mm) develop on 10% (w/v) marine salts solid medium after 2 d incubation at 37 °C. No pigments. Broth culture is uniformly turbid. The optimum marine salts concentration for growth is 10% (w/v) at 37 °C; grows at marine salts concentrations between 0.5 and 20% (w/v). No growth in the absence of NaCl. Growth at 5–45 °C and pH 5–10 (optimal growth at 37 °C and pH 7.5).

Facultative anaerobe. Catalase and oxidase are produced. Acid is produced from D-glucose, maltose and D-trehalose; acid is not produced from D-arabinose, inositol, lactose or D-xylose. Gelatin and casein are hydrolysed; starch is not hydrolysed. Voges–Proskauer and arginine decarboxylase tests are positive. Indole, β-galactosidase, lysine and ornithine decarboxylase tests are negative. Nitrates usually not reduced to nitrites; no reduction of nitrite. The following compounds are utilized as sole carbon and energy sources: D-trehalose, glycerol, acetate, N-acetylglucosamine, fumarate, glutamate, lactate, DL-malate, pyruvate, propionate and succinate. The following compounds are not utilized as carbon and energy sources: amygdalin, D-arabinose, D-cellobiose, aesculin, D-galactosamine, D-gluconolactone, lactose, D-melibiose, L-rhamnose, D-xylose, erythritol, *cis*-aconitate, benzoate, *p*-hydroxybenzoate, hippurate, malonate, quinate, salicylate, suberate and D-tartrate. The following compounds are utilized as sole carbon, nitrogen and energy sources: L-alanine, L-asparagine, L-glutamine, L-ornithine, L-proline and L-serine. The following compounds are not utilized as sole carbon, nitrogen and energy sources: L-allantoin, L-aspartic acid, betaine, creatine, ethionine, L-isoleucine, L-methionine, putrescine, sarcosine, L-tryptophan and L-valine.

Isolated from hypersaline habitats and from salted food. The G + C content of the DNA is between 49.4 and 50.5 mol %. The type strain is NCIMB 701^T.

The description of this strain is the same as that given above for the species, except that acid is produced

Table 1. Divergence of phenotypic characteristics between *S. costicola* and strain DV^T

Data for *S. costicola* were taken from Garcia *et al.* (1987) and Farmer & Hickman-Brenner (1992).

Characteristic	Strain DV ^T	<i>S. costicola</i>
Growth without NaCl	+	—
Growth in the presence of 20% NaCl	—	+
Anaerobic gas production from glucose	+	—
Hydrolysis of starch	+	—
Growth at 50 °C	+	—
Fermentation of:		
Galactose	—	+
Mannose	—	+
Mannitol	—	+
Sucrose	—	+
Aerobic growth on:		
L-Alanine	—	+
Lactate	—	+
Mannose	+	—
Mannitol	—	+

from D-xylose, casein is hydrolysed and D-malate and propionate are utilized as sole sources of carbon and energy. The G + C content of the DNA of the type strain is 50.0 (CsCl) or 49.9 (*T_m*) mol %.

Description of *Salinivibrio costicola* subsp. *vallismortis* subsp. nov.

Salinivibrio costicola subsp. *vallismortis* (val.lis. mor'tis. L. gen. n. *vallis* of the valley; L. gen. n. *mortis* of death; M. L. fem. n. *vallismortis* of the valley of death, named after Death Valley, California, USA).

Cells are Gram-negative, non-sporulating, short, curved rods, 1.0–1.8 × 0.5–0.6 µm, highly motile by means of one or rarely two subpolar flagella. Cells occur singly, in pairs or rarely in chains. Colonies (up to 3 mm) are round with entire edges, smooth, convex, opaque and creamy white. Halotolerant. Growth occurs at NaCl concentration of 0–12.5%. Optimal growth occurs at 2.5% NaCl. Mesophilic. Optimum temperature for growth is 37 °C, growth occurs at 20–50 °C. Growth occurs at pH 5.5–8.2; optimum pH for growth is 7.3. No growth factors are required for growth, but yeast extract enhances growth. Either organic or inorganic nitrogen sources are sufficient. Resistant to streptomycin (200 µg ml⁻¹), penicillin-G (250 µg ml⁻¹), D-cycloserine and tetracycline (both 600 µg ml⁻¹).

Facultative anaerobe. Chemoorganotrophic. Oxidase and catalase-positive. Gelatin, starch and lipid (Tween 80) are hydrolysed. Arginine dihydrolase and Voges–Proskauer tests are positive. Aesculin and urea are not hydrolysed. Indole, lysine and ornithine decarboxylase

and β-galactosidase tests are negative. Fumarate, nitrate and sulfate are not reduced. No growth occurs with H₂/CO₂.

D-Glucose, D-mannose, sucrose, trehalose, starch, adipate, caprate, D-gluconate, pyruvate, L-glutamic acid and yeast extract are metabolized aerobically. Slight growth occurs on D-xylose, L-histidine and L-proline. The following compounds are not used: L-arabinose, cellobiose, D-galactose, D-galacturonate, lactose, maltose, L-rhamnose, salicin, citrate, 2-oxoglutarate, DL-lactate, L-malate, malonate, phenylacetate, propionate, valerate, ethanol, D-mannitol, *m*-inositol, D-sorbitol, DL-α-alanine, β-alanine, L-leucine, L-serine, L-tyrosine, benzoate, *p*-hydroxybenzoate, betaine, sarcosine and spermine.

Cellobiose, fructose, D-glucose, maltose, mannose, trehalose, *N*-acetylglucosamine, glycogen, starch and pyruvate are fermented. The major products from glucose fermentation are ethanol, isobutyrate, propionate, lactate, formate and CO₂. The following compounds are not fermented: arabinose, galactose, lactose, melibiose, raffinose, rhamnose, ribose, sucrose, xylose, adonitol, dulcitol, erythritol, glycerol, inositol, mannitol, methanol, sorbitol, formate, acetate, butyrate, propionate, ascorbate, citrate, fumarate, lactate, oxalate, succinate, tartrate, betaine, methylamine, trimethylamine, sarcosine, Casamino acids, cellulose, chitin and pectin.

The G + C content of DNA is 50 mol % (HPLC). The type strain is strain DV^T (= DSM 8285^T), which was isolated from Death Valley, California, USA.

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