Anaerovirgula multivorans gen. nov., sp. nov., a novel spore-forming, alkaliphilic anaerobe isolated from Owens Lake, California, USA

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A novel, alkaliphilic, obligately anaerobic bacterium, strain SCA^T, was isolated from mud sediments of a soda lake in California, USA. The rod-shaped cells were motile, Gram-positive, formed spores and were 0.4–0.5 × 2.5–5.0 µm in size. Growth occurred within the pH range 6.7–10.0 and was optimal at pH 8.5. The temperature range for growth was 10–45 °C, with optimal growth at 35 °C. NaCl was required for growth. Growth occurred at 0.5–9.0 % (w/v) NaCl and was optimal at 1–2 % (w/v). The novel isolate was a catalase-negative chemo-organoheterotroph that fermented sugars, proteolysis products, some organic and amino acids, glycerol, D-cellobiose and cellulose. It was also capable of growth by the Stickland reaction. Strain SCA^T was sensitive to tetracycline, chloramphenicol, rifampicin and gentamicin, but it was resistant to ampicillin and kanamycin. The G + C content of the genomic DNA was 34.2 mol%. Major fatty acid components were C_{14:0}, iso-C_{15:0}, C_{16:1ω9c} and C_{16:0}. 16S rRNA gene sequence analysis of strain SCA^T showed a similarity of approximately 97 % with the type strains of *Clostridium formicaceticum* and *Clostridium acetivum* in clostridial cluster XI and a similarity of less than 94.2 % to any other recognized *Clostridium* species and those of related genera in this cluster. Strain SCA^T was clearly differentiated from *C. formicaceticum* and *C. acetivum* based on comparison of their phenotypic properties and fatty acid profiles, as well as low levels of DNA–DNA relatedness between strain SCA^T and the type strains of these two species. Therefore, strain SCA^T is considered to represent a novel species of a new genus, *Anaerovirgula multivorans* gen. nov., sp. nov., in clostridial cluster XI. The type strain is SCA^T (= ATCC BAA-1084^T = JCM 12857^T = DSM 17722^T = CIP 107910^T).

Aerobic and anaerobic micro-organisms hydrolysing cellulose in alkaline ecosystems have not been well characterized (Lynd *et al.*, 2002). In our laboratory, the process of cellulose degradation was studied with anaerobic sediments from alkaline Owens Lake, California, USA. At room temperature, a piece of filter paper was completely dissolved over a period of 7–10 days. The dominant bacterial forms observed microscopically were motile rods

and spirochaetes (Pikuta *et al.*, 2005). Subsequent isolation of both of these morphotypes led to the characterization of two saccharolytic strains, SCA^T and ASpC2^T. The study showed that in addition to the hydrolysis of cellulose, strain ASpC2^T was able to ferment sugars, but strain SCA^T exhibited a much broader fermentative metabolism; it fermented sugars, some organic acids, amino acids and proteolysis products. In pure or binary cultures, these isolates exhibited a weak ability to hydrolyse cellulose, but only in the presence of yeast extract. Here we present a taxonomic description of the obligately anaerobic strain SCA^T which, based on its phenotypic and genotypic features, is considered to represent a novel species of a new genus within clostridial cluster XI.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain SCA^T is AB201750.

Images of cells of strain SCA^T and graphs showing growth of the strain under varying salinity, pH and temperature conditions are available as supplementary material in IJSEM Online.

Black mud sediments with a pH of 10.0 and a strong smell of hydrogen sulfide were collected anaerobically from beneath shallow water (temperature 30 °C, salinity 3 ‰) on the eastern side of Owens Lake in August 2000. Sampling, maintenance, transportation and storage were as previously described by Hoover *et al.* (2003) and Pikuta *et al.* (2003a). Enrichment cultures were obtained using an anaerobic technique and the medium contained (per litre): 30 g NaCl, 2.76 g Na₂CO₃, 24.0 g NaHCO₃, 0.2 g KCl, 0.2 g K₂HPO₄, 0.1 g MgCl₂·6H₂O, 1.0 g NH₄Cl, 0.4 g Na₂S·9H₂O, 0.001 g resazurin, 0.1 g yeast extract, 2 ml vitamin solution (Wolin *et al.*, 1963) and 1 ml trace mineral solution (Whitman *et al.*, 1982); the final pH was adjusted to 9.5. Filter paper or microcrystalline cellulose (5 g l⁻¹; Sigma) was used as the substrate. The gas phase was composed of high-purity nitrogen gas. Serial dilutions were performed in Hungate tubes with a filter paper substrate. Cultures on the 10⁻⁹ dilution with one morphotype observed microscopically were chosen for subsequent 'roll-tube' serial dilution purification. Upon inoculation of 'roll-tubes' containing a medium with 3 ‰ (w/v) agar and D-glucose, colonies appeared after 3–4 days incubation at 35 °C. These colonies were white to yellowish-cream in colour and were round to convex lens shaped (in deep agar) with a diameter of 0.5–1.3 mm. The colonies were also circular with smooth, thin edges and a darker coloured centre that had a denser consistency. One of the colonies from the 10⁻⁹ dilution was chosen as the type strain and was designated SCA^T. Unless specified otherwise, all subsequent incubations were performed at 35–37 °C. During the substrate utilization tests, the purity of the culture was checked by reverse inoculation to the first substrate. The culture purity was also routinely monitored microscopically.

Cell morphology of the novel isolate was examined under a Fisher Micromaster phase-contrast microscope. Cells of strain SCA^T were long, straight rods with rounded ends and ranged from 0.4 to 0.5 µm in width and from 2.5 to 5.0 µm in length. Cells were motile and Gram-positive. Spores were round and located terminally without swelling sporangium. Cells occurred singly, in pairs or in short slightly curved chains. Images of cells of strain SCA^T viewed with an Olympus BX41 light microscope equipped with a CytoViva 150 Illumination System (Vodyanoy, 2005) are available as Supplementary Fig. S1 in IJSEM Online.

Growth of the culture was determined by direct cell counting under a microscope or by measuring the optical density at 595 nm (Genesis 5; Spectronic Instruments). Catalase activity was determined based on the reaction with hydrogen peroxide (Gerhardt *et al.*, 1994). Substrates were added to the medium at concentrations of 3 g l⁻¹. The medium also contained 0.1 g yeast extract l⁻¹. Gases were measured using a Varian 3700 gas chromatograph equipped with a Porapak Q column and thermal conductivity/flame ionization detector. Nitrogen was used as the gas carrier.

The novel isolate was an obligate anaerobe and grew exclusively under anaerobic conditions. Strain SCA^T was

catalase-negative. It was obligately dependent upon Na⁺ ions and could not grow without NaCl (the experiment was performed on medium in which all sodium salts were substituted by potassium salts and all Cl⁻-containing salts were replaced by SO₄²⁻ salts). At a concentration of 0.5 ‰ (w/v) NaCl, growth was observed after a significantly longer lag phase than at optimal growth conditions. Optimum growth was at 1 ‰ NaCl on the medium for which the total salinity (with carbonate and bicarbonate salts) was 2 ‰ (w/v). The NaCl range for growth was 0.5–9.0 ‰ (w/v) (see Supplementary Fig. S2 in IJSEM Online). No growth was observed within 60 days at 0 or 10 ‰ NaCl. At 1 ‰ NaCl, strain SCA^T grew in the pH range from 6.7 to 10.0, with optimum growth at pH 8.5 (see Supplementary Fig. S2). At a salt concentration of 3 ‰ NaCl and 1 ‰ other salts in the test medium, strain SCA^T grew at pH 7.5, but not at pH 7.0. In a medium without carbonate-containing salts at pH 7.5 (a medium used routinely for the growth of neutrophilic bacteria), growth was very poor; cell yield was low and extensive spore formation was observed, confirming that the novel isolate was truly alkaliphilic. Growth was not dependent on CO₃ ions; good growth was observed on glycine-buffered medium at pH 9.0 without carbonate-containing salts (three subsequent passages demonstrated good growth). Strain SCA^T was mesophilic. Growth was observed at 10–45 °C, with optimal growth at 35 °C (Supplementary Fig. S2 in IJSEM Online).

Strain SCA^T had a chemo-organoheterotrophic metabolism and was capable of growth on a range of substrates (see Table 1 and the species description below). Growth on cellulose and filter paper was slow and weak and required 0.1 g yeast extract l⁻¹; filter paper was never completely hydrolysed. Hence strain SCA^T could be considered as a facultatively cellulolytic bacterium.

Strain SCA^T was capable of respiration by the Stickland reaction on the amino acid pairs listed in the species description. The only gaseous metabolic product of the culture grown on D-glucose was CO₂; no hydrogen was detected. In the liquid phase, only acetate was detected. The same metabolic products were detected during growth with cellulose, glycerol and pyruvate. Therefore, the novel isolate is an acetogen. Vitamins and yeast extract (0.1–0.5 g l⁻¹) stimulated growth.

Antibiotic inhibition was tested at a concentration of 250 µg ml⁻¹ (except for chloramphenicol, which was tested at 125 µg ml⁻¹) and the results are given in the species description. Growth with ampicillin exhibited a prolonged lag phase (3–4 days).

For extraction of fatty acid methyl esters, strain SCA^T was incubated for 4 days at 22 °C on the medium described above (with D-fructose as a substrate). Other reference strains (*Clostridium formicaceticum* ATCC 27076^T and *Clostridium aceticum* ATCC 35044^T) received from the ATCC for comparative study were incubated at the same conditions on ATCC 1612 medium, which is very close in

Table 1. Characteristics of strain SCA^T and its phylogenetically closest related taxa

Taxa: 1, strain SCA^T; 2, *Clostridium formicaceticum* ATCC 27076^T (data from Andreessen *et al.*, 1970); 3, *Clostridium aceticum* ATCC 35044^T (Wieringa, 1940; Braun *et al.*, 1981). +, Positive; –, negative; ND, no data. All three taxa are able to grow on D-fructose, D-ribose and pyruvate; none is able to use glycine, lactose or D-mannose. None of the taxa produces hydrogen.

Characteristic	1	2	3
Gram staining	+	–	–
Cell size (µm)	0.4–0.5 × 2.5–5.0	1.2–2 × 5–12	0.8–1.0 × 5.0
pH optimum (range)	8.5 (6.7–10.0)	7.8 (7.0–8.0)	8.3 (7.5–9.5)
NaCl optimum (range) (%)	2 (0.5–9.0)	ND	ND
Temperature optimum (range) (°C)	35 (10–45)	37 (28–44)	30 (22–45)
Substrate use:			
D-Glucose	+	–	–
D-Maltose	+	ND	–
Sucrose	+	–	–
D-Mannitol	+	+	–
Starch	+	–	–
Pectin	+	+	ND
D-Trehalose	+	ND	ND
D-Arabinose	–	–	ND
Methanol	–	+	–
Ethanol	–	+	+
Glycerol	+	+	–
Lactate	+	+	–
Formate	–	–	+
L-Serine	+	ND	+
L-Threonine	–	ND	–
Products of glucose fermentation:			
Formate	–	+	ND
Acetate	+	+	+*
Lactate	–	ND	ND
DNA G+C content (mol%)	34.2	34	33
DNA–DNA hybridization value with strain SCA ^T (%)	100	13	11

*Forms acetate from H₂ + CO₂ (classical acetogen).

composition to that used for the novel isolate. The extraction and analysis procedures were as described previously (Pikuta *et al.*, 2003b). The major fatty acids of strain SCA^T were C_{14:0}, iso-C_{15:0}, C_{16:1}ω_{9c} and C_{16:0}, whereas those of the two reference strains were C_{14:0}, C_{16:1}ω_{9c} and C_{16:0} (no iso-C_{15:0}; Table 2).

The G+C content of the genomic DNA of strain SCA^T was determined as described in Mesbah *et al.* (1989), except that 2 units of S1 nuclease were used instead of P1 nuclease and the nuclease degradation was performed in a 20 mM sodium acetate buffer, pH 5.0. The total G+C content of the purified genomic DNA for strain SCA^T was 34.2 ± 0.2 mol% (mean ± SD, n = 12).

For 16S rRNA gene sequence analysis, cells were lysed by sonication and the genomic DNA was isolated following phenol/chloroform extraction and ethanol precipitation (Sambrook *et al.*, 1989). The 16S rRNA genes were PCR amplified with primers EB-10F (5'-AGTTTGATCCTGGCTC, positions 10–25 according to

the *Escherichia coli* numbering system) and EB-1530R (5'-AAGGAGGTGATCCAGCC, positions 1541–1525).

The amplified 16S rRNA genes were sequenced directly in an ABI PRISM 310 Genetic Analyser (Applied Biosystems) with the 16S rRNA internal primers (Namwong *et al.*, 2005). In addition, to confirm the sequence of both end regions, the product purified by recovery from agarose gels using a GenElute Minus EtBr spin column (Sigma) was cloned into pT7 Blue vectors (Novagene), transformed in competent cells (*E. coli* JCM 109) and the plasmid DNAs were sequenced with the vector primers. Multiple alignments of the sequence were performed with CLUSTAL X (Thompson *et al.*, 1997). The alignment was manually verified and edited. After gaps and ambiguous bases were eliminated, evolutionary distances were calculated (see below) and a phylogenetic tree (Fig. 1) was constructed by using the neighbour-joining method (Saitou & Nei, 1987). Confidence values of branches of the phylogenetic tree were determined using bootstrap analyses (Felsenstein, 1985) based on 1000 resamplings.

Table 2. Cellular fatty acid content of strain SCA^T and related taxa

Taxa: 1, strain SCA^T; 2, *Clostridium acetivum* ATCC 35044^T; 3, *Clostridium formicaceticum* ATCC 27076^T. Values are percentages (w/w) of total fatty acids. DMA, dimethyl acetate; FAME, fatty acid methyl ester.

Fatty acid	1	2	3
iso-C _{14:0} FAME	2.32	0.00	0.00
C _{14:0} FAME	12.67	23.99	28.68
iso-C _{15:0} FAME	16.99	0.00	0.00
anteiso-C _{15:0} FAME	2.29	8.13	4.19
Summed feature 4*	3.22	12.12	5.09
C _{16:0} ALDE	3.07	9.02	11.03
C _{15:0} FAME	1.04	0.00	0.00
iso-C _{15:0} DMA	0.61	0.00	0.00
iso-C _{16:0} FAME	1.05	0.00	0.00
C _{16:1} ω7c FAME	3.24	0.00	0.00
C _{16:1} ω9c FAME	21.12	30.02	22.49
C _{16:1} ω11c FAME	1.1	0.00	0.00
C _{16:0} FAME	20.93	8.32	18.99
C _{16:1} ω9c FAME	2.82	3.62	1.95
C _{16:0} DMA	3.78	4.77	5.7
iso-C _{17:0} FAME	2.02	0.00	0.00
UN17.103 iso-C _{17:0} DMA	1.01	0.00	0.00
C _{18:0} FAME	0.72	0.00	0.00

*Summed feature 4 represents unresolved C_{15:2} FAME and/or C_{15:1}ω7c.

The sequence determined (1495 nt) was compared with all sequences currently available in the GenBank database and appeared to be highly similar to those from species of clostridial cluster XI (Collins *et al.*, 1994). The phylogenetic tree was constructed with representative strains of clostridial cluster XI by comparing 1202 nucleotide positions as shown

in Fig. 1. Strain SCA^T formed a cluster with the type strains of *C. acetivum* and *C. formicaceticum*, with sequence similarity values, based on pairwise comparison, of 97.0 % (1479 positions) and 96.6 % (1366 positions), respectively. Strain SCA^T was distantly related to other members of clostridial cluster XI (<94.2 % 16S rRNA gene sequence similarity).

DNA–DNA hybridization studies, performed according to the method of Ezaki *et al.* (1989), revealed that strain SCA^T was not a member of either of these two species, showing low hybridization values to *C. formicaceticum* ATCC 27076^T and *C. acetivum* ATCC 35044^T (13 and 11 %, respectively).

Strain SCA^T was isolated from a sample of anaerobic sediments of soda Owens Lake, which is a highly alkaline, evaporitic, carbonate-saturated lake remnant that has undergone the complete cycle of synthesis and degradation of organic matter. Photosynthetic prokaryotes (cyanobacteria and purple bacteria linked to the sulfur cycle) represent the primary producers of organic matter in the lake; the decomposers are represented by cellulolytic, sugarlytic, proteolytic, bacteriolytic, sulfate-reducers, together with other secondary anaerobes (Pikuta *et al.*, 2005).

The novel isolate occurs within a group of obligately anaerobic, alkalitolerant and alkaliphilic species of clostridial cluster XI. The role of this novel bacterium within the community studied is polyfunctional. The organism participates in cellulose decomposition as a primary anaerobe, it also performs hydrolysis of bimeric and monomeric sugars and products of proteolysis as a dissimilatory and finally it performs the role of a secondary anaerobe by using low-energy molecules of organic acids such as lactic, pyruvic and citric acid.

While this paper was in preparation, the anaerobic, alkaliphilic bacterium *Clostridium alkaliphilum* Z-7026^T (capable of growing exclusively on cellulose, xylan and

Fig. 1. Unrooted phylogenetic tree derived from 16S rRNA gene sequences showing the relationship of strain SCA^T to other taxa of clostridial cluster XI (Collins *et al.*, 1994). The tree was constructed by the neighbour-joining method. Numbers indicate bootstrap values based on 1000 resamplings. Roman numerals to the right designate clostridial clusters as defined by Collins *et al.* (1994). Bar, 1 % sequence divergence.

Table 3. Differential characteristics of the genus *Anaerovirgula* gen. nov. and other alkaliphilic, acetogenic, anaerobic, mesophilic genera

Genera: 1, *Anaerovirgula*; 2, *Tindallia* (data from Kevbrin *et al.*, 1998); 3, *Clostridium* (Hippe *et al.*, 1992); 4, *Natronincola* (Zhilina *et al.*, 1998); 5, *Alkaliphilus* (Takai *et al.*, 2001); 6, *Anoxynatronum* (Garnova *et al.*, 2003); 7, *Natroniella* (Zhilina *et al.*, 1996). +, Positive; –, negative; +/–, variable; ND, no data. An (atl), aerotolerant anaerobe; Oblig an, obligate anaerobe.

Characteristic	1	2	3	4	5	6	7
Motility	+	+/–	+	+	+	+	+
Gram reaction	+	+	+/–	+	+	+	–
Spores	+	+	+	+/–*	–	–	+
Metabolism	Oblig an	Oblig an	An (atl)	Oblig an	Oblig an	An (atl)	Oblig an
Reduction of:							
SO_4^{2-} to H_2S	–	–	–	–	–†	–	–
NO_3^{2-} to NO_2^-	–	–	+/–	ND	–	–	ND
Activity of:							
Catalase	–	–	–	ND	ND	+	ND
Oxidase	–	–	–	ND	ND	ND	ND
NaCl (3–12 %) requirement	–	+	+/–	+	–	+	+
CO_3^{2-} requirement	–	–	–	+	–	+	+
Use of substrates:							
Sugars	+	–	+/–	–	–	+	–
Proteolysis products and amino acids	+	+	+/–	+	+	+	+‡
Acetogenesis	+	+	+/–	+	ND	+	+§

*Oligospore-forming.

†Reduction of sulfur, thiosulfate and fumarate.

‡Using only glutamate.

§Homoacetogen.

cellobiose) was described by Zhilina *et al.* (2005). This bacterium belongs to clostridial cluster III. This highly specialized cellulose- and xylan-decomposer is incapable of fermenting monosaccharides, disaccharides other than cellobiose, other polymeric sugars or proteins. These properties are quite different from those of strain SCA^T.

It is interesting that strain SCA^T is capable of growth on L-isomers of sugars, which suggests the possible presence of alkaliphilic isomerases in its metabolism. The distinguishing features of the novel isolate and closely related species *C. formicaceticum* and *C. aceticum* are shown in Table 1. Phenotypic differences between the novel isolate and *C. formicaceticum* ATCC 27076^T are as follows: cell size (diameter of cells of strain SCA^T is three times smaller), ability to ferment D-glucose, sucrose, D-mannitol, methanol and lactate. Furthermore, *C. aceticum* ATCC 35044^T is a classical homoacetogen that is capable of growth on $\text{H}_2 + \text{CO}_2$, but strain SCA^T cannot grow on hydrogen or $\text{H}_2 + \text{CO}_2$ and the G+C content of the genomic DNA is different by 1 mol%.

The fatty acid content of strain SCA^T is also very different from that of the two phylogenetically closest related species. The $\text{C}_{14:0}$ fatty acid content of strain SCA^T is less than half that of *C. formicaceticum* ATCC 27076^T and *C. aceticum* ATCC 35044^T, and strain SCA^T contains only one-third the

content of $\text{C}_{16:0}$ aldehydes present in the latter two strains. Finally, iso-branched fatty acids are abundant in strain SCA^T (24.0 % in total), but completely absent in the other two organisms (Table 2).

Strain SCA^T can also be differentiated from recognized genera in clostridial cluster XI, such as *Natronincola*, *Tindallia*, *Alkaliphilus* and *Caminicella*, with less than 94.2 % 16S rRNA sequence similarity to the type species of these genera. In addition to its phylogenetic distinctiveness, strain SCA^T can be differentiated from these non-*Clostridium* genera based on its phenotypic properties (Table 3). Members of the genus *Natronincola* are halophilic (>4 %) and do not ferment sugars; representatives of the genus *Tindallia* have higher genomic DNA G+C contents and no iso- C_{15} fatty acids and species of the genus *Alkaliphilus* have less $\text{C}_{16:1}$ fatty acid methyl esters. On the basis of these data, strain SCA^T should be classified in a new genus in clostridial cluster XI.

Our 16S rRNA gene sequence analysis reveals that strain SCA^T represents a member of clostridial cluster XI and is closely related to *C. formicaceticum* and *C. aceticum*. However, the type species of the genus *Clostridium* (*Clostridium butyricum*) is located in clostridial cluster I, a completely separate phylogenetic cluster (as shown in Fig. 1). Thus, strain SCA^T (as well as *C. formicaceticum*

and *C. aceticum*) should in principle be accommodated within a separate new genus. However, given the differences in metabolism, low DNA–DNA relatedness value and the significantly different fatty acid profiles, we do not consider it appropriate at this time to move the latter two species to a new genus. Further comparative studies will need to be carried out to clarify the taxonomic status of *C. formicaeceticum* and *C. aceticum*, which may subsequently be reclassified and included in the new genus *Anaerovirgula*.

On the basis of phenotypic and genotypic characteristics, including Gram-positive cell walls, spore formation, obligately anaerobic and fermentative metabolism, mesophilic and alkaliphilic physiology, dependence on NaCl for growth, fatty acid content, 16S rRNA gene sequence divergence and levels of DNA–DNA relatedness, strain SCA^T is proposed as the type strain of a novel species in a new genus, *Anaerovirgula multivorans* gen. nov., sp. nov.

Description of *Anaerovirgula* gen. nov.

Anaerovirgula (An.aer.o.vir'gu.la. Gr. pref. *an* not; Gr. n. *aer* aeros, air; L. fem. n. *virgula* a small rod; N.L. fem. n. *Anaerovirgula* an anaerobic small rod).

Gram-positive, spore-forming, small (2.5–5.0 µm) rod-shaped cells that occur alone, in pairs or in short chains. Obligately anaerobic, and catalase-negative. Alkaliphilic and mesophilic. Chemo-organotrophic, with a fermentative metabolism (sugars and proteolysis products) and capable of respiration by the Stickland reaction. The fatty acid profile differs from that of members of the same clostridial cluster in the following respects: presence of iso-C_{15:0} fatty acid methyl esters, reduced quantity of C_{14:0} fatty acid methyl esters and significantly reduced quantity of C_{16:0} aldehydes. The G+C content of the genomic DNA is around 34 mol%. Belongs to clostridial cluster XI as defined by Collins *et al.* (1994).

The type and only species is *Anaerovirgula multivorans*.

Description of *Anaerovirgula multivorans* sp. nov.

Anaerovirgula multivorans (mul.ti.vo'rans. L. adj. *multus* many; L. part. adj. *vorans* devouring; N.L. part. adj. *multivorans* devouring numerous kinds of substrates).

Cells are motile, straight rods with rounded ends, 0.4–0.5 × 2.5–5.0 µm in size. Spores are terminally located. Growth occurs between 10 and 45 °C (optimum 35 °C), and at pH^{22 °C} 6.7–10.0 (optimum pH 8.5). Range of NaCl for growth is 0.5–9% (w/v), with optimum growth at 1–2% (w/v) NaCl. Heterotrophic growth occurs with lactate, pyruvate, citrate, glycerol, D-mannitol, D-glucose, D-fructose, D-maltose, D-trehalose, D-ribose, L-ribose, L-arabinose, sucrose, pectin, starch, D-cellobiose, cellulose, filter paper, triethylamine, peptone, yeast extract, Casamino acids, chitin, L-cysteine, L-aspartic acid, L-arginine, L-histidine, *trans*-4-hydroxy-L-proline, L-serine, L-alanine, L-lysine and

L-glutamine. No growth on H₂ + CO₂, H₂ alone, formate, acetate, propionate, butyrate, acetone, ethanol, methanol, lactose, D-arabinose, L-mannose, L-glucose, D-fucose, L-fucose, betaine, trimethylamine, D-arginine, L-proline, D-proline, D-lysine, D-serine, glycine, L-tyrosine, L-threonine, D-threonine, L-cystine or L-valine. Produces CO₂ but not H₂ during fermentation. Capable of respiration by the Stickland reaction on the following amino acid pairs: L-proline + L-isoleucine and L-proline + L-leucine. No growth on L-proline + L-valine, glycine + L-leucine, glycine + L-isoleucine, glycine + L-valine or L-tryptophan + L-valine. Resistant to ampicillin and kanamycin, but sensitive to gentamicin, tetracycline, rifampicin and chloramphenicol. The G + C content of the genomic DNA is 34.2 mol%.

The type strain, SCA^T (=ATCC BAA-1084^T=JCM 12857^T=DSM 17722^T=CIP 107910^T), was isolated from anaerobic, alkaline mud sediments of Owens Lake, California, USA.

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