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Shewanella irciniae sp. nov., a novel member of the family *Shewanellaceae*, isolated from the marine sponge *Ircinia dendroides* in the Bay of Villefranche, Mediterranean Sea

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Strain UST040317-058^T, comprising non-pigmented, rod-shaped, facultatively anaerobic, Gram-negative cells that are motile by means of single polar flagella, was isolated from the surface of a marine sponge (*Ircinia dendroides*) collected from the Mediterranean Sea. Comparative 16S rRNA gene sequence-based phylogenetic analysis placed the strain in a separate cluster with the recognized bacterium *Shewanella algae* IAM 14159^T, with which it showed a sequence similarity of 95·0 %. The sequence similarity between strain UST040317-058^T and its other (six) closest relatives ranged from 91·6 to 93·8 %. Strain UST040317-058^T showed oxidase, catalase and gelatinase activities. The typical respiratory quinones for shewanellas, menaquinone MK-7 and ubiquinones Q-7 and Q-8, were also detected. The predominant fatty acids in strain UST040317-058^T were i15 : 0, 16 : 0, 17 : 1ω8*c* and summed feature 3 (comprising i15 : 0 2-OH and/or 16 : 1ω7*c*), altogether representing 56·9 % of the total. The DNA G+C content was 39·9 mol%. The strain could be differentiated from other *Shewanella* species by its inability to reduce nitrate or produce H_2S and by 10–22 additional phenotypic characteristics. On the basis of the phylogenetic and phenotypic data presented in this study, strain UST040317-058^T represents a novel species in the genus *Shewanella*, for which the name *Shewanella irciniae* sp. nov. is proposed. The type strain is UST040317-058^T (=JCM 13528^T=NRRL B-41466^T).

The family *Shewanellaceae* was established from the emended description of a group of marine *Alteromonas*-like bacteria because of their deep phylogenetic branching and lack of association with any other genus in the family *Alteromonadaceae* (Ivanova *et al.*, 2004c). At present, the family *Shewanellaceae* includes only one genus, *Shewanella* (MacDonell & Colwell, 1985), which was created from the reclassification of two species previously assigned to the genus *Alteromonas*, namely [*Alteromonas*] *putrefaciens* (Lee *et al.*, 1981) and [*Alteromonas*] *hanedai* (Jensen *et al.*, 1980). *Shewanella* species comprise Gram-negative, straight or curved rod-shaped, aerobic or facultatively anaerobic and readily cultivated gammaproteobacteria isolated from diverse sources, including activated sludge (Xu *et al.*, 2005), marine invertebrates (Ivanova *et al.*, 2004b), red algae (Simidu *et al.*, 1990), a tidal flat (Yoon *et al.*, 2004a), seawater (Ivanova *et al.*, 2001, 2004a; Yoon *et al.*, 2004b), sediments (Venkateswaran *et al.*, 1998) and clinical samples (Levin, 1972; Debois *et al.*, 1975; Holmes *et al.*, 1975). In the last decade, the number of recognized species in this genus has increased; they have been studied extensively because of their capacity for dissimilatory reduction of manganese and iron oxides (Myers & Nealson, 1988; Bowman *et al.*, 1997; Venkateswaran *et al.*, 1998), for co-metabolization of

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain UST040317-058^T is DQ180743.

A scanning electron micrograph of cells of strain UST040317-058^T is available as a supplementary figure in IJSEM Online.

halogenated organic pollutants (Petrovskis *et al.*, 1994), for the destructive souring of crude petroleum (Semple & Westlake, 1987) and for the production of tetrodotoxin (Simidu *et al.*, 1990) and large proportions of polyunsaturated fatty acids (Bowman *et al.*, 1997; Russell & Nichols, 1999; Ivanova *et al.*, 2004a). At the time of writing, there are more than 30 *Shewanella* species with validly published names. On the basis of the polyphasic taxonomic data presented in this study, we propose a novel member of this genus, strain UST040317-058^T, isolated in March 2004 from the surface of a marine sponge (*Ircinia dendroides*) found associated with sea-grass (*Posidonia*) in the Bay of Villefranche in the Mediterranean Sea.

Strain UST040317-058^T was isolated using a standard dilution plating technique on a marine agar medium containing 3 g yeast extract (Oxoid), 5 g peptone (Oxoid) and 12 g bacteriological agar (Oxoid) in 1 l 0·22 μm-filtered seawater at 32 ‰ salinity after 48 h incubation at 28 °C. Unless otherwise indicated, all characteristics described hereafter are based on cultures grown on marine agar under these conditions. Colonies of strain UST040317-058^T were milky, raised and circular (0·8–1·5 mm in diameter) with entire edges and a smooth surface (as observed under a Leica MZ6 light microscope at 40 × magnification). Gram stain was determined using light microscopy according to Smibert & Krieg (1994). Cell morphology was examined using scanning electron microscopy (6700F; JEOL) according to Neu *et al.* (2001) and the presence of flagella was determined by transmission electron microscopy according to Allan *et al.* (2002). Gliding motility was observed under a phase-contrast light microscope (BX51; Olympus) at 100 × magnification using cells grown on quarter-strength marine broth 2216 (Oxoid) solidified with 1·2 % agar according to Bowman (2000). Strain UST040317-058^T comprised Gram-negative, rod-shaped cells that were motile by means of a single polar flagellum (see Supplementary Fig. S1 available in IJSEM Online).

The almost-complete 16S rRNA gene sequence of strain UST040317-058^T (1462 bp) was obtained bidirectionally with three replicates, as described by Lau *et al.* (2004). Comparative analysis of the 16S rRNA gene sequence with sequences deposited in GenBank using BLAST indicated that the strain belonged to the family *Shewanellaceae* and showed the highest sequence similarity (95·0 %) with *Shewanella algae* IAM 14159^T (Simidu *et al.*, 1990). The 16S rRNA gene sequence was automatically, and then manually, aligned with a database of >30 000 previously aligned 16S rRNA gene sequences by using the ARB software package (Ludwig *et al.*, 2004). Phylogenetic trees were constructed using three different methods: neighbour-joining, maximum-likelihood and maximum-parsimony. The neighbour-joining phylogenetic tree (Fig. 1) placed strain UST040317-058^T

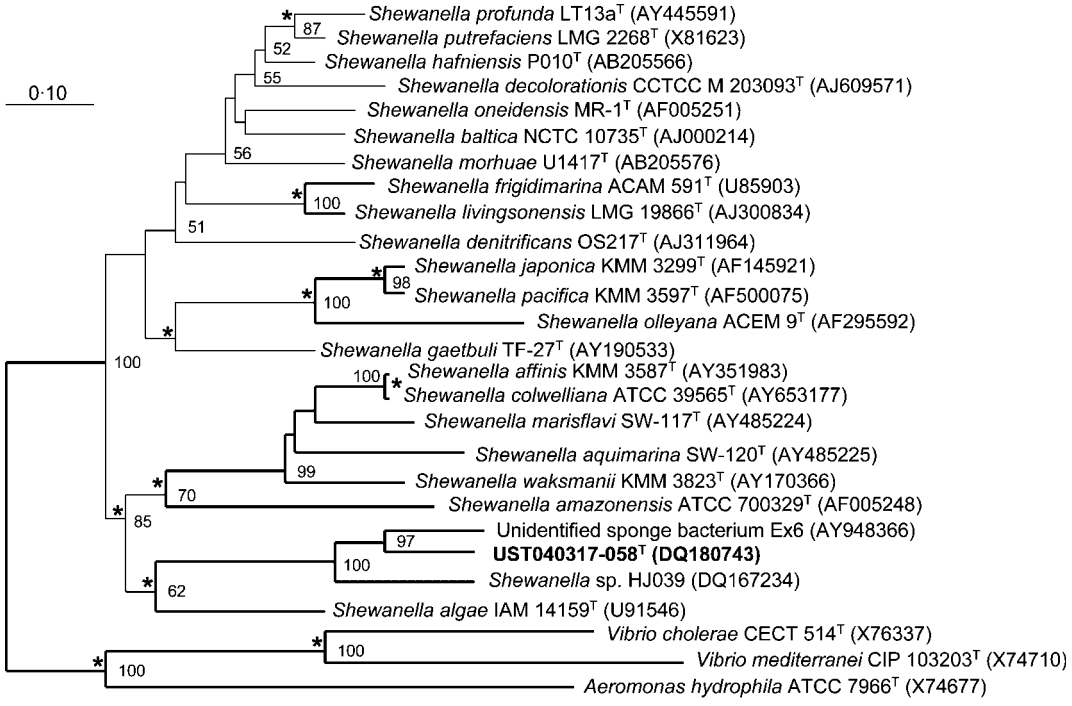

Fig. 1. Neighbour-joining tree, based on 16S rRNA gene sequence comparisons, showing the estimated phylogenetic relationships between UST040317-058^T and related species. Strains belonging to the genera *Vibrio* and *Aeromonas* were chosen as the outgroups. Asterisks indicate nodes that are also found in the maximum-parsimony tree; bold lines indicate branches that are also found in the maximum-likelihood tree. Bootstrap values greater than 50 %, expressed as a percentage of 500 replicates, are shown at the nodes. GenBank accession numbers are shown in parentheses. Bar, 1 nucleotide substitution per 100 nucleotides.

within a cluster of two undescribed bacteria which were also isolated from marine sponges: an unidentified sponge bacterium, strain Ex6 (Wichels *et al.*, 2006), and *Shewanella* species strain HJ039 (GenBank accession no. DQ167234). This cluster, together with the recognized species *S. algae* IAM 14159^T, formed a distinct clade that clustered robustly with another clade comprising six other *Shewanella* species with validly published names, including *Shewanella amazonensis* SB2B^T (Venkateswaran *et al.*, 1998), *Shewanella waksmanii* KMM 3823^T (Ivanova *et al.*, 2003), *Shewanella aquimarina* SW-120^T (Yoon *et al.*, 2004b), *Shewanella marisflavi* SW-117^T (Yoon *et al.*, 2004b), *Shewanella colwelliana* ATCC 39565^T (Coyne *et al.*, 1989) and *Shewanella affinis* KMM 3587^T (Ivanova *et al.*, 2004b). These species shared 91·6–93·8 % 16S rRNA gene sequence similarity with strain UST040317-058^T. The maximum-likelihood and maximum-parsimony trees based on cladistic methods (i.e. character-based) showed similar topography for the novel strain and the *Shewanella* species. These results support the inclusion of UST040317-058^T as a novel species in the genus *Shewanella*.

The cellular fatty acid profile of strain UST040317-058^T was determined using the Sherlock Microbial Identification System (MIDI) according to the manufacturer's protocol. Strain UST040317-058^T had a cellular fatty acid profile dominated by the saturated straight-chain fatty acid 16 : 0 (13·0 %), the saturated branched-chain fatty acid i15 : 0 (14·1 %), the unsaturated straight-chain fatty acid 17 : 1ω8*c* (13·3 %) and summed feature 3 (comprising i15 : 0 2-OH and/or 16 : 1ω7*c*) (16·5 %), which together constituted 56·9 % of the total fatty acid content (Table 1). These fatty acids are common to *Shewanella* species, supporting the inclusion of strain UST040317-058^T in the genus. However, some fatty acids that were common in some *Shewanella* species, e.g. 15 : 0, 16 : 1ω7*c* (Table 1) and polyunsaturated fatty acids (Bowman *et al.*, 1997; Skerratt *et al.*, 2002; Ivanova *et al.*, 2004a), were not observed in strain UST040317-058^T, suggesting that this novel isolate is unique.

The DNA G+C content of UST040317-058^T was 40·0$\pm$0·1 mol% (n=3) as determined using an HPLC method according to Mesbah *et al.* (1989). This value is within the range of G+C contents observed among members of the genus *Shewanella* (39·0–54·0 mol%) (Table 2). The presence of respiratory quinones was checked using an HPLC method according to Collins (1994). Menaquinones extracted from *Cellulophaga lytica* (Nakagawa & Yamasato, 1993) and *Pedobacter heparinus* (Steyn *et al.*, 1998) served as references for MK-6 and MK-7, respectively, while ubiquinones extracted from *Escherichia coli* strain XL1-Blue (Gao *et al.*, 2004) served as references for Q-7 and Q-8. MK-7, Q-7 and Q-8, but not MK-6, were detected in strain UST040317-058^T.

The oxygen requirement for growth was investigated using the Oxoid Anaerobic System. Growth at different temperatures (4, 12, 20, 28, 36, 44 and 52 °C) and pH (5, 6, 7, 8, 9 and 10) was monitored on marine agar incubated for up to 10 days. The NaCl requirement for growth was tested on a 1·2 % agar medium containing 5 g peptone, 5 g $MgCl_2$, 2 g $MgSO_4$, 1 g KCl, 0·5 g $CaCl_2$ and different amounts of NaCl (from 0 up to 180 g) and the pH was adjusted to 7·5 using KOH (Isnansetyo & Kamei, 2003). Haemolytic activity was studied on blood agar containing 40 g blood agar base (Oxoid), 50 ml rabbit blood and 950 ml 0·22 μm-filtered seawater (Ivanova *et al.*, 2004b). Susceptibility to the antibiotics streptomycin, benzylpenicillin, chloramphenicol, ampicillin, tetracycline and kanamycin was tested using standard agar disc diffusion assays according to Acar (1980). The amounts of antibiotic tested ranged from 1·0 to 100·0 μg per disc. The hydrolysis of casein and cellulose was investigated according to Norris *et al.* (1985) and Bowman (2000), respectively. The hydrolysis of Tweens 20, 40 and 80 and of chitin was tested as described in Baumann & Baumann (1981). The hydrolysis of agar, DNA and starch and the production of oxidase and catalase were determined according to Smibert & Krieg (1994). Other enzymic activities, the utilization of (and acid production from) different carbon sources, the reduction of nitrate and the production of H_2S, indole and acetoin were tested using the commercial systems API 20E, API 20NE, API 50 CH, API ZYM (bioMérieux) and MicroLog 3 (Biolog) according to the manufacturers' manuals, except that the cells used for the API system were suspended in sterile seawater at 22 ‰ salinity before inoculation (MacDonell *et al.*, 1982). Growth on glycerol, D-glucose, sucrose, D-mannitol, D-galactose, starch, D-sorbitol, D-arabinose and D-melibiose as sole carbon sources was also tested on a 1·2 % agar medium containing 0·2 g $NaNO_3$, 0·2 g NH_4Cl, 0·05 g yeast extract and 4 % (w/v) carbon source in 1 l seawater at 35 ‰ salinity (Nedashkovskaya *et al.*, 2003). Detailed physiological and biochemical characteristics of UST040317-058^T are given in the species description below.

Strain UST040317-058^T can be differentiated from its closest relative, *S. algae* IAM 14159^T, by means of several phenotypic characteristics, including the inability of the novel strain to reduce nitrate, produce H_2S, grow at 8 % NaCl and 40 °C, produce lipase or utilize D-maltose, DL-lactate, DL-malate, succinate, fumarate and L-serine and its ability to utilize D-galactose, D-glucose, D-mannitol and D-sorbitol as sole carbon sources. The novel strain is differentiated from other selected *Shewanella* species in Table 2. On the basis of the phylogenetic evidence together with the phenotypic characteristics presented in this study, strain UST040317-058^T represents a novel species within the genus *Shewanella*, for which the name *Shewanella irciniae* sp. nov. is proposed.

Description of *Shewanella irciniae* sp. nov.

Shewanella irciniae (ir.ci′ni.ae. N.L. gen. n. *irciniae* of/from *Ircinia*, isolated from the marine sponge *Ircinia dendroides*).

Cells are Gram-negative, short, straight rods (1·3–2·0 μm in length and 0·5 μm in width) and are motile by means of a

Table 1. Cellular fatty acid content of strain UST040317-058^T and its close relatives in the genus *Shewanella*

Strains: 1, UST040317-058^T; 2, *S. algae* IAM 14159^T (data from Simidu *et al.*, 1990); 3, *S. amazonensis* SB2B^T (Venkateswaran *et al.*, 1998); 4, *S. waksmanii* KMM 3823^T (Ivanova *et al.*, 2003); 5, *S. aquimarina* SW-120^T (Yoon *et al.*, 2004b); 6, *S. marisflavi* SW-117^T (Yoon *et al.*, 2004b); 7, *S. colwelliana* ATCC 39565^T (Coyne *et al.*, 1989); 8, *S. affinis* KMM 3587^T (Ivanova *et al.*, 2004b). Values given are mean percentages of the total fatty acid content. The prefixes 'i' and 'a' indicate iso-branched and anteiso-branched fatty acids, respectively. Fatty acids representing $<0{\cdot}2$ % in all rows have been excluded. –, Not detected.

Fatty acid	1	2	3	4	5	6	7	8
Saturated straight-chain fatty acids								
12 : 0	1·8	–	–	2·0	1·9	1·6	1·0	3·3
13 : 0	1·2	–	–	–	0·9	0·5	3·1	2·1
14 : 0	2·0	1·3	1·4	1·7	1·3	1·5	1·9	1·6
15 : 0	–	6·5	9·2	5·3	4·4	4·1	14·0	6·7
16 : 0	13·0	16·8	6·1	6·2	11·6	14·5	8·7	11·0
17 : 0	4·4	4·1	4·0	–	1·7	1·9	5·1	4·2
18 : 0	0·9	0·4	0·1	0·3	–	–	0·5	0·6
Unsaturated straight-chain fatty acids								
15 : 1ω8*c*	0·6	–	–	–	–	–	1·0	0·7
15 : 1ω6*c*	1·1	0·2	0·8	–	–	–	2·4	1·1
16 : 1ω9*c*	1·5	2·8	0·7	–	0·7	0·7	–	–
16 : 1ω7*c*	–	15·3	14·7	9·8	–	–	19·1	26·5
17 : 1ω8*c*	13·3	10·9	23·5	–	6·7	5·3	17·0	19·0
17 : 1ω6*c*	1·9	0·9	2·4	–	0·8	0·6	0·7	2·1
18 : 1ω9*c*	4·2	5·0	1·4	–	1·6	1·7	0·9	2·8
18 : 1ω7*c*	3·7	5·2	4·5	2·0	3·4	4·1	0·8	6·1
20 : 4ω5*c*	–	–	–	6·7	–	–	–	–
20 : 5ω3*c*	–	–	–	–	–	–	0·4	–
Hydroxy straight-chain fatty acids								
11 : 0 3-OH	0·7	–	–	–	–	–	–	–
12 : 0 3-OH	4·3	–	–	–	0·8	1·3	–	–
Saturated branched fatty acids								
i11 : 0	0·3	–	–	–	0·1	2·0	–	–
i13 : 0	2·3	0·5	4·7	10·0	7·4	5·9	6·6	1·9
i14 : 0	1·0	1·4	1·6	–	0·9	0·7	0·5	1·8
i15 : 0	14·1	27·4	26·7	32·5	27·5	22·9	13·8	25·2
a15 : 0	0·1	–	–	–	–	–	0·2	0·3
i16 : 0	0·4	0·5	1·4	–	0·4	0·2	–	0·6
i17 : 0	0·8	1·4	1·8	–	1·9	1·6	1·9	0·8
Unsaturated branched fatty acids								
a15 : 1	–	–	–	–	–	–	1·0	–
Hydroxy branched fatty acids								
i13 : 0 3-OH	5·0	–	–	–	5·1	4·5	–	–
Summed feature 1*	1·9	–	–	–	0·6	0·5	–	–
Summed feature 2†	1·2	–	–	–	0·9	0·9	–	–
Summed feature 3‡	16·5	–	–	–	15·9	18·6	–	–

*Summed feature 1 comprises 13 : 0 3-OH and/or i15 : 1.

†Summed feature 2 comprises 14 : 0 3-OH and/or i16 : 1.

‡Summed feature 3 comprises i15 : 0 2-OH and/or 16 : 1ω7*c*.

single polar flagellum. Facultatively anaerobic. When cultivated on marine agar at 28 °C for 48 h, colonies are milky, 0·8–1·5 mm in diameter, circular and raised with a smooth surface and an entire edge. Does not produce diffusible pigments. Optimal growth occurs at 20–28 °C, but no growth occurs at temperatures lower than 12 °C or higher than 36 °C. Growth occurs at pH 6–10, but no growth occurs at or below pH 5. Requires NaCl (2·0–6·0 %; optimum, 2·0–4·0 %) for growth. MK-7, Q-7 and Q-8 are the predominant respiratory quinones detected. The predominant fatty acids are i15 : 0, 16 : 0, 17 : 1ω8*c* and summed feature 3 (comprising i15 : 0 2-OH and/or

Table 2. Phenotypic characteristics that differentiate strain UST040317-058^T from the seven most closely related members of the genus *Shewanella*

Strains: 1, UST040317-058^T; 2, *S. algae* IAM 14159^T (data from Simidu *et al.*, 1990); 3, *S. amazonensis* SB2B^T (Venkateswaran *et al.*, 1998); 4, *S. waksmanii* KMM 3823^T (Ivanova *et al.*, 2003); 5, *S. aquimarina* SW-120^T (Yoon *et al.*, 2004b); 6, *S. marisflavi* SW-117^T (Yoon *et al.*, 2004b); 7, *S. colwelliana* ATCC 39565^T (Coyne *et al.*, 1989); 8, *S. affinis* KMM 3587^T (Ivanova *et al.*, 2004b). All strains are straight rods, Gram-negative, facultatively anaerobic and motile by means of a single polar flagellum. All are positive for haemolytic activity and the production of gelatinase, oxidase and catalase. All are negative for the production of arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase and indole and for the utilization of lactose. +, Positive; −, negative; ND, not determined.

Characteristic	1	2	3	4	5	6	7	8
DNA G+C content (mol%)	40	54	52	43	54	51	46	45
Pigment of cell biomass	−	−	−	+*	−	−	−	−
Growth at/with:								
4 °C	−	−	+	+	−	+	+	−
28 °C	+	+	+	+	+	+	ND	+
40 °C	−	+	+	−	+	+	−	−
0 % NaCl	−	−	+	−	−	+	−	−
6 % NaCl	+	+	−	+	+	+	−	+
8 % NaCl	−	+	−	−	+	+	−	−
pH range	6–10	6–10	6–10	6–10	5–8	5–8	ND	ND
Reduction of NO_3 to NO_2	−	+	+	+	+	+	+	+
Hydrolysis of:								
Casein	−	ND	ND	−	+	+	ND	+
Chitin	−	−	−	−	ND	ND	−	−
Tween 40	−	ND	−	+	−	−	ND	+
Tween 80	−	ND	−	−	+	+	ND	+
Production of:								
Amylase	−	−	−	−	+	−	+	−
H_2S	−	+	+	+	+	+	ND	+
Lipase	−	+	−	+	+	+	+	+
Utilization of:								
D-Cellobiose	−	−	ND	−	−	+	ND	ND
D-Fructose	−	−	+	−	−	−	−	−
Fumarate	−	+	+	−	−	−	+	−
D-Galactose	+	−	+	−	+	−	−	−
D-Glucose	+	−	ND	+	−	+	ND	+
Glycerol	+	+	−	−	−	−	−	−
DL-Lactate	−	+	+	−	+	+	−	−
DL-Malate	−	+	−	ND	+	+	−	ND
D-Maltose	−	+	−	ND	+	+	−	ND
D-Mannitol	+	−	−	ND	−	−	−	+
D-Melibiose	+	−	ND	ND	−	−	ND	ND
L-Serine	−	+	+	ND	ND	ND	ND	+
D-Sorbitol	+	−	−	ND	−	−	−	ND
Succinate	−	+	+	−	+	+	−	−
Sucrose	+	+	−	−	−	−	−	−

*The colour of the pigment was brown/greenish.

16 : 1ω7c), together constituting 56·9 % of the total. Susceptible to 1·0 μg benzylpenicillin, 1·0 μg chloramphenicol, 1·0 μg ampicillin, 10·0 μg tetracycline and 100·0 μg streptomycin, but resistant to kanamycin (up to 100·0 μg tested). Gelatin is hydrolysed, but casein, agar, starch, chitin, cellulose and Tweens 20, 40 and 80 are not. Acetoin, indole and H_2S are not produced. Nitrate is not reduced. Citrate is not utilized. Positive for haemolytic activity, DNase, oxidase, catalase, alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase and α-fucosidase. Negative for urease, lipase (C14), cystine arylamidase, trypsin, α-chymotrypsin, α- and β-galactosidases, β-glucuronidase, α- and β-glucosidases, *N*-acetyl-β-glucosaminidase, α-mannosidase, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase and tryptophan deaminase. Utilizes glycerol, D-glucose, sucrose, D-mannitol, D-galactose, starch, D-sorbitol, D-arabinose and D-melibiose as sole carbon sources on agar medium supplemented with 4 % (w/v) carbon source. Utilizes D-glucose, L-arabinose, aesculin ferric citrate and potassium 2-ketogluconate in the API 50 CH and 20NE systems. Utilizes α- and β-hydroxybutyric acids, methyl pyruvate and D-psicose in the MicroLog 3 system. Other carbon sources included in the MicroLog 3, API 20NE and 50 CH systems are not utilized. No acid production is observed from the carbon sources in the API 50 CH and 20E systems.

The type strain, UST040317-058^T (=JCM 13528^T=NRRL B-41466^T), was isolated from the surface of a marine sponge (*Ircinia dendroides*) associated with *Posidonia* sea-grass in the Bay of Villefranche, Mediterranean Sea.

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