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Haliea salexigens gen. nov., sp. nov., a member of the Gammaproteobacteria from the Mediterranean Sea

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Abstract:

A novel aerobic, Gram-negative bacterium, designated 3X/A02/235^T, was isolated from the surface of coastal waters in the north-western Mediterranean Sea. Cells were motile, straight rods, 1.6 µm long and 0.5 µm wide, and formed cream colonies on marine agar medium. The G+C content of the genomic DNA was 61 mol%. Phylogenetic analysis of the 16S rRNA gene sequence placed the strain in the class Gammaproteobacteria and within the family Alteromonadaceae. On the basis of 16S rRNA gene sequence comparisons and physiological and biochemical characteristics, this isolate represents a novel species of a novel genus, for which the name Haliea salexigens gen. nov., sp. nov. is proposed. The type strain of Halieasalexigens is 3X/A02/235^T (=DSM 19537^T =CIP 109602^T =MOLA 286^T).

The genus Microbulbifer was proposed by Gonzalez et al. (1997) for a Gram-negative, aerobic strain that was catalaseandoxidase-positive and possessed numerous vesicles derived from the outer membrane. Four species belonging to this genus have been described: Microbulbifer hydrolyticus (Gonzalez et al., 1997), Microbulbifer salipaludis (Yoon et al., 2003a), Microbulbifer maritimus (Yoon et al., 2004) and Microbulbifer elongatus (Yoon et al., 2003b). The latter species was originally named Pseudomonas elongate (Humm, 1946; Palleroni, 1984), but was transferred to the genus Microbulbifer on the basis of 16S rRNA gene sequence similarity, cellular fatty acid composition and respiratory quinone content. Consequently, major amounts of iso-15 : 0 and iso-17 : 1v9c as well as ubiquinone Q-8 were defined as chemical markers of this genus (Yoon et al., 2004). Microbulbifer species have been isolated from various saline and marine environments, including salt marshes, intertidal sediments and coastal waters. In this work, a strain named 3X/A02/235T was isolated from seawater. On the basis of its 16S rRNA gene sequence, the closest relatives were species of the genus Microbulbifer, but the similarity values were only 90–91 %. Thus, the taxonomic position and characteristics of this novel strain were analysed in more detail. On the basis of the findings, a novel genus belonging to the family Alteromonadaceae, close to the genus Microbulbifer, is proposed for this strain.

Samples of the surface microlayer of seawater in the bay of Banyuls-sur-Mer (42u 299 N 3u 089 E) were collected in October 2002 by submerging a metal screen (Agogué et al., 2004). Subsamples were spread on marine agar 2216 plates (Difco) and incubated at 25 uC for 2 weeks. Colonies were picked and purified after three subcultures. An isolate that formed cream-coloured colonies was obtained and designated strain 3X/A02/235T (Agogué et al., 2005).

Microscopic observations (AX70; Olympus) showed that cells from isolate 3X/A02/235T were motile rods, approximately 1.6±0.3 mm long and 0.5±0.2 mm wide. Cells were negatively stained for transmission electron microscopy (Raguénès et al., 1997) and were shown to possess single polar flagella (see Supplementary Fig. S1, available in IJSEM Online). The Ryu KOH reaction (Powers, 1995) led to immediate cell lysis that was confirmed by microscopy (AX70; Olympus). This positive reaction indicated that the strain was Gram-negative.

Strain 3X/A02/235T was grown in marine broth 2216 (Difco). To determine the salinity range, marine broth 2216 was prepared according to the composition provided by the manufacturer, with the appropriate NaCl concentration. For determination of the pH range, MES, PIPES, AMPSO or MOPS (Sigma) was added to marine broth 2216 to achieve the appropriate pH. Cultures were incubated at 30 uC under aerobic conditions. The methods used for the determination of growth parameters were as reported by Wery et al. (2001b). Growth was observed at 10–37 uC, the optimum temperature being between 25 and 30 uC (see Supplementary Fig. S2, available in IJSEM Online). The strain grew at NaCl concentrations ranging from 7 to 70 g l21, and an optimum concentration could be defined at 40 g l21 (Supplementary Fig. S2). Growth occurred at pH 5.0–9.0, the optimum being pH 8.0. Growth decreased by 61% at pH 9.0 relative to the value obtained at pH 8.0, whereas a relative decrease of only 19% was observed at pH 6.0 (Supplementary Fig. S2). Anaerobic growth was checked using marine agar 2216 plates in an anaerobic jar under these optimal conditions. No growth was observed after 10 days and thus the strain should be considered as strictly aerobic.

The ability of isolate 3X/A02/235T to use different substrates was investigated using Biolog GN2 microplates (Tang et al., 1998) according to the manufacturer's instructions. Positive reactions were observed for Tweens 40 and 80, pyruvic acid methyl ester, succinic acid methyl ester, b-hydroxybutyric acid, a-ketovaleric acid, succinic acid, glutamic acid, glycyl L-glutamic acid and glycerol. DMannose, a-cyclodextrin, acetate, aspartate, succinamic acid and L-proline produced weakly positive reactions. A comparison between strain 3X/A02/235T and its closest relatives is presented in Table 1.

Enzyme activities were investigated using the API ZYM system (bioMérieux) according to the manufacturer's instructions. Alkaline phosphatase, leucine arylamidase, valine arylamidase, acid phosphatase and naphthol-AS-BIphosphohydrolase exhibited positive reactions (Table 1).

An analysis of the fatty acid methyl esters was performed by the Identification Service of the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ, Braunschweig, Germany). The composition for isolate 3X/A02/235T was as follows: 16 : 1v7c (21.2 %), 18 : 1v7c (17.5 %), 15 : 1v6c (5.8 %), 17 : 1v6c (2.7 %), 17 : 1v8c (23.9 %), 10 : 0 3-OH (1.8 %), 11 : 0 3-OH (3.3 %), 12 : 0 3- OH (1.1 %), iso-11 : 0 3-OH (3.3 %), 11 : 0 (1.0 %), 12 : 0 (1.6 %), 13 : 0 (1.3 %), 14 : 0 (1.3 %), 15 : 0 (4.5 %), 16 : 0 (2.0 %) and 17 : 0 (9.3 %) (Table 2). Two major fatty acids found in all Microbulbifer strains, iso-15 : 0 and iso- 17 : 1v9c, were not detected in strain 3X/A02/235T, while 17 : 1v8c, which is the predominant fatty acid in strain 3X/ A02/235T, was poorly represented, or not detected, within the four Microbulbifer species. Fatty acids 11 : 0, 12 : 0, 13 : 0, 15 : 1v6c and 12 : 0 3-OH, which were found in strain 3X/A02/235T, were not detected in any Microbulbifer species. In contrast, fatty acids 10 : 0, iso-11 : 0, iso-15 : 1 and iso-17 : 0, which are always present in Microbulbifer species, were not detected in strain 3X/A02/235T. Analyses of respiratory quinones and polar lipids were carried out by the Identification Service of the DSMZ and Brian Tindall (DSMZ). Strain 3X/A02/235T had a ubiquinone (Q-8) system and the polar lipids were diphosphatidylglycerol, phosphatidylglycerol and an undefined aminophospholipid.

Genomic DNA was extracted as described by Wery et al. (2001a). The G+C content was determined by thermal denaturation using the method of Marmur & Doty (1962) and the conditions described by Raguénès et al. (1997). The DNA G+C content of strain 3X/A02/235T was 61.4±0.2 mol%. The 16S rRNA gene was amplified and sequenced as described by Agogué et al. (2005) and the sequence was analysed as described by Urios et al. (2006).

Strain 3X/A02/235T was found to be phylogenetically affiliated to the family Alteromonadaceae in the class Gammaproteobacteria (Fig. 1). The closest relative was Microbulbifer salipaludis SM-1T, with a similarity value of 91 %. Strain 3X/A02/235T is distinguishable from its closest phylogenetic relatives on the basis of differences in several phenotypic properties, as shown in Tables 1 and 2. Thus we propose that strain 3X/A02/235T represents a novel species of a novel genus belonging to the Alteromonadaceae. Because of the marine origin of strain 3X/A02/235T and its salinity requirement, the name Haliea salexigens gen. nov., sp. nov. is proposed.

Description of Haliea gen. nov.

Haliea (Ha9lie.a. N.L. fem. n. Haliea named after Halie, a sea nymph in Greek mythology, referring to the marine source of the first strain). Motile Gram-negative rods. The major fatty acids are 17 : 1v8c, 16:1v7c, 18:1v7c and 17 : 0. The ubiquinone is Q-8 and the polar lipids are diphosphatidylglycerol and phosphatidylglycerol. Phylogenetically affiliated with the class Gammaproteobacteria within the family Alteromonadaceae. The type species is Haliea salexigens.

Description of Haliea salexigens sp. nov.

Haliea salexigens (sa.le9xi.gens. L. n. sal, salis salt, seawater; L. v. exigo to demand; N.L. part. adj. salexigens seawaterdemanding). Displays the following properties in addition to those given in the genus description. Produces cream colonies on marine agar 2216. Cells are 1.6±0.3 mm long and 0.5±0.2 mm wide, with single polar flagella. The G+C content of the type strain is 61 mol%. Growth occurs at 10–37 uC (optimum, 25–30 uC), at pH 5.0–9.0 (optimum, pH 8.0) and at salinities in the range 7–70 g NaCl l21 (optimum, 42 g l21). Positive reactions with Biolog GN2 plates are obtained for Tweens 40 and 80, pyruvic acid methyl ester, succinic acid methyl ester, b-hydroxybutyric acid, a-ketovaleric acid, succinic acid, glutamic acid, glycyl

Table 1. Characteristics that distinguish strain 3X/A02/235T from type strains of Microbulbifer species Strains: 1, M. maritimus JCM 12187T; 2, M. hydrolyticus DSM 11525T; 3, M. salipaludis KCCM 41586T; 4, M. elongatus DSM 6810T; 5, strain 3X/ A02/235T. The quinone in all of the strains was Q-8. Data for reference type strains were taken from Gonzalez et al. (1997) and Yoon et al. (2003a, b, 2004). +, Positive; 2, negative; (+), weakly positive; ND, no data available.

Characteristic	1	2	3	4	5
Sampling environment	Marine sediment	Salt marsh	Salt marsh	Sand and seawater	Seawater
DNA G+C content (mol%)	59.9	57.7	59	ND	61.4
Motility	−	−	−	+	+
Temperature range (optimum) (°C)	15–48 (37)	10–41 (37)	10–45 (37)	(25–30)	10–37 (25–30)
Salinity range (optimum) (g NaCl l^{-1})	<10 (2–4)	<60 (6–30)	<10 (2–3)	(2–3)	7–70 (42)
pH range (optimum)	>5 (6.5–7.5)	6.5–8.5 (7.5)	>5 (7–8)	ND	5–9 (8)
Oxidase	+	+	+	ND	+
Catalase	ND	ND	+	+	+
API ZYM tests					
Alkaline phosphatase	+	ND	+	ND	+
Leucine arylamidase	+	ND	+	ND	+
Valine arylamidase	−	ND	−	ND	+
Acid phosphatase	+	ND	+	ND	+
Naphthol-AS-BI-phosphohydrolase	+	ND	+	ND	+
N-Acetyl-β-glucosaminidase	−	ND	+	ND	−
Esterase (C4)	+	ND	+	ND	−
Lipase (C8)	+	ND	+	ND	(+)
Substrates					
Arabinose	ND	ND	−	+	−
Cellobiose	+	+	+	+	−
Fructose	−	−	−	+	−
Galactose	ND	ND	−	+	−
Glucose	+	+	+	+	−
Lactose	−	−	−	+	−
Maltose	ND	ND	+	+	−
Mannose	−	−	−	+	(+)
Rhamnose	−	−	+	−	−
Raffinose	−	ND	−	−	−
Sucrose	−	−	−	+	−
Adonitol	−	ND	−	ND	−
Glycerol	ND	−	ND	−	+
Acetate	+	+	ND	+	−
Aspartate	ND	−	ND	(+)	+
Citrate	−	−	ND	−	−
Glutamate	−	+	ND	+	(+)
β-Hydroxybutyrate	ND	+	ND	ND	+
Lactate	−	ND	ND	+	−
Propionate	ND	+	ND	+	−
Pyruvate	+	+	ND	ND	+
Succinate	+	+	ND	+	+
Alanine	ND	+	ND	(+)	−
Leucine	ND	+	ND	+	+
Proline	ND	+	ND	(+)	+
Serine	+	+	ND	−	−
Tween 80	ND	−	+	ND	+

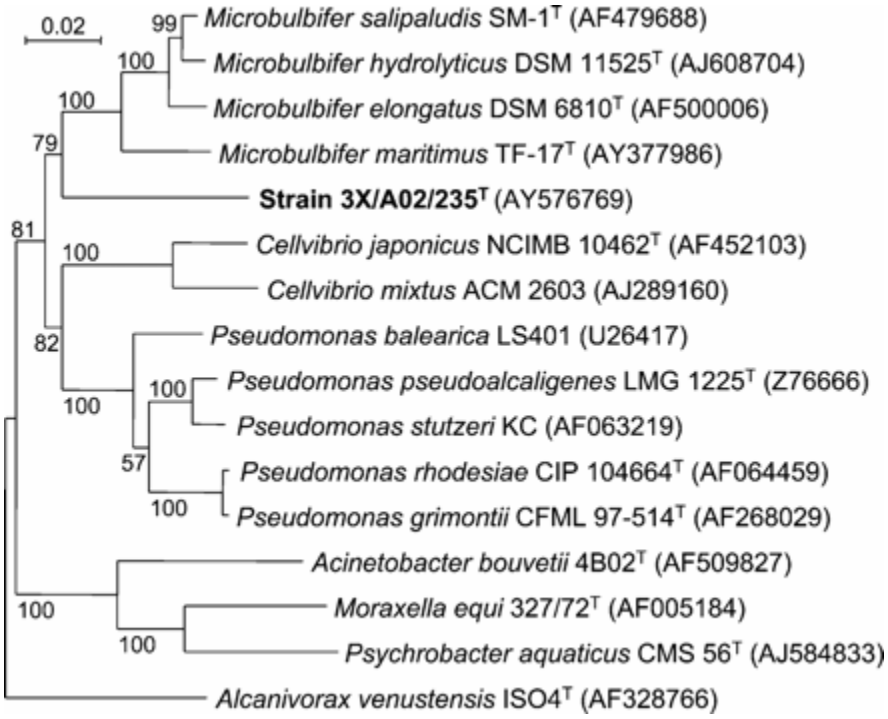
L-glutamic acid and glycerol. Positive API ZYM reactions are obtained for the following enzyme activities: alkaline phosphatase, leucine arylamidase, valine arylamidase, acid phosphatase and naphthol-AS-BI-phosphohydrolase. Oxidase- and catalase-positive. The major fatty acids also include 15 : 1ω6c and 15 : 0.

Table 2. Cellular fatty acid compositions (%) of strain 3X/ A02/235$_T$ and type strains of the genus Microbulbifer Strains: 1, M. maritimus JCM 12187T; 2, M. hydrolyticus DSM 11525T; 3, M. salipaludis KCCM 41586T; 4, M. elongatus DSM 6810T; 5, strain 3X/A02/235T. –, Not detected. Data for reference type strains were taken from Yoon et al. (2004).

Fatty acid	1	2	3	4	5
10 : 0	1.3	1.7	2.4	1.6	–
11 : 0	–	–	–	–	1.0
12 : 0	–	–	–	–	1.6
13 : 0	–	–	–	–	1.3
14 : 0	1.0	1.2	2.6	0.7	1.3
15 : 0	1.6	1.5	1.7	0.9	4.5
16 : 0	8.7	11.4	16.3	7.1	2.0
17 : 0	1.3	2.9	2.2	2.5	9.3
18 : 0	–	1.6	1.4	1.2	–
17 : 0 cyclo	2.3	5.7	–	–	–
19 : 0ω8c	1.4	1.0	–	–	–
iso-11 : 0	10.0	5.7	4.8	6.5	–
iso-15 : 0	25.9	24.4	19.4	20.7	–
iso-15 : 1	0.8	1.0	0.7	1.0	–
iso-16 : 0	–	–	–	0.5	–
iso-17 : 0	6.9	10.4	5.5	9.9	–
anteiso-17 : 0	–	–	–	0.8	–
iso-17 : 1ω9c	12.6	10.1	9.5	11.3	–
15 : 1ω6c	–	–	–	–	5.8
17 : 1ω6c	–	–	–	–	2.7
17 : 1ω8c	–	0.5	1.0	1.8	23.9
18 : 1ω5c	–	–	0.7	–	–
18 : 1ω7c	5.6	8.9	11.8	16.3	17.5
10 : 0 3-OH	1.7	1.0	1.2	1.6	1.8
11 : 0 3-OH	–	–	–	–	3.3
12 : 0 3-OH	–	–	–	–	1.1
16 : 0 2-OH	–	–	0.9	–	–
iso-11 : 0 3-OH	14.2	6.2	5.7	7.7	3.3
iso-17 : 0 3-OH	–	–	0.9	–	–
16 : 1ω7c/iso-15 : 0 2-OH	2.2	2.7	7.1	6.0	21.2

The type strain, 3X/A02/235T (5DSM 19537T 5CIP 109602T 5MOLA 286T), was isolated from the surface microlayer of seawater from the bay of Banyuls-sur-Mer.

Fig. 1. Unrooted neighbour-joining phylogenetic tree (Kimura corrections), based on 16S rRNA gene sequences, showing the position of strain 3X/A02/235T. Bootstrap percentages (based on 100 replications) are shown at branch points. Bar, 2 substitutions per 100 nucleotide positions.



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