Arenicella xantha gen. nov., sp. nov., a gammaproteobacterium isolated from a marine sandy sediment

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A Gram-negative, aerobic, yellow-pigmented, rod-shaped, non-motile bacterium, strain KMM 3895^T, was isolated from a marine sandy sample collected offshore from the Sea of Japan. Phylogenetic analysis based on the 16S rRNA gene sequence placed strain KMM 3895^T in the class *Gammaproteobacteria*, forming a separate branch, sharing 89.5 % sequence similarity with *Nitrincola laxisaponensis* 4CA^T and 88–87 % similarity with the other members of the cluster, including members of *Kangiella*, *Spongiibacter*, *Alcanivorax* and *Microbulbifer*. The major isoprenoid quinone was Q-8. Polar lipids consisted of phosphatidylethanolamine, phosphatidylglycerol, diphosphatidylglycerol, aminophospholipid and an unknown phospholipid. Fatty acid analysis revealed C_{16:1}ω7, iso-C_{16:0}, iso-C_{18:0} and C_{18:1}ω7 as dominant components. The DNA G + C content was determined to be 48.1 mol%. Based on its unique phenotypic characteristics and phylogenetic distance, the marine coastal sediment isolate KMM 3895^T should be classified as a representative of a novel genus and species, for which the name *Arenicella xantha* gen. nov., sp. nov. is proposed. The type strain of *Arenicella xantha* is KMM 3895^T (=NRIC 0759^T =JCM 16153^T).

In the course of a survey of the biodiversity of micro-organisms that are associated with coastal marine environments, a Gram-negative, aerobic, yellow-pigmented, non-motile bacterium, strain KMM 3895^T, was isolated from a sandy sediment sample collected offshore on the coast of the Sea of Japan, Russia, and characterized by phenotypic and phylogenetic analyses.

Strain KMM 3895^T was isolated from a sediment sample taken at a depth of 2 m, as described previously (Romanenko *et al.*, 2004). The bacterium was grown aerobically on marine 2216 agar (MA) or marine broth (MB) at 25–28 °C, and was stored at –80 °C in liquid MB supplemented with 30 % (v/v) glycerol. Motility was observed by the hanging-drop method as described by Gerhardt *et al.* (1994). Gram staining, oxidase and catalase activities and hydrolytic reactions for gelatin, casein, chitin, starch, CM-cellulose, DNA and Tweens 20, 40 and 80 were tested according to the standard methods described by

Smibert & Krieg (1994). Formation of H₂S from thiosulfate was tested using a lead acetate paper strip. Acid production from carbohydrates was examined using the oxidation/fermentation medium of Leifson (1963) for marine bacteria. The ability of strain KMM 3895^T to grow in the presence of organic substrates as sole carbon and energy sources was tested for 4 weeks on an artificial seawater (ASW)-based medium supplemented with 2 g NH₄Cl and 0.5 g yeast extract l^{–1} and 0.4 % carbon source. The ASW contained (per litre distilled water) 30 g NaCl, 4.9 g MgCl₂, 3.9 g Na₂SO₄, 1.1 g CaCl₂, 0.66 g KCl, 0.2 g NaHCO₃, 0.096 g KBr, 0.026 g H₃BO₃, 0.024 g SrCl₂ and 0.003 g NaF. Requirements for organic growth factors and sodium ions were tested on the basal medium of Baumann & Baumann (1981) supplemented with 0.1 % glycerol, 0.1 % potassium acetate or 0.1 % potassium succinate, and on basal medium in which the Na⁺ salts had been replaced by equimolar amounts of K⁺ salts, respectively. Requirement for and tolerance of NaCl were tested on ASW-based medium using various concentrations of NaCl in the range 0–20 %, supplemented with (l^{–1}) 10.0 g Bacto peptone, 2.0 g yeast extract, 0.028 g FeSO₄ and 15.0 g agar. Growth at different temperatures and pH and antibiotic resistance were studied as described previously (Romanenko *et al.*,

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain KMM 3895^T is AB500096.

Results of 2D TLC of polar lipids of strain KMM 3895^T and a 16S rRNA gene sequence-based maximum-parsimony tree are available as supplementary material with the online version of this paper.

2004, 2005). In addition, biochemical tests were carried out using API 20 NE, API 20 E and API ZYM test kits (bioMérieux) according to the manufacturer's instructions, except that the culture was suspended in ASW.

For polar lipid and fatty acid analyses, strain KMM 3895^T was cultivated on MA at 28 °C for 3 days and lipids were extracted using the chloroform/methanol extraction method of Bligh & Dyer (1959). Polar lipids were analysed as described by Vaskovsky & Terekhova (1979). Fatty acid methyl esters were obtained by alkaline methanolysis (15 % NaOH/methanol). The resultant fatty acid methyl esters were extracted with hexane and analysed using a GLC-MS Hewlett Packard model 6890 gas chromatograph equipped with an HP 5 MS 5 % phenyl methyl siloxane capillary column (30 m × 250 µm × 0.25 µm) and connected to a Hewlett Packard model 5973 mass spectrometer. Cellular biomass of strain KMM 3895^T for respiratory lipoquinone analysis was obtained from an MB culture grown at 28 °C. Isoprenoid quinones were extracted using chloroform/methanol (2:1, v/v), purified by preparative TLC on silica gel 60 ADAMANT plates (Fluka) and analysed by HPLC (Agilent 1100 Series) using a reversed-phase column (Hypersil ODS, 5 µm; 40 × 250 mm). Methanol/2-propanol (65:35) was used as a mobile phase and quinones were detected at 270 nm. The DNA base composition was determined as described by Marmur & Doty (1962) and Owen *et al.* (1969). A 16S rRNA gene sequence of 1506 nucleotides was determined for strain KMM 3895^T as described by Shida *et al.* (1997). The sequence obtained was compared with 16S rRNA gene sequences retrieved from the EMBL/GenBank/DBJ databases by using the FASTA program (Pearson & Lipman, 1988). Phylogenetic analysis of 16S rRNA gene sequences was performed using the software package MEGA 4 (Tamura *et al.*, 2007) after multiple alignment of data by CLUSTAL_X (version 1.83; Thompson *et al.*, 1997). Phylogenetic trees were constructed by the neighbour-joining and maximum-parsimony methods and distances were calculated according to Kimura's two-parameter model. The robustness of phylogenetic trees was estimated by bootstrap analysis of 1000 replicates.

Cultural, physiological and biochemical properties of strain KMM 3895^T are listed in Table 1 and in the genus and species descriptions. An analysis of respiratory lipoquinones revealed ubiquinone Q-8 to be the major compound. Polar lipids of strain KMM 3895^T included phosphatidylethanolamine, phosphatidylglycerol and diphosphatidylglycerol, an aminophospholipid and an unknown phospholipid (Supplementary Fig. S1, available in IJSEM Online). The predominance of isoprenoid quinone Q-8 has been reported for the related gammaproteobacteria *Kangiella koreensis* and *Kangiella aquimarina* (Yoon *et al.*, 2004b) and *Microbulbifer hydrolyticus* (González *et al.*, 1997; Tang *et al.*, 2008). The presence of phosphatidylethanolamine, phosphatidylglycerol and diphosphatidylglycerol is in accordance with the polar lipids found in some other marine gammaproteobacteria (Romanenko *et al.*, 2003, 2004, 2005). Polar lipids and

ubiquinones have not been reported for the phylogenetic neighbours *Nitricola laciaponensis* (Dimitriu *et al.*, 2005), *Spongiibacter marinus* (Graeber *et al.*, 2008) and *Alcanivorax borkumensis* (Yakimov *et al.*, 1998; Fernández-Martínez *et al.*, 2003), and polar lipids have not been reported for *Kangiella* species (Yoon *et al.*, 2004b).

The fatty acid composition of strain KMM 3895^T was characterized by the predominance of C_{16:1}ω7c, iso-C_{16:0}, iso-C_{18:0} and C_{18:1}ω7c. The full profile consisted of iso-C_{14:0} (4.5 %), C_{14:1}ω7 (7.5 %), iso-C_{15:1} (3.2 %), C_{15:1} (3.5 %), iso-C_{16:0} (18.6 %), C_{16:1}ω7c (21.1 %), C_{16:0} (6.9 %), iso-C_{17:1} (4.7 %), C_{17:1}ω5 (3.0 %), iso-C_{18:1} (2.8 %), iso-C_{18:0} (12.8 %) and C_{18:1}ω7c (11.9 %). Strain KMM 3895^T could be distinguished readily from *Kangiella* species (Yoon *et al.*, 2004b), *M. hydrolyticus* (González *et al.*, 1997; Tang *et al.*, 2008), *Alcanivorax borkumensis* (Yakimov *et al.*, 1998; Fernández-Martínez *et al.*, 2003) and *S. marinus* (Graeber *et al.*, 2008) by the presence of iso-C_{16:0}, iso-C_{18:0}, iso-C_{18:1}, iso-C_{14:0} and C_{14:1}ω7 and from *Kangiella* species and *S. marinus* by the absence of hydroxy branched fatty acids. In contrast to *K. koreensis*, *K. aquimarina* and *M. hydrolyticus*, the novel isolate KMM 3895^T did not contain iso-C_{15:0} or iso-C_{11:0}, but contained C_{16:1}ω7c as a major fatty acid. Thus, the fatty acid composition of isolate KMM 3895^T can be considered to be unique compared with profiles obtained for related taxa, including *S. marinus*, *K. koreensis*, *K. aquimarina*, *Alcanivorax borkumensis* and *M. hydrolyticus*.

The DNA G + C content of strain KMM 3895^T was determined to be 48.1 mol%. This mean was close to the G + C content of 47.4 mol% found for *N. laciaponensis*, but clearly distinguished the strain from *Kangiella* species (44 mol%), *S. marinus* (69.1 mol%), *M. hydrolyticus* (57.7 mol%) and *Alcanivorax borkumensis* (53–54 mol%) (Table 1).

Comparative 16S rRNA gene sequence analysis showed that the novel marine sediment isolate KMM 3895^T was affiliated to the Gammaproteobacteria as a separate line adjacent to the members of the genus *Kangiella*, *K. koreensis* and *K. aquimarina* (Fig. 1 and Supplementary Fig. S2), and displayed highest sequence similarity of 89.5 % to the type strain of *N. laciaponensis* and 87–88 % sequence similarity to the members of recognized genera *Kangiella*, *Spongiibacter*, *Alcanivorax*, *Saccharophagus* and *Microbulbifer*.

The isolated phylogenetic position of strain KMM 3895^T was supported by its unique physiological and biochemical properties. Cells of strain KMM 3895^T were Gram-negative, aerobic, non-fermentative, heterotrophic, stenohaline, deep-yellow-pigmented, rod-shaped and non-motile. Strain KMM 3895^T exhibited hydrolytic activities in routine and API ZYM tests as described in Table 1 and the species description. Novel isolate KMM 3895^T could be distinguished from its close relatives belonging to the genera *Kangiella*, *Spongiibacter* and *Nitricola* and from *Alcanivorax borkumensis* and *M. hydrolyticus* in minimum and maximum temperatures that support growth, salinity range for growth and metabolic properties (Table 1).

Table 1. Differential characteristics of strain KMM 3895^T (*Arenicella xantha* gen. nov., sp. nov.) and related gammaproteobacteria

Taxa: 1, KMM 3895^T (data from the present study); 2, *Kangiella koreensis* (Yoon *et al.*, 2004b); 3, *Kangiella aquimarina* (Yoon *et al.*, 2004b); 4, *Spongiibacter marinus* (Graeber *et al.*, 2008); 5, *Alcanivorax borkumensis* (Yakimov *et al.*, 1998; Fernández-Martínez *et al.*, 2003); 6, *Nitrincola laciaponensis* (Dimitriu *et al.*, 2005); 7, *Microbulbifer hydrolyticus* (González *et al.*, 1997; Yoon *et al.*, 2004a; Tang *et al.*, 2008). +, Positive; –, negative; s, slow; w, weak; ND, no data available.

Characteristic	1	2	3	4	5	6	7
Pigmentation*	Y	YB	YB	BG	—	WHBG	C
Motility	—	—	—	+	—	+	—
Growth at 4 °C	—	+	—	—	+	—	—
Maximal growth temperature (°C)	35	43	48	40	35	ND	41
NaCl range for growth (%)	1–5	0.5–13	0.5–13	1–7	1–12.5	1–7.5	0.6–6
Nitrate reduction	—	—	—	ND	+	+	—
Carbohydrate utilization	w	—	—	+	—	—	+
Hydrolysis of:							
Aesculin	+	—	—	ND	—	ND	+
Gelatin	+	+	+	ND	—	—	+
Starch	+	—	—	ND	—	—	+
Tween 80	+	+	+	+	+	ND	+
Tyrosine	—	+	+	ND	ND	ND	ND
Casein	s	+	+	ND	ND	ND	+
DNA	—	ND	ND	ND	ND	—	ND
Urea	—	—	—	ND	—	—	ND
Enzyme activity (API ZYM)							
Alkaline phosphatase	+	+	+	+	ND	ND	ND
Esterase (C4)	+	+	+	—	ND	ND	ND
Esterase lipase (C8)	+	+	+	+	ND	ND	ND
Lipase (C14)	—	—	—	—	ND	ND	ND
Leucine arylamidase	+	+	+	+	ND	ND	ND
Valine arylamidase	+	+	+	—	ND	ND	ND
Cystine arylamidase	—	—	—	—	ND	ND	ND
Trypsin	+	+	+	—	ND	ND	ND
α-Chymotrypsin	—	—	—	—	ND	ND	ND
Acid phosphatase	—	—	+	+	ND	ND	ND
Naphthol-AS-BI-phosphohydrolase	—	+	+	+	ND	ND	ND
N-Acetyl-β-glucosaminidase	—	—	—	+	ND	ND	—
β-Glucosidase	+	—	—	—	ND	ND	ND
Major ubiquinone	Q-8	Q-8	Q-8	ND	ND	ND	Q-8
Main fatty acid(s)†	C _{16:1} ω7c, i-C _{16:0} , i-C _{18:0} , C _{18:1} ω7c	i-C _{15:0}	i-C _{15:0}	C _{17:1} ω8, C _{17:0} , C _{18:1} ω7	C _{16:0} , C _{16:1} ω7c, C _{18:1} ω7c	ND	i-C _{15:0} , C _{16:0}
DNA G + C content (mol%)	48.1	44	44	69.1	53–54	47.4	57.7

*BG, Beige; C, cream; WHBG, whitish beige; Y, yellow; YB, yellowish brown.

†i, iso-branched.

Characteristics that differentiate strain KMM 3895^T from related gammaproteobacteria are listed in Table 1. On the basis of phenotypic and phylogenetic evidence, it is proposed to classify strain KMM 3895^T within a novel genus and species, *Arenicella xantha* gen. nov., sp. nov.

Description of *Arenicella xantha* gen. nov.

Arenicella xantha (A.re.ni.cel'la. L. n. *arena* sand; L. fem. n. *cella* a chamber, a cell; N.L. fem. n. *Arenicella* a cell from sand).

Gram-negative, aerobic, oxidase- and catalase-positive, rod-shaped bacteria. Chemo-organoheterotrophic. Sodium ions are essential for growth. The predominant isoprenoid quinone is Q-8. Polar lipids include phosphatidylethanolamine, phosphatidylglycerol, diphosphatidylglycerol, an aminophospholipid and an unknown phospholipid. Major fatty acids are C_{16:1}ω7c, iso-C_{16:0}, iso-C_{18:0} and C_{18:1}ω7c. The only known strain has been isolated from the marine environment. On the basis of 16S rRNA gene sequence analysis, the genus represents a separate branch within the *Gammaproteobacteria*, related to the genera *Kangiella*,

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences available from the GenBank/EMBL/DBJ databases (accession numbers in parentheses) showing the relationship of isolate KMM 3895^T and related members of the Gammaproteobacteria. Phylogenetic analysis was performed using the software package MEGA 4 (Tamura *et al.*, 2007) after multiple alignment of data by CLUSTAL_X (version 1.83; Thompson *et al.*, 1997). Bootstrap values (greater than 70%) based on 1000 replications are given as percentages at branching points. Bar, 0.02 substitutions per nucleotide position.

Sphingobacter, *Alcanivorax*, *Nitriicola* and *Microbulbifer*. The type species is *Arenicella xantha*.

Description of *Arenicella xantha* sp. nov.

Arenicella xantha (xan'tha. N.L. fem. adj. *xantha* from Gr. adj. *xanthos* yellow-coloured).

In addition to properties given in the genus description, the species is characterized as follows. Cells are 0.5–0.6 µm in diameter and 3.0–4.0 µm long. Non-motile. Produces deep-yellow-pigmented, smooth, shiny colonies with regular edges, 2–3 mm in diameter, on MA. Requires NaCl for growth; growth occurs at 1–5 % (w/v) NaCl and is optimal with 2–3 %. Grows well in/on peptone medium containing NaCl alone without the addition of any of the components of sea salts (MgCl₂, KCl, CaCl₂, NaNO₃, K₂HPO₄, KCl, NaSO₄, NaHCO₃, NaF and FeSO₄). No growth on basal medium supplemented with 0.1 % glycerol, 0.1 % potassium acetate or 0.1 % potassium succinate or on basal medium when the Na⁺ salts are replaced by equimolar amounts of K⁺ salts. Shows weak growth on D-glucose, melibiose, L-arabinose, D-ribose, D-xylose and L-alanine and no growth on maltose, sucrose, D-galactose, N-acetylglucosamine, lactose, raffinose, L-rhamnose, D-mannose, cellobiose, glycerol, acetate, glutamic acid, DL-methionine, D-mannitol, citrate, L-asparagine, L-arginine, L-phenylalanine, L-valine, L-lysine, L-leucine and L-histidine. Psychrotolerant; the temperature range for growth is 5–35 °C, with optimum growth at 25–28 °C. No growth is observed at 4 °C or above 35 °C. The pH range for growth is 6.0–11.0 with an optimum at pH 8.5–9.5. Negative for H₂S production. Positive for hydrolysis of gelatin, starch and Tweens 20, 40 and 80. Casein is hydrolysed in 3–5 days. Does not degrade CM-cellulose, agar, chitin or DNA. On L-tyrosine-containing medium, does not produce melanin-like pigments and/or clearance zones. No acid production is observed from D-glucose, maltose, sucrose, lactose, D-galactose, D-mannose, cellobiose, D-xylose, L-arabinose, L-rhamnose, D-sorbitol or D-mannitol. According to API 20 NE tests, positive for

aesculin hydrolysis, gelatin hydrolysis and the PNPG test (β-glucosidase) and negative for nitrate reduction, indole production, D-glucose acidification under anaerobic conditions, production of arginine dihydrolase and urease and assimilation of D-glucose, L-arabinose, D-mannose, D-mannitol, N-acetylglucosamine, maltose, D-gluconate, caprate, adipate, L-malate, citrate and phenylacetate. According to API 20 E tests, positive for gelatin hydrolysis and the PNPG test and negative for arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, citrate utilization, H₂S production under anaerobic conditions, urease, tryptophan deaminase, indole production, acetoin production (Voges–Proskauer reaction) and oxidation or fermentation of D-glucose, D-mannitol, inositol, D-sorbitol, L-rhamnose, sucrose, melibiose, amygdalin and L-arabinose. According to API ZYM tests, positive for alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, trypsin and β-glucosidase and negative for lipase (C14), cystine arylamidase, α-chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, N-acetyl-β-glucosaminidase, α-galactosidase, β-galactosidase, α-glucosidase, β-glucuronidase, α-mannosidase and α-fucosidase. The fatty acid profile includes (in decreasing order of abundance) C_{16:1}ω7c, iso-C_{16:0}, iso-C_{18:0}, C_{18:1}ω7c, C_{14:1}ω7, C_{16:0}, iso-C_{17:1}, iso-C_{14:0}, C_{15:1}, iso-C_{15:1}, C_{17:1}ω5 and iso-C_{18:1}. Susceptible to (content per disc) ampicillin (10 µg), benzylpenicillin (10 U), carbenicillin (100 µg), gentamicin (10 µg), oleandomycin (15 µg), rifampicin (5 µg), streptomycin (30 µg), kanamycin (30 µg), nalidixic acid (30 µg), neomycin (30 µg), ofloxacin (5 µg), erythromycin (15 µg), cephazolin (30 µg), cephalixin (30 µg) and chloramphenicol (30 µg) and resistant to vancomycin (30 µg), lincomycin (15 µg), oxacillin (10 µg), polymyxin (300 U) and tetracycline (30 µg). The DNA G + C content of the type strain is 48.1 mol% (thermal denaturation method).

The type strain, KMM 3895^T (=NRIC 0759^T =JCM 16153^T), was isolated from a sandy sediment sample collected offshore from the Sea of Japan, Russia.

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