Colwellia aestuarii sp. nov., isolated from a tidal flat sediment in Korea

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A novel *Colwellia*-like bacterial strain, SMK-10^T, was isolated from a tidal flat sediment in Korea and subjected to a polyphasic taxonomic analysis. Cells of strain SMK-10^T were Gram-negative, motile, greyish yellow-pigmented, curved rods. Optimal growth occurred at 25–30 °C and in the presence of 2–3 % (w/v) NaCl. Strain SMK-10^T contained Q-8 as the predominant ubiquinone and C_{16:1}ω₇C and/or iso-C_{15:0} 2-OH, C_{17:1}, C_{15:1} and iso-C_{16:0} as major fatty acids. The DNA G + C content was 39.3 mol%. Phylogenetic trees based on 16S rRNA gene sequence analysis showed that strain SMK-10^T belonged to the genus *Colwellia*. 16S rRNA gene sequence similarity values (94.7–96.7 %) to the type strains of all other *Colwellia* species and various differential phenotypic properties were sufficient to distinguish strain SMK-10^T from recognized *Colwellia* species. On the basis of its phenotypic properties and phylogenetic distinctiveness, strain SMK-10^T (=KCTC 12480^T=DSM 17314^T) is classified as the type strain of a novel *Colwellia* species, for which the name *Colwellia aestuarii* sp. nov. is proposed.

The family *Colwelliaceae*, which belongs to the *Gamma-proteobacteria* (Ivanova *et al.*, 2004), includes the genera *Colwellia* and *Thalassomonas*. This family accommodates bacteria with cells that are Gram-negative, motile or non-motile, chemo-organotrophic, curved rods or straight rods, psychrophilic and/or halophilic, and contain ubiquinone-8 as the predominant isoprenoid quinone (Yumoto *et al.*, 1998; Nogi *et al.*, 2004; Yi *et al.*, 2004; Ivanova *et al.*, 2004). At the time of writing, the genus *Colwellia* comprises eight species, *Colwellia psychrerythraea* and *C. hadaliensis* (Deming *et al.*, 1988), *C. demingiae*, *C. hornerae*, *C. psychrotropica* and *C. rossensis* (Bowman *et al.*, 1998), *C. maris* (Yumoto *et al.*, 1998) and *C. piezophila* (Nogi *et al.*, 2004). The genus *Thalassomonas* comprises two species, *Thalassomonas viridans* (Macián *et al.*, 2001) and *T. ganghwensis* (Yi *et al.*, 2004). Recently, a bacterial strain, SMK-10^T, cells of which were Gram-negative, greyish-yellow-pigmented, curved rods, was isolated from a tidal flat sediment in Korea. 16S rRNA gene sequence comparison indicated that strain SMK-10^T is phylogenetically related to the family *Colwelliaceae*. Accordingly, the aim of the present study was to determine the exact taxonomic position of strain SMK-10^T by using a combination of polyphasic taxonomic data.

Tidal flat sediment collected from Saemankum, Pyunsan, Korea, was used as the source for the isolation of bacterial

strains. Strain SMK-10^T was isolated by the dilution plating technique on marine agar 2216 (MA; Difco) at 30 °C. *C. hornerae* CIP 105821^T and *C. maris* CIP 106458^T, which were obtained from the Collection de l'Institut Pasteur (CIP), Paris, France, were used as reference strains for fatty acid analysis. Cell morphology and presence of flagella were examined by light microscopy (Nikon E600) and transmission electron microscopy (TEM) by using cells grown on MA. The Gram reaction was determined by using the bio-Mérieux Gram Stain kit according to the manufacturer's instructions. Growth at various temperatures from 4 to 40 °C was measured on MA, and tolerance to various NaCl concentrations was measured in marine broth 2216 (MB; Difco). The pH range for growth and optimal pH were determined in MB that was adjusted to various pH values (initial pH 4.5–9.0 at intervals of 0.5 pH units). Growth in the absence of NaCl was investigated in R2A agar (Difco) and trypticase soy broth prepared according to the formula for the Difco medium except that no NaCl was used. Growth under anaerobic conditions was determined after incubation in an anaerobic chamber on MA and on MA supplemented with nitrate, both of which had been prepared anaerobically using nitrogen. Catalase and oxidase activity and hydrolysis of casein and starch were determined as described by Cowan & Steel (1965). Hydrolysis of hypoxanthine, tyrosine and xanthine was investigated on MA with the substrate concentrations described by Cowan & Steel (1965). Hydrolysis of aesculin, gelatin and urea, and nitrate reduction were studied as described by Lanyi (1987) with the modification that artificial sea water (containing 23.6 g NaCl, 0.64 g KCl, 4.53 g MgCl₂·6H₂O, 5.94 g MgSO₄·7H₂O

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and 1.3 g $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ per litre distilled water; Bruns *et al.*, 2001) was used for the preparation of media. Hydrolysis of Tweens 20, 40, 60 and 80 was determined as described by Cowan & Steel (1965) with the modification that artificial sea water was used for the preparation of media. Acid production from carbohydrates was determined as described by Leifson (1963). Utilization of various substrates as sole carbon and energy sources was tested according to the method of Baumann & Baumann (1981), using supplementation with 2% (v/v) Hutner's mineral base (Cohen-Bazire *et al.*, 1957), 1% (v/v) vitamin solution (Staley, 1968) and 0.005% (w/v) yeast extract. The API ZYM system (bioMérieux) was used to determine enzyme activities. Antibiotic sensitivity was tested by spreading bacterial suspension on MA and applying discs impregnated with the following antibiotics (concentration per disc): ampicillin (10 µg), carbenicillin (100 µg), cephalothin (30 µg), chloramphenicol (100 µg), gentamicin (30 µg), lincomycin (15 µg), kanamycin (30 µg), neomycin (30 µg), novobiocin (5 µg), oleandomycin (15 µg), penicillin G (20 U), polymyxin B (100 U), streptomycin (50 µg) and tetracycline (30 µg).

Strain SMK-10^T was cultivated for 3 days in MB at 30 °C to obtain the cell mass required for isoprenoid quinone analysis and DNA extraction. Isoprenoid quinones were extracted according to the method of Komagata & Suzuki (1987) and analysed using reversed-phase HPLC and a YMC ODS-A (250 × 4.6 mm) column. For fatty acid methyl ester analysis, cell mass of strain SMK-10^T was harvested from agar plates after cultivation for 3 days at 30 °C on MA; cell mass of *C. maris* CIP 106458^T was obtained from 5 days cultivation at 15 °C on PYSE agar (Yumoto *et al.*, 1998) and cell mass of *C. hornerae* CIP 105821^T was obtained from 7 days cultivation at 10 °C on MA. The fatty acid methyl esters were extracted and prepared according to the standard protocol of the MIDI/Hewlett Packard Microbial Identification System (Sasser, 1990). Chromosomal DNA was extracted and purified by the procedure described by Yoon *et al.* (1996). The DNA G + C content was determined by the method of Tamaoka & Komagata (1984) with a modification that DNA was hydrolysed and the resulting nucleotides were analysed by reversed-phase HPLC. Amplification of the 16S rRNA gene was performed according to the method described by Yoon *et al.* (1998) using two universal primers. Sequencing of the amplified 16S rRNA gene and phylogenetic analysis were performed as described by Yoon *et al.* (2003).

Morphological, cultural, physiological and biochemical characteristics of strain SMK-10^T are shown in Table 1 or are given in the species description below. The predominant ubiquinone detected in strain SMK-10^T was Q-8 at a peak area ratio of approximately 98%. The cellular fatty acid profile of strain SMK-10^T is given in Table 2, together with those of some other *Colwellia* species. The profile of strain SMK-10^T was characterized by large amounts of unsaturated, straight-chain, branched and hydroxy fatty acids; the major components (> 10% of the total) were $\text{C}_{16:1}\omega 7\text{c}$ and/

or iso- $\text{C}_{15:0}$ 2-OH, $\text{C}_{17:1}$, $\text{C}_{15:1}$ and iso- $\text{C}_{16:0}$. The DNA G + C content of strain SMK-10^T was 39.3 mol%.

The 16S rRNA gene sequence of strain SMK-10^T determined in this study comprised 1496 nt, representing approximately 96% of the *Escherichia coli* 16S rRNA gene sequence. In the phylogenetic tree based on the neighbour-joining algorithm, strain SMK-10^T joined the cluster comprising *Colwellia* species at a bootstrap resampling value of 93.4% (Fig. 1). Strain SMK-10^T exhibited 16S rRNA gene sequence similarity values of 94.7–96.7% to the type strains of seven *Colwellia* species and of 94.1–95.7% to the type strains of the two *Thalassomonas* species. Sequence similarities to the other species included in the phylogenetic analysis were below 90.0% (Fig. 1).

Phylogenetic analyses based on the neighbour-joining, maximum-likelihood and maximum-parsimony algorithms revealed that strain SMK-10^T and *Colwellia* species form a clade that is independent of the genus *Thalassomonas* (Fig. 1). In the neighbour-joining phylogenetic tree, the relationship between this clade and the cluster comprising the two *Thalassomonas* species was supported by a bootstrap confidence value of 100% (Fig. 1). Phylogenetic discrimination, supported by high bootstrap resampling values, between the genera *Colwellia* and *Thalassomonas* was also shown in previous studies (Yi *et al.*, 2004; Ivanova *et al.*, 2004). These results suggest two possibilities for the classification of strain SMK-10^T: that it is a member of the genus *Colwellia* or a member of a new genus. There were no distinct phenotypic, particularly chemotaxonomic, properties to differentiate strain SMK-10^T from the genus *Colwellia* (Bowman *et al.*, 1998; Yumoto *et al.*, 1998; Nogi *et al.*, 2004). The predominant respiratory lipoquinone (Q-8) of strain SMK-10^T was the same as that of the genus *Colwellia* (Yumoto *et al.*, 1998; Nogi *et al.*, 2004). The fatty acid profile of strain SMK-10^T was similar to those of *Colwellia* species, although there were differences in the proportion of each fatty acid, possibly as a result of the different analytical conditions used, e.g. cultivation and apparatus for the analysis. In particular, two fatty acids, $\text{C}_{16:1}\omega 7\text{c}$ and iso- $\text{C}_{15:0}$ 2-OH, could not be separated by GLC with the MIDI system in the present study. When the type strains of two *Colwellia* species, *C. maris* CIP 106458^T and *C. hornerae* CIP 105821^T, were analysed in this study, their fatty acid profiles were similar to that of strain SMK-10^T. Based on our combined phylogenetic and chemotaxonomic analyses, it seems appropriate that strain SMK-10^T be considered as a member of the genus *Colwellia*. Strain SMK-10^T was distinguishable from recognized *Colwellia* species by several phenotypic characteristics (Table 1). Despite the fact that we did not undertake any DNA–DNA hybridization experiments, the phylogenetic distinctiveness of strain SMK-10^T is sufficient to separate it from other recognized *Colwellia* species (Stackebrandt & Goebel, 1994). Therefore, on the basis of the data presented, strain SMK-10^T should be placed in the genus *Colwellia* as a member of a novel species, for which the name *Colwellia aestuarii* sp. nov. is proposed.

Table 1. Differential phenotypic characteristics of *Colwellia aestuarii* sp. nov. and other *Colwellia* species

Species: 1, *C. aestuarii* sp. nov.; 2, *C. psychrerythraea* (data from Bowman *et al.*, 1998; D'Aoust & Kushner, 1972); 3, *C. demingiae* (Bowman *et al.*, 1998); 4, *C. hornerae* (Bowman *et al.*, 1998); 5, *C. psychrotropica* (Bowman *et al.*, 1998); 6, *C. rossensis* (Bowman *et al.*, 1998); 7, *C. maris* (Yumoto *et al.*, 1998); 8, *C. piezophila* (Nogi *et al.*, 2004). *n*, Number of strains; +, positive; –, negative; v, variable; ND, not determined; NG, no growth on test medium. Data in parentheses are for the type strain. All species are positive for oxidase, catalase, nitrate reduction and growth at 4 °C. All species are negative for Gram-stain, growth at 37 °C and acid production (not determined for *C. maris*) from D-mannitol, D-sorbitol and myo-inositol.

Characteristic	1 (<i>n</i> =1)	2 (<i>n</i> =4)	3 (<i>n</i> =2)	4 (<i>n</i> =1)	5 (<i>n</i> =1)	6 (<i>n</i> =1)	7 (<i>n</i> =1)	8 (<i>n</i> =2)
Cell shape	Curved rod	Rod	Curved rod	Curved rod	Curved rod	Curved rod	Curved rod	Rod
Cell size (µm)	0.4–0.5 × 1.8–3.1	0.5 × 2.5–3.5	0.4–0.6 × 1.5–4.5	0.4–0.8 × 1.5–3.0	0.4–0.8 × 1.5–3.0	0.4–0.8 × 1.5–3.0	0.6–1.0 × 2.0–4.0	0.8–1.0 × 2.0–4.0
Motility	+	+	+	+	+	–	+	+
Growth at 25 °C	+	–	–	–	+	–	–	–
Urea	–	v (+)	–	–	+	+	ND	ND
Hydrolysis of:								
Aesculin	–	+	+	+	–	NG	ND	ND
Casein	+	+	+	+	+	–	–	–
Gelatin	–	v (+)	–	–	–	–	+	+
Starch	–	+	+	+	–	+	–	–
Tween 80	+	+	–	+	+	NG	+	ND
Utilization of:								
L-Arabinose	+	–	–	–	–	+	–	–
D-Fructose	–	–	–	–	–	+	–	–
D-Galactose	+	–	–	–	–	–	ND	–
D-Glucose	+	v (+)	–	–	–	+	+	ND
Maltose	+	–	–	–	–	–	–	ND
D-Cellobiose	+	–	–	–	–	–	ND	–
D-Trehalose	+	–	–	–	–	–	ND	–
Acetate	–	+	+	+	+	+	ND	ND
Citrate	–	–	+	+	–	+	ND	ND
L-Malate	+	–	–	–	+	+	ND	ND
Acid production from:								
D-Mannose, D-fructose,	+	–	–	–	–	–	–	–
L-arabinose, sucrose								
D-Trehalose, lactose	+	–	–	–	–	–	ND	–
Melibiose	+	–	–	–	–	–	–	ND
DNA G+C content (mol%)	39.3	35–38	37	39	42	38	39.4	38.5–39.1

Table 2. Cellular fatty acid composition of *Colwellia aestuarii* SMK-10^T and some *Colwellia* species

Taxa: 1, *C. aestuarii* SMK-10^T (data from this study); 2, *C. maris* CIP 106458^T (this study); 3, *C. maris* JCM 10085^T (Nogi *et al.*, 2004); 4, *C. hornerae* CIP 105821^T (this study); 5, *C. hornerae* ACAM 607^T (Bowman *et al.*, 1998); 6, *C. psychrerythraea* (Bowman *et al.*, 1998); 7, *C. demingiae* (Bowman *et al.*, 1998); 8, *C. psychrotropica* ACAM 179^T (Bowman *et al.*, 1998); 9, *C. rossensis* ACAM 608^T (Bowman *et al.*, 1998); 10, *C. piezophila* Y223G^T and Y251E (Nogi *et al.*, 2004). Values are percentages of total fatty acids. —, Not detected or not described. tr, Trace fatty acid component making up 0.1% or less of total fatty acid content; fatty acids that represented <1.0% in all strains are not indicated.

Fatty acid	1	2	3	4	5	6	7	8	9	10
Straight-chain										
C _{10:0}	1.7	2.0	—	0.9	—	—	—	—	—	—
C _{11:0}	—	1.6	—	0.2	—	—	—	—	—	—
C _{12:0}	0.5	1.9	2	—	—	—	—	—	—	1–2
C _{14:0}	2.3	1.0	—	0.9	3.0	5.1–7.8	7.6–8.0	0.8	4.6	9
C _{15:0}	4.8	8.0	4	5.3	2.9	1.7–11.0	0.9–1.4	2.7	2.9	3
C _{16:0}	9.9	15.0	25	7.7	13.5	26.8–33.2	21.9–23.6	21.9	27.1	31–33
C _{17:0}	2.5	2.6	3	1.0	—	0–1.3	tr	1.5	0.1	—
C _{18:0}	—	—	—	—	2.0	0.1–2.4	0.2–0.5	0.4	tr	—
Branched										
iso-C _{16:0}	10.4	—	—	15.4	10.3	0–0.2	—	—	—	—
Unsaturated										
C _{14:1} *	—	—	2	—	2.8	5.1–7.3	9.1–9.3	2.0	2.8	2
C _{15:1}	10.9	13.4	6	23.7	21.4	0–2.7	1.9–2.6	4.3	4.1	3
C _{16:1} †	—	—	51	—	15.4	31.4–36.3	37.5–37.8	56.8	43.4	48–50
C _{16:1} ω7c and/or iso-C _{15:0} 2-OH‡	23.6	32.8	—	24.0	—	—	—	—	—	—
C _{16:1} ω9c	5.6	4.2	—	1.8	2.0	6.2–8.8	9.5–11.8	—	1.8	—
C _{17:1}	11.8	6.2	4	6.2	7.5	0–2.2	tr	5.4	0.5	—
C _{18:1} ω9c	0.6	—	—	—	1.4	0–1.7	tr–0.2	0.3	0.8	—
C _{18:1} ω7c	1.2	0.7	—	0.8	—	0.3–2.1	1.3–1.4	1.9	4.2	—
C _{20:5} ω3c	—	—	—	—	—	0–1.5	—	0.1	tr†	—
C _{22:6} ω3c	—	—	—	—	2.1	5.5–8.0	1.7–2.2	0.7	6.0	—
Hydroxy										
C _{11:0} 3-OH	2.6	1.9	—	1.9	—	—	—	—	—	—
C _{12:0} 3-OH	2.5	3.0	3	0.8	—	—	—	—	—	0–1
C _{16:0} 3-OH	—	1.1	—	—	—	—	—	—	—	—
iso-C _{12:0} 3-OH	5.3	—	—	2.7	—	—	—	—	—	—

*Indicated as C_{14:1}ω7c by Bowman *et al.* (1998).

†Indicated as C_{16:1}ω7c by Bowman *et al.* (1998).

‡Could not be separated by GLC with the MIDI system.

Fig. 1. Neighbour-joining tree showing the phylogenetic positions of strain SMK-10^T and other related taxa based on 16S rRNA gene sequences. Only bootstrap values (expressed as percentages of 1000 replications) of >50% are shown at the branch points. Dots indicate that the corresponding nodes are also recovered in trees generated with the maximum-likelihood and maximum-parsimony algorithms. Bar, 0.01 substitutions per nucleotide position.

Description of *Colwellia aestuarii* sp. nov.

Colwellia aestuarii (aes.tu.a'ri.i. L. gen. n. *aestuarii* of a tidal flat, from where the type strain was isolated).

Cells are Gram-negative, curved rods, 0.4–0.5 × 1.8–3.1 µm. Motile by means of a single polar flagellum. Growth occurs under anaerobic conditions on MA and on MA supplemented with nitrate. Growth occurs at 4 and 32 °C with an optimum temperature range of 25–30 °C. Optimal pH range for growth is 7.0–8.0; growth occurs weakly at pH 5.0 but not at 4.5. Optimal growth occurs in the presence of 2–3 % (w/v) NaCl; growth occurs in the presence of 6 % (w/v) NaCl but not in the presence of >7 % NaCl. Tweens 20, 40 and 60 are hydrolysed, but hypoxanthine, xanthine and L-tyrosine are not. In assays with the API ZYM system, alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, acid phosphatase, naphthol-AS-BI phosphohydrolase and β-glucosidase are present, but lipase (C14), valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, N-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase are absent. D-Xylose and salicin are utilized as sole carbon and energy sources. Benzoate, formate, L-glutamate, D-mannose, pyruvate, succinate and sucrose are not utilized. Acid is produced from D-cellobiose, D-galactose, D-glucose, D-ribose, L-rhamnose, D-xylose and maltose. Acid is not produced from D-melezitose or D-raffinose. Susceptible to carbenicillin, cephalothin, chloramphenicol, gentamicin, kanamycin, neomycin, novobiocin, oleandomycin, penicillin G, polymyxin B and streptomycin, but not to ampicillin, lincomycin or tetracycline. The major cellular fatty acids are C_{16:1}ω7c and/or iso-C_{15:0} 2-OH, C_{17:1}, C_{15:1} and iso-C_{16:0}. The predominant ubiquinone is Q-8. The DNA G+C content is 39.3 mol%.

The type strain, SMK-10^T (=KCTC 12480^T = DSM 17314^T), was isolated from a tidal flat sediment from Saemankum, Korea.

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