Colwellia asteriadis sp. nov., a marine bacterium isolated from the starfish *Asterias amurensis*

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A marine bacterial strain, KMD 002^T, was isolated from an Amur starfish, *Asterias amurensis*, collected in the East Sea of Korea. Strain KMD 002^T was a Gram-negative, beige-pigmented, rod-shaped bacterium. The strain was capable of growth at relatively low temperatures (4–25 °C) and over a broad pH range (pH 4.0–10.0). The major fatty acids were C_{16:1}ω7c and/or iso-C_{15:0} 2-OH and C_{16:0} and the predominant isoprenoid quinone was Q-8. The DNA G+C content of strain KMD 002^T was 40.3 mol%. Phylogenetic analysis using 16S rRNA gene sequences revealed that strain KMD 002^T belonged to the genus *Colwellia*. However, various phenotypic properties as well as low 16S rRNA gene sequence similarities to members of the genus *Colwellia* (94.1–96.7%) suggested that strain KMD 002^T is a representative of a novel species, for which the name *Colwellia asteriadis* sp. nov. is proposed. The type strain is KMD 002^T (=KCCM 90077^T =JCM 15608^T).

A culture-dependent investigation of the microbial diversity associated with marine creatures led to the isolation of 18 culturable bacterial strains from a specimen of the Amur starfish, *Asterias amurensis*, collected from the Korean East Sea. The investigation of these strains led to the identification of the new genus and species *Kistimonas asteriae* (type strain KMD 001^T; Choi *et al.*, 2010) and strain KMD 002, belonging to the class *Gammaproteobacteria*. Phylogenetic analysis of the 16S rRNA gene sequence of strain KMD 002 revealed a close affinity with the genus *Colwellia*. Members of *Colwellia* are psychrophiles and facultative anaerobes belonging to the order *Alteromonadales* and the family *Colwelliaceae* (Ivanova *et al.*, 2004). At the time of writing, the genus *Colwellia* included 10 species, *Colwellia psychrerythraea*, *C. hadaliensis* (Deming *et al.*, 1988), *C. demingiae*, *C. hornerae*, *C. psychrotropica*, *C. rossensis* (Bowman *et al.*, 1998), *C. maris* (Yumoto *et al.*, 1998), *C. piezophila* (Nogi *et al.*, 2004), *C. aestuarii* (Jung *et al.*, 2006) and *C. polaris* (Zhang *et al.*, 2008).

Strain KMD 002^T was isolated from the skin of an *A. amurensis* specimen, which was collected at a depth of 10 m near the Im-Won port in Korea in April 2007. The skin was macerated and mixed with seawater (1:10) and an aliquot (100 µl) of the suspension was spread on A1 agar, a seawater-based medium [containing (per litre filtered seawater) 10.0 g starch (Difco), 4.0 g peptone (Difco), 2.0 g yeast extract (Bacto) and 18.0 g agar (Junsei)]. After incubation at 25 °C for 2 weeks, single colonies were selected and subcultured on A1 agar to achieve purity. Stocks of each strain were generated by incubation in A1 broth (25 ml) at 25 °C for 7 days and stored at –80 °C in A1 broth containing 15% glycerol.

For morphological study, strain KMD 002^T was cultured at 20 °C for 7 days on A1 agar and marine agar (MA; Difco). Cell morphology was observed using scanning electron microscopy (S-3500N; Hitachi). Cell motility was examined using light microscopy (Eclipse TE2000 U; Nikon) and the presence of flagella was determined using transmission electron microscopy (CM 20; Philips) using sections cut with a Reichert Ultracut E (Leica).

Growth of strain KMD 002^T at 4, 10, 15, 20, 25, 30, 37 and 42 °C was examined on A1 agar and MA and in A1 broth and at 4, 10 and 25 °C on nutrient agar (Difco) and tryptic soy broth agar (TSA; Bacto). The requirement for and

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A scanning electron micrograph of strain KMD 002^T is available with the online version of this paper.

tolerance of NaCl was examined on A1 agar containing 0–15 % (w/v) NaCl (in increments of 1 %). Growth at pH 4–10 (in increments of 1 pH unit) was tested in A1 broth after adjustment of the pH. Gram staining was performed according to the method described by Süßmuth *et al.* (1987). Anaerobic growth was evaluated on A1 agar and MA in an anaerobic system (Glove Box; Three-Shine). Hydrolysis of starch, casein, gelatin and Tween 80 was tested using A1 medium as described by Smibert & Krieg (1994). Chitin hydrolysis was tested on chitin agar as described by Hsu & Lockwood (1975). Catalase activity was determined according to Nogi & Kato (1999). Utilization of carbon sources was investigated using the API 50CH system (bioMérieux) according to the manufacturer's recommendations. The API 20E, API 20NE and API ZYM systems (bioMérieux) were used to determine additional biochemical properties.

Fatty acid methyl esters were extracted and prepared according to the standard protocol of the Sherlock Microbial Identification System (Sasser, 1990). The DNA G+C content was determined by HPLC as described by Martin *et al.* (1997). Isoprenoid quinones were extracted according to the method described by Bligh & Dyer (1959) and separated by HPLC as described by Collins & Jones (1981).

Chromosomal DNA of strain KMD 002^T was isolated using the G-spin genomic DNA extraction kit (iNtRON Biotechnology). The 16S rRNA gene was amplified by PCR using the universal primers 27f and 1492r, which anneal to positions 8–27 and 1492–1513, respectively (*Escherichia coli* numbering; Brosius *et al.*, 1978). 16S rRNA gene sequence similarities were determined by performing BLAST searches of public databases (Altschul *et al.*, 1990). A multiple sequence alignment of representative sequences was performed using CLUSTAL W version 1.8 (Thompson *et al.*, 1994). A neighbour-joining analysis (Kimura, 1980) and a bootstrap analysis of 1000 data resamplings were performed to determine the robustness of the topology. A phylogenetic tree was reconstructed using MEGA version 4 (Tamura *et al.*, 2007).

Strain KMD 002^T was a Gram-negative, facultatively anaerobic, beige-pigmented bacterium after incubation at 20 °C on A1 agar and MA. The strain was motile with a single polar flagellum (Fig. 1). Scanning electron microscope observations of strain KMD 002^T revealed short rod-shaped cells, 0.4–0.5 µm in diameter and 0.7–1.0 µm long (Supplementary Fig. S1, available in IJSEM Online). Strain KMD 002^T grew at 4–25 °C (optimum 15 °C) on A1 agar and MA and in A1 broth. Strain KMD 002^T grew on TSA at 4 and 25 °C and on nutrient agar at 15 and 25 °C. In general, members of the genus *Colwellia* are psychrophilic bacteria that have optimal growth at ≤20 °C and no growth over 30 °C (Bowman *et al.*, 1998; Zhang *et al.*, 2008; Jung *et al.*, 2006). Strain KMD 002^T grew with 0–11 % (w/v) NaCl, but not with 12–15 % (w/v) NaCl, and at pH 4–10. With the API ZYM gallery, the phenotypic

Fig. 1. Transmission electron micrograph of a cell of strain KMD 002^T after growth on A1 medium at 20 °C for 4 days.

profile of strain KMD 002^T was similar to that of *C. aestuarii* SMK-10^T (Jung *et al.*, 2006) except that *N*-acetyl-β-glucosaminidase activity was detected and β-glucosidase was not detected in strain KMD 002^T. The cellular fatty acid profile of strain KMD 002^T is presented in Table 1 together with those of other *Colwellia* species (Jung *et al.*, 2006). The predominant fatty acids of strain KMD 002^T were C_{16:1ω7c} and/or iso-C_{15:0} 2-OH and C_{16:0}. The characteristics of strain KMD 002^T were similar to those reported for the genus *Colwellia* in previous studies (Yumoto *et al.*, 1998), i.e. marine habitat, sodium ion requirement for good growth, non-fermentation of glucose, anaerobic respiration and major isoprenoid quinone Q-8.

The 16S rRNA gene sequence from strain KMD 002^T clustered with those from strains of nine *Colwellia* species (Fig. 2) and shared 94.1–96.7 % similarity with these sequences. In particular, the 16S rRNA gene sequence of strain KMD 002^T showed 96.6 and 96.7 % similarity to sequences from *C. demingiae* ACAM 459^T and *C. psychrerythraea* ATCC 27364^T, respectively. However, strain KMD 002^T is significantly distinguishable from these phylogenetic neighbours by utilization of aesculin, casein and D-fructose, colony colour and ability to grow at 25 °C on MA (Table 2). Even though we did not carry out DNA–DNA hybridization experiments, the phylogenetic and phenotypic distinctiveness of strain KMD 002^T is sufficient to separate it from other recognized *Colwellia* species (Stackebrandt & Goebel, 1994). In addition, strain KMD 002^T showed similar physiological and chemotaxonomic characteristics to those of other genera such as

Table 1. Cellular fatty acid compositions of strain KMD 002^T and *Colwellia* species

Strains: 1, *Colwellia asteriadis* sp. nov. KMD 002^T; 2, *C. psychrerythraea* (four strains) (data from Bowman *et al.*, 1998); 3, *C. demingiae* (two strains) (Bowman *et al.*, 1998); 4, *C. aestuarii* SMK-10^T (Jung *et al.*, 2006); 5, *C. polaris* 537^T (Zhang *et al.*, 2008); 6, *C. hornerae* ACAM 607^T (Bowman *et al.*, 1998); 7, *C. piezophila* (two strains) (Nogi *et al.*, 2004); 8, *C. maris* JCM 10085^T (Nogi *et al.*, 2004); 9, *C. psychrotropica* ACAM 179^T (Bowman *et al.*, 1998); 10, *C. rossensis* ACAM 608^T (Bowman *et al.*, 1998). Values are percentages of total fatty acids; fatty acids that represented <0.1 % of total content in all strains are not shown. tr, Trace (≤0.1 % of total content); –, not detected/not reported.

Fatty acid	1	2	3	4	5	6	7	8	9	10
Straight-chain										
C _{10:0}	1.3	—	—	1.7	2.3	—	—	—	—	—
C _{11:0}	0.5	—	—	—	1.2	—	—	—	—	—
C _{12:0}	3.2	—	—	0.5	tr	—	1–2	2	—	—
C _{13:0}	0.2	—	—	—	—	—	—	—	—	—
C _{14:0}	2	5.1–7.8	7.6–8.0	2.3	1.5	3	9	—	0.8	4.6
C _{15:0}	4	1.7–11.0	0.9–1.4	4.8	—	2.9	3	4	2.7	2.9
C _{16:0}	27.3	26.8–33.2	21.9–23.6	9.9	7.6	13.5	31–33	25	21.9	27.1
C _{17:0}	1.7	0–1.3	tr	2.5	2.1	—	—	3	1.5	0.1
C _{18:0}	0.9	0.1–2.4	0.2–0.5	—	tr	2	—	—	0.4	tr
Branched										
iso-C _{16:0}	0.7	0–0.2	—	10.4	5.7	10.3	—	—	—	—
Unsaturated										
C _{14:1} *	—	5.1–7.3	9.1–9.3	—	—	2.8	2	2	2	2.8
C _{15:1}	—	0–2.7	1.9–2.6	10.9	17.6	21.4	3	6	4.3	4.1
C _{15:1} ω8 <i>c</i>	3.0	—	—	—	—	—	—	—	—	—
C _{15:1} ω6 <i>c</i>	0.2	—	—	—	—	—	—	—	—	—
C _{16:1} †	—	31.4–36.3	37.5–37.8	—	—	15.4	48–50	51	56.8	43.4
C _{16:1} ω9 <i>c</i>	4.1	6.2–8.8	9.5–11.8	5.6	3.9	2.0	—	—	—	1.8
C _{17:1}	—	0–2.2	tr	11.8	13.7	7.5	—	4	5.4	0.5
C _{17:1} ω8 <i>c</i>	3.9	—	—	—	—	—	—	—	—	—
C _{18:1} ω9 <i>c</i>	0.6	0–1.7	tr–0.2	0.6	tr	1.4	—	—	0.3	0.8
C _{18:1} ω7 <i>c</i>	1.8	0.3–2.1	1.3–1.4	1.2	—	—	—	—	1.9	4.2
C _{20:5} ω3 <i>c</i>	—	0–1.5	—	—	—	—	—	—	0.1	tr†
C _{22:6} ω3 <i>c</i>	—	5.5–8.0	1.7–2.2	—	—	2.1	—	—	0.7	6
Hydroxy										
C _{10:0} 3-OH	0.5	—	—	0.5	—	—	—	—	—	—
C _{11:0} 3-OH	0.8	—	—	2.6	4.8	—	—	—	—	—
C _{12:0} 3-OH	4.2	—	—	2.5	3.1	—	0–1	3	—	—
C _{12:1} 3-OH	0.1	—	—	—	—	—	—	—	—	—
iso-C _{12:0} 3-OH	—	—	—	5.3	6.5	—	—	—	—	—
C _{16:0} 3-OH	0.7	—	—	—	—	—	—	—	—	—
Summed features‡										
C _{16:1} ω7 <i>c</i> and/or iso-C _{15:0} 2-OH	36.5	—	—	23.6	23.0	—	—	—	—	—
C _{13:0} 3-OH and/or iso-C _{15:1}	0.4	—	—	—	—	—	—	—	—	—
C _{14:0} 3-OH and/or iso-C _{16:1}	0.3	—	—	—	—	—	—	—	—	—

*Reported as C_{14:1}ω7*c* by Bowman *et al.* (1998).

†Reported as C_{16:1}ω7*c* by Bowman *et al.* (1998).

‡Summed features represent two or three fatty acids that cannot be separated by the Microbial Identification System.

Alteromonas, *Pseudoalteromonas* and *Shewanella* (Yumoto *et al.*, 1998). However, strain KMD 002^T shared low 16S rRNA gene sequence similarity (<90.0 %) with these genera and the phylogenetic tree showed that members of these genera were not closely related to strain KMD 002^T. Although strain KMD 002^T was also closely related to type strains of species in the genus *Thalassomonas* (94.4–95.8 % 16S rRNA gene sequence similarity), strain KMD 002^T was

distinct from the genus *Thalassomonas* in physiological and chemotaxonomic characters, i.e. growth at 4 °C, nitrate reduction and enzyme activities (Yi *et al.*, 2004).

On the basis of the phenotypic and phylogenetic data, it is proposed that strain KMD 002^T represents a novel species of the genus *Colwellia*, for which the name *Colwellia asteriadis* sp. nov. is proposed.

Fig. 2. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the position of strain KMD 002^T among strains of other *Colwellia* species and representing other genera. Bootstrap values based on 1000 resamplings are shown as percentages at branch nodes. Bar, 0.01 substitutions per nucleotide position.

Table 2. Differential phenotypic characteristics of strain KMD 002^T and other *Colwellia* species

Strains: 1, *C. asteriadis* sp. nov. KMD 002^T; 2, *C. psychrerythraea* (four strains) (data from Bowman *et al.*, 1998; D'Aoust & Kushner, 1972); 3, *C. demingiae* (two strains) (Bowman *et al.*, 1998); 4, *C. aestuarii* SMK-10^T (Jung *et al.*, 2006); 5, *C. polaris* 537^T (Zhang *et al.*, 2008); 6, *C. hornerae* ACAM 607^T (Bowman *et al.*, 1998); 7, *C. piezophila* (two strains) (Nogi *et al.*, 2004); 8, *C. maris* JCM 10085^T (Yumoto *et al.*, 1998); 9, *C. psychrotropica* ACAM 179^T (Bowman *et al.*, 1998); 10, *C. rossensis* ACAM 608^T (Bowman *et al.*, 1998). +, Positive; –, negative; v, variable (data in parentheses are for the type strain); ND, no data available, NG, no growth.

Characteristic	1	2	3	4	5	6	7	8	9	10
Cell shape*	R	R	CR	CR	CR	CR	R	CR	CR	CR
Colony colour†	B	R	OW	GY	WH	OW	ND	F	OW	CW
Motility	+	+	+	+	+	+	+	+	+	–
Growth at 25 °C	+	–	–	+	+	–	–	–	+	–
Urea	–	v (+)	–	–	–	–	ND	ND	+	+
Hydrolysis of:										
Aesculin	–	+	+	–	+	+	ND	ND	–	NG
Casein	–	+	+	+	+	+	–	–	+	–
Gelatin	–	v (+)	–	–	+	–	+	+	–	–
Starch	+	+	+	–	+	+	–	–	–	+
Tween 80	–	+	–	+	+	+	ND	+	+	NG
Utilization of:										
L-Arabinose	–	–	–	+	+	–	–	–	–	+
D-Fructose	+	–	–	–	+	–	–	–	–	+
D-Galactose	–	–	–	+	+	–	–	ND	–	–
D-Glucose	–	v (+)	–	+	+	–	ND	+	–	+
Maltose	–	–	–	+	+	–	ND	–	–	–
Cellobiose	–	–	–	+	–	–	–	ND	–	–
Trehalose	–	–	–	+	ND	–	–	ND	–	–
Acetate	+	+	+	–	+	+	ND	ND	+	+
Citrate	–	–	+	–	–	+	ND	ND	–	+
L-Malate	–	–	–	+	–	–	ND	ND	+	+
Acid production from:										
L-Arabinose, sucrose	–	–	–	+	–	–	–	–	–	–
D-Fructose	–	–	–	+	–	–	–	–	–	–
Lactose	–	–	–	+	–	–	–	ND	–	–
D-Mannose	–	–	–	+	+	–	–	–	–	–
Melibiose	–	–	–	+	+	–	ND	–	–	–
DNA G + C content (mol%)	40.3	35–38	37	39.3	38.9	39	38.5–39.1	39.4	42	38

*CR, Curved rod; R, rod.

†B, Beige; CW, chalky white; F, flesh tint; GY, greyish yellow; OW, off white; R, red; WH, white.

Description of *Colwellia asteriadis* sp. nov.

Colwellia asteriadis (as.te'ri.adis. N.L. n. *Asterias* a zoological genus name; N.L. gen. n. *asteriadis* of *Asterias*, referring to the isolation of the type strain from the starfish *Asterias amurensis*).

Cells are Gram-negative, facultatively anaerobic, motile rods ($0.4\text{--}0.5 \times 0.7\text{--}1.0\ \mu\text{m}$) with a single polar flagellum. Colonies are beige, circular, flat and smooth. Grows with 1–10% (w/v) NaCl (weak without NaCl and no growth with 12–15% NaCl), at 4–25 °C (optimum 15 °C) and at pH 4–10. Hydrolyses starch and chitin but not casein, Tween 80 or gelatin. Catalase-positive. Utilizes ribose, D-fructose, acetate, L-sorbose, starch, turanose, D-tagatose and potassium 5-ketogluconate, but not L-arabinose, D-galactose, D-glucose, maltose, cellobiose, trehalose, citrate or L-malate. Reduces nitrates to nitrites and is positive for cytochrome *c* oxidase. With API ZYM, alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase and *N*-acetyl- β -glucosaminidase are detected, but lipase (C14), valine arylamidase, cystine arylamidase, trypsin, α -chymotrypsin, α -galactosidase, β -galactosidase, β -glucuronidase, α -glucosidase, β -glucosidase, α -mannosidase and α -fucosidase are not detected. The major cellular fatty acids are C_{16:1 ω 7c} and/or iso-C_{15:0} 2-OH and C_{16:0}. The predominant ubiquinone is Q-8. The DNA G + C content of the type strain is 40.3 mol%.

The type strain, KMD 002^T (=KCCM 90077^T =JCM 15608^T), was isolated from the skin of a specimen of the starfish *Asterias amurensis* collected at the Im-Won port in Samcheok on the East Sea of Korea.

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