Algoriphagus jejuensis sp. nov., isolated from seawater

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A Gram-negative, pink-pigmented, non-motile, strictly aerobic rod, designated CNU040^T, was isolated from seawater from the coast of Jeju Island in Korea. The temperature, pH and NaCl ranges for growth were 4–30 °C, pH 5.5–10.0 and 0–5.0 % NaCl. Phylogenetic analysis based on 16S rRNA gene sequences indicated that strain CNU040^T belonged to a distinct lineage in the genus *Algoriphagus* and exhibited high sequence similarity with *Algoriphagus terrigena* DS-44^T (98.3 %) and *Algoriphagus alkaliphilus* AC-74^T (96.6 %) and lower sequence similarity (<96.0 %) with all other members of the genus *Algoriphagus*. DNA–DNA relatedness between strain CNU040^T and *A. terrigena* KCTC 12545^T was 44.5 %. The DNA G + C content of the isolate was 48.5 mol% and the major respiratory quinone was menaquinone-7. The major cellular fatty acids were iso-C_{15:0} (28.6 %) and summed feature 3 (consisting of iso-C_{15:0} 2-OH and/or C_{16:1ω7c}; 24.0 %). The polar lipids consisted of diphosphatidylglycerol, phosphatidylethanolamine, one unknown amino lipid, one unknown aminophospholipid and three unknown polar lipids. On the basis of phenotypic, phylogenetic and genotypic data, strain CNU040^T represents a novel species within the genus *Algoriphagus*, for which the name *Algoriphagus jejuensis* sp. nov. is proposed. The type strain is CNU040^T (=KCTC 22647^T =JCM 16112^T).

The genus *Algoriphagus*, in the family *Cyclobacteriaceae*, was first proposed by Bowman *et al.* (2003) with *Algoriphagus ratkovskyi* as the type species. Members of this genus are heterotrophic, strictly aerobic, saccharolytic, pink-/orange-pigmented, non-motile, Gram-negative rods from marine environments, Antarctic and saline lakes, and soil. The genus now includes species from the genera *Hongiella* (Yi & Chun, 2004) and *Chimaericella* (Tiago *et al.*, 2006), which were reclassified on the basis of phylogenetic data and phenotypic and genotypic traits (Nedashkovskaya *et al.*, 2004, 2007). At the time of writing, the genus *Algoriphagus* comprises 19 species (Bowman *et al.*, 2003; Nedashkovskaya *et al.*, 2004, 2007; Van Trappen *et al.*, 2004; Yoon *et al.*, 2005a, b, 2006; Copa-Patiño *et al.*, 2008; Liu *et al.*, 2009; Young *et al.*, 2009; Park *et al.*, 2010).

Strain CNU040^T was isolated from surface seawater collected from the coast of Jeju Island in Korea using a standard dilution-plating technique on marine agar 2216

(MA; Difco) at 25 °C for 5 days. Subcultivation was routinely performed on MA at 30 °C for 3 days under aerobic conditions and the isolate was preserved at –80 °C in marine broth 2216 (MB, Difco) supplemented with 20 % glycerol. For phenotypic and genotypic comparison, *Algoriphagus terrigena* KCTC 12545^T was used as a reference strain.

Genomic DNA of strain CNU040^T was extracted and purified using a Wizard genomic extraction kit (Promega), and the nearly complete 16S rRNA gene was amplified using bacterial universal primers 27F and 1522R (Johnson, 1994). The PCR product was purified using a QIAquick PCR purification kit (Qiagen) and ligated into the pGEM-T Easy vector (Promega), according to the manufacturers' instructions. The ligation product was transformed into competent *Escherichia coli* JM109 cells. The cloned 16S rRNA gene sequence was analysed with an automated sequencer (ABI 3730XL, Applied Biosystems) at Solgent (Daejeon, Korea). An almost-complete 16S rRNA gene sequence (1519 bp) of strain CNU040^T was obtained. The resulting 16S rRNA gene sequence was compared with those available in GenBank using the BLAST program (<http://blast.ncbi.nlm.nih.gov/>

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain CNU040^T is EF217418.

A supplementary figure is available with the online version of this paper.

Blast.cgi) to determine approximate phylogenetic affiliations. Sequences of closely related organisms were aligned using CLUSTAL X (Thompson *et al.*, 1997). Sequence similarity values were computed using the EzTaxon server (<http://www.eztaxon.org/>; Chun *et al.*, 2007). Gaps at the 5' and 3' ends and ambiguous bases were removed from the alignment using BioEdit (Hall, 2007). Phylogenetic trees based on comparison of 1330 bases were constructed using the neighbour-joining and maximum-parsimony algorithms in MEGA version 4.0 (Tamura *et al.*, 2007) and bootstrap values were calculated on the basis of 1000 replications (Felsenstein, 1985). Evolutionary distance matrices were calculated according to the algorithm of Kimura's two-parameter model (Kimura, 1983).

Phylogenetic analysis showed that strain CNU040^T was closely related to the genus *Algoriphagus* in the family *Cyclobacteriaceae* and formed a distinct phyletic line with *A. terrigena* DS-44^T. This branching pattern was supported by a high bootstrap value (100 %) (Fig. 1). The tree based on maximum parsimony showed essentially the same topology (not shown). Sequence comparisons with strains of species with validly published names showed that strain CNU040^T shared high similarity with *A. terrigena* DS-44^T (98.3 % 16S rRNA gene sequence similarity) and *Algoriphagus alkaliphilus* AC-74^T (96.6 %) and lower similarity (<96.0 %) with all other members of the genus *Algoriphagus*.

DNA–DNA hybridization was carried out between strain CNU040^T and *A. terrigena* KCTC 12545^T using the membrane filter hybridization method described by Brown (2005). Probe labelling and hybridization were conducted using the non-radioactive DIG-High Prime system and the hybridized DNA was visualized using the DIG luminescent

detection kit (Roche), according to the manufacturer's instructions. DNA–DNA relatedness was quantified using a densitometer (Bio-Rad). DNA–DNA relatedness between strain CNU040^T and *A. terrigena* KCTC 12545^T was 44.5 %. According to the criteria for differentiation of bacterial species (Wayne *et al.*, 1987), this strongly indicated that the isolate was a member of a novel species of the genus *Algoriphagus*.

Cells grown aerobically on MA at 30 °C for 3 days were used for the examination of colony morphology, size and colour. Cell morphology and size were examined by light (FDX-35; Nikon) and transmission electron (JEM-1010; JEOL) microscopy using cells from exponentially growing cultures. Growth at 4, 10–35 (at intervals of 5 °C), 37 and 42 °C was tested on MA. Growth with 0–10 % (w/v) NaCl (at intervals of 1 % NaCl) was tested on synthetic ZoBell agar (per litre: 5 g Bacto peptone, 1 g yeast extract, 0.1 g ferric citrate, 15 g Bacto agar; ZoBell, 1941) using modified artificial seawater (per litre: 5.94 g MgSO₄·7H₂O, 4.53 g MgCl₂·6H₂O, 0.64 g KCl, 1.3 g CaCl₂). Growth at pH 4.0–11.0 (at intervals of 0.5 pH units) was determined in MB with the pH adjusted with 1 M NaOH or 1 M HCl after sterilization.

The Gram reaction was determined using the bioMérieux Gram-stain kit, according to the manufacturer's instructions. Gliding motility was investigated by phase-contrast light microscopy with the hanging drop method, as described by Bernardet *et al.* (2002). Anaerobic growth was tested on MA incubated for up to 15 days in the AnaeroPack-Anaero system (Mitsubishi Gas Chemical). Catalase and oxidase activities were assessed using standard methods (Tindall *et al.*, 2007). The degradation of agar,

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the relationships of strain CNU040^T and the type strains of species of the genus *Algoriphagus*. Bootstrap values (>50 %) based on 1000 replicates are shown at branch nodes. *Persicobacter psychroavidus* Asr22-19^T was used as an outgroup. Bar, 0.02 substitutions per nucleotide position.

DNA, starch and CM-cellulose was assessed according to the methods of Smibert & Krieg (1994). Casein hydrolysis was assessed according to the method of Lányi (1987). Hydrolysis of chitin and Tweens 20, 40, 60 and 80 was assessed according to Baumann & Baumann (1981). To detect flexirubin-type pigments, cell mass was subjected to the KOH test, as described by Bernardet *et al.* (2002). Antibiotic sensitivity was assessed after 3 days of incubation at 30 °C on MA by the disc diffusion method with commercial discs (BBL Becton Dickinson) impregnated with the following antibiotics (µg per disc unless otherwise indicated): ampicillin (10), carbenicillin (100), erythromycin (15), gentamicin (10), kanamycin (30), lincomycin (15), nalidixic acid (30), neomycin (30), novobiocin (5), oleandomycin (15), penicillin (10 IU), polymyxin B (300), streptomycin (10) and tetracycline (30). The results were interpreted according to the guidelines set forth by CLSI (2003). Other phenotypic and enzymic characterizations of strain CNU040^T and *A. terrigena* KCTC 12545^T were conducted using the API 20 E, API 20 NE and API 50 CH kits at 30 °C for 3 days and the API ZYM kit at 30 °C for 8 h (bioMérieux). Utilization of carbon sources was tested using GN2 MicroPlates (Biolog), according to the manufacturer's instructions. All commercial kits were inoculated with bacterial suspensions in 3 % sea salts. Detailed results of the morphological, physiological and biochemical tests are given in the species description and Table 1. Briefly, the isolate could be distinguished from *A. terrigena* KCTC 12545^T by hydrolysis of agar, casein and gelatin, utilization of D-fructose, D-glucose, gentiobiose and malate, enzyme activities and susceptibility to carbenicillin, lincomycin, oleandomycin and tetracycline.

For cellular fatty acid analysis, cells of strain CNU040^T and *A. terrigena* KCTC 12545^T were grown aerobically on MA at 30 °C for 3 days. Fatty acid methyl esters were obtained by saponification, methylation and extraction using standard protocol of the MIDI (Sherlock Microbial Identification System, version 4.5). Fatty acids were analysed by GC (6890N; Agilent) and identified using the TSBA50 database of the Microbial Identification System (Sasser, 1990). The major respiratory quinones were analysed by HPLC (ZQ 4000; Waters) with a reversed-phase column (300 × 3.9 mm Nova-Pak 130 C18; Waters) as described by Komagata & Suzuki (1987). Polar lipids were extracted according to the procedures described by Minnikin *et al.* (1984) and separated by two-dimensional TLC using 6.5 × 6.5 cm silica gel 60 F₂₅₄ plates (Merck). Individual phospholipids were identified by spraying with the appropriate detection reagents and also co-migration with authentic standards (Sigma) (Komagata & Suzuki, 1987). The DNA G+C content of strain CNU040^T was determined by the thermal denaturation method (Marmur & Doty, 1962) using an Ultrospec 2100 spectrophotometer (Pharmacia Biotech) and DNA from *Escherichia coli* K-12 as a control.

The major cellular fatty acids (>10 % of total) of strain CNU040^T and *A. terrigena* KCTC 12545^T were iso-C_{15:0} (28.6 and 39.0 %) and summed feature 3 (consisting of

Table 1. Differential phenotypic characteristics of strain CNU040^T and *Algoriphagus terrigena* KCTC 12545^T

All data were obtained in this study unless otherwise indicated. Both strains grow in the presence of 0–5 % (w/v) NaCl.

Characteristic	CNU040 ^T	<i>A. terrigena</i> KCTC 12545 ^T
Temperature range (°C)	4–30	10–30
pH range	5.5–10	6–9
Nitrate reduction	–	+
Hydrolysis of:		
Agar	+	–
Casein	–	+
Gelatin	+	–
Enzyme activity		
Esterase (C4)	–	+
Esterase lipase (C8)	–	+
Leucine arylamidase	–	+
Valine arylamidase	–	+
α-Galactosidase	+	–
Utilization of:		
D-Fructose	–	+
D-Glucose	–	+
Gentiobiose	+	–
Malate	+	–
Susceptibility to:		
Carbenicillin	+	–
Lincomycin	+	–
Oleandomycin	+	–
Tetracycline	+	–
DNA G+C content (mol%)	48.5	49.0*

*Data from Yoon *et al.* (2006).

iso-C_{15:0} 2-OH and/or C_{16:1}ω7; 24.0 and 20.9 %, respectively). The fatty acid compositions were similar in the two strains, though there were differences in the proportions of some fatty acids (Table 2). The major respiratory quinone of strain CNU040^T was menaquinone 7 (MK-7), which has been found in other *Algoriphagus* species (Nedashkovskaya *et al.*, 2004, 2007; Young *et al.*, 2009; Park *et al.*, 2010). The polar lipids in strain CNU040^T were diphosphatidylglycerol, phosphatidylethanolamine, one unknown amino lipid, one unknown aminophospholipid and three unknown polar lipids. The polar lipid profile of strain CNU040^T was similar to that of *A. terrigena* KCTC 12545^T (Supplementary Fig. S1, available in IJSEM Online). The G+C content of the genomic DNA of strain CNU040^T was 48.5 mol%, which is within the range reported for the genus *Algoriphagus* (35–49 mol%; Young *et al.*, 2009).

In conclusion, the 16S rRNA gene sequence data, DNA–DNA relatedness and phenotypic characteristics distinguished strain CNU040^T from *A. terrigena* KCTC 12545^T. Thus, strain CNU040^T should be assigned to a novel species of the genus *Algoriphagus*, for which the name *Algoriphagus jejuensis* sp. nov. is proposed.

Table 2. Cellular fatty acid compositions of strain CNU040^T and *Algoriphagus terrigena* KCTC 12545^T

All data were obtained in this study. Values are percentages of total fatty acids; fatty acids that represented <0.5 % in both strains are not shown. tr, Trace amount (<0.5 %); –, not detected.

Fatty acid	CNU040 ^T	<i>A. terrigena</i> KCTC 12545 ^T
iso-C _{11:0}	–	1.0
anteiso-C _{11:0}	1.6	2.0
iso-C _{14:0}	1.2	1.2
C _{14:0}	0.6	tr
iso-C _{15:1} G	1.6	0.6
iso-C _{15:0}	28.6	39.0
anteiso-C _{15:0}	4.8	3.8
C _{15:1} ω6c	2.2	0.7
C _{15:0}	3.7	0.7
iso-C _{16:1} H	3.9	2.5
iso-C _{16:0}	3.8	1.5
C _{16:1} ω5c	4.0	2.7
C _{16:0}	1.5	0.7
iso-C _{15:0} 3-OH	2.1	3.5
iso-C _{17:1} ω9c	4.5	10.0
C _{17:1} ω6c	tr	0.7
C _{17:1} ω6c	1.9	tr
iso-C _{16:0} 3-OH	1.2	tr
C _{16:0} 3-OH	1.3	tr
iso-C _{17:0} 3-OH	4.7	4.0
Summed features*		
3	24.0	20.9
4	1.5	2.1

*Summed features represent groups of two or three fatty acids that cannot be separated by the Microbial Identification System. Summed feature 3 consisted of iso-C_{15:0} 2-OH and/or C_{16:1}ω7c. Summed feature 4 consisted of iso-C_{17:1} I and/or anteiso-C_{17:1} B.

Description of *Algoriphagus jejuensis* sp. nov.

Algoriphagus jejuensis (je.ju.en'sis. N.L. masc. adj. *jejuensis* of or belonging to Jeju Island in the Republic of Korea, where the type strain was isolated).

Cells are Gram-negative, non-motile rods (0.3–0.5 × 0.5–3.0 µm). Colonies on MA are viscous, pink, circular and convex with smooth surfaces and entire margins (0.5–3.0 mm in diameter). Catalase- and oxidase-positive. Flexirubin-type pigments are not produced. Growth occurs at 4–30 °C (optimum 25 °C), at pH 5.5–10.0 and with 0–5 % NaCl. Anaerobic growth does not occur on MA. Nitrate is not reduced. Indole and H₂S are not produced. Hydrolyses agar, aesculin, gelatin and Tweens 20, 40, 60 and 80, but not casein, CM-cellulose, starch or urea. Acid is produced from D-arabinose, D-xylose, D-glucose, cellobiose, maltose, trehalose and raffinose. Oxidizes mannose, N-acetylglucosamine and maltose. Positive for alkaline phosphatase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α- and β-galactosidases, α- and β-glucosidases, N-acetyl-β-glucosaminidase

and α-mannosidase, but negative for esterase (C4), esterase lipase (C8), lipase (C14), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, arginine dihydrolase, β-glucuronidase, α-fucosidase, lysine decarboxylase, ornithine decarboxylase, tryptophan deaminase and urease. With API 50 CH, acid is produced from D-arabinose, D-xylose, D-galactose (weak), D-fructose (weak), D-mannose (weak), amygdalin, arbutin, aesculin ferric citrate, salicin (weak), cellobiose (weak), maltose (weak), lactose (weak) and sucrose. With GN2 MicroPlates, utilizes glycogen, N-acetyl-D-galactosamine, N-acetyl-D-glucosamine, D-arabitol, cellobiose, L-fucose, gentiobiose, lactose, lactulose, D-mannitol, D-mannose, melibiose, methyl β-D-glucoside, D-psicose (weak), raffinose (weak), L-rhamnose (weak), D-sorbitol, sucrose, trehalose, *cis*-aconitic acid, D-glucosaminic acid, α-hydroxybutyric acid (weak), DL-lactic acid (weak), malonic acid (weak), propionic acid, quinic acid, sebacic acid, succinamic acid, L-alanine, glycyl L-aspartic acid, hydroxy-L-proline, L-proline, L-pyrogutamic acid, DL-carnitine (weak), γ-aminobutyric acid, inosine (weak), thymidine (weak), putrescine (weak), 2-aminoethanol, DL-α-glycerol phosphate, glucose 1-phosphate and D-glucose 6-phosphate; no other carbon sources in the GN MicroPlate system are utilized. Resistant to gentamicin, kanamycin, neomycin, polymyxin B and streptomycin, but sensitive to ampicillin, carbenicillin, chloramphenicol, erythromycin, lincomycin, nalidixic acid, novobiocin, oleandomycin, penicillin and tetracycline. The major respiratory quinone is MK-7. The predominant fatty acids are iso-C_{15:0} and summed feature 3 (consisting of iso-C_{15:0} 2-OH and/or C_{16:1}ω7c). The polar lipids are diphosphatidylglycerol, phosphatidylethanolamine, one unknown amino lipid, one unknown aminophospholipid and three unknown polar lipids. The DNA G+C content of the type strain is 48.5 mol%.

The type strain, CNU040^T (=KCTC 22647^T =JCM 16112^T), was isolated from coastal seawater of Jeju Island, Korea.

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