Algoriphagus yeomjeoni sp. nov., isolated from a marine solar saltern in the Yellow Sea, Korea

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Two Gram-negative, rod-shaped bacterial strains, MSS-160^T and MSS-161, were isolated from a marine solar saltern in the Yellow Sea, Korea, and subjected to a polyphasic taxonomic study. The two strains grew optimally in the presence of 2% (w/v) NaCl and at 25–30 °C. Strains MSS-160^T and MSS-161 contained MK-7 as the predominant menaquinone and large amounts of fatty acids C_{16:1}ω7c and/or iso-C_{15:0} 2-OH and iso-C_{15:0}. The DNA G+C content of the two strains was 41 mol%. Phylogenetic analyses based on 16S rRNA gene sequences showed that the two strains were phylogenetically affiliated to the genus *Algoriphagus*. Strains MSS-160^T and MSS-161 exhibited no difference in their 16S rRNA gene sequences and possessed a mean DNA–DNA relatedness level of 91%; they exhibited 16S rRNA similarity levels of 96.6–99.3% to the type strains of *Algoriphagus* species with validly published names. DNA–DNA relatedness levels between the two strains and the type strains of five *Algoriphagus* species were lower than 46%. On the basis of phenotypic, phylogenetic and genetic data, strains MSS-160^T and MSS-161 were classified in the genus *Algoriphagus* as members of a novel species, for which the name *Algoriphagus yeomjeoni* sp. nov. (type strain, MSS-160^T=KCTC 12309^T=JCM 12598^T) is proposed.

The genus *Algoriphagus* was proposed by Bowman *et al.* (2003) with the single species *Algoriphagus ratkowskyi*. Subsequently, four further *Algoriphagus* species, *Algoriphagus aquimarinus*, *Algoriphagus chordae* and *Algoriphagus winogradskyi* (Nedashkovskaya *et al.*, 2004) and *Algoriphagus antarcticus* (Van Trappen *et al.*, 2004) have been described. *Hongiella halophila* was transferred to the genus *Algoriphagus* as *Algoriphagus halophilus* by Nedashkovskaya *et al.* (2004). The genus *Algoriphagus* accommodates strictly aerobic, Gram-negative, non-motile, pink-pigmented, oxidase-positive and rod-shaped bacteria (Bowman *et al.*, 2003; Nedashkovskaya *et al.*, 2004; Van Trappen *et al.*, 2004). Phylogenetic analyses based on 16S rRNA gene sequences showed that the genus is phylogenetically related to the *Cytophaga–Flavobacterium–Bacteroides* group (Bowman *et al.*, 2003; Nedashkovskaya *et al.*, 2004; Van Trappen *et al.*, 2004). The genus *Algoriphagus* is characterized chemotaxonomically by having MK-7 as the predominant menaquinone and by a DNA G+C content of 35–42 mol% (Bowman *et al.*, 2003; Nedashkovskaya *et al.*, 2004; Van Trappen *et al.*, 2004). In the study presented here,

we report on the taxonomic characterization of two slightly halophilic vivid orange-pigmented *Algoriphagus*-like bacterial strains, MSS-160^T and MSS-161, which were isolated from a marine solar saltern in the Yellow Sea, Korea.

Strains MSS-160^T and MSS-161 were isolated by the standard dilution plating technique at 25 °C on marine agar 2216 (MA; Difco). *A. ratkowskyi* CIP 107452^T was obtained from the Collection de l'Institut Pasteur (CIP), Paris, France. *A. aquimarinus* LMG 21971^T, *A. chordae* LMG 21970^T, *A. winogradskyi* LMG 21969^T and *A. antarcticus* LMG 21980^T were obtained from the Laboratorium voor Microbiologie Universiteit Gent (LMG), Gent, Belgium. To investigate their morphological and physiological characteristics, strains MSS-160^T and MSS-161 were routinely cultivated at 30 °C on MA. Cell morphology was examined by light microscopy (Nikon E600) and transmission electron microscopy (TEM). Presence of flagella was examined by TEM using cells from exponentially growing cultures. Gliding motility was determined as described by Bowman (2000). Gram reaction was determined using the bioMérieux Gram Stain kit according to the manufacturer's instructions. Growth at various temperatures (4–40 °C) was measured on MA. The pH range for growth was determined in marine broth 2216 (MB; Difco) that was adjusted to various pH values (pH 4.5–9.5 at intervals of 0.5 pH units). Growth under anaerobic conditions was

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determined after incubation in a Forma anaerobic chamber on MA and MA supplemented with nitrate, both of which had been prepared anaerobically using nitrogen. Catalase and oxidase activities and hydrolysis of casein, starch and Tweens 20, 40, 60 and 80 were determined as described by Cowan & Steel (1965). Hydrolysis of hypoxanthine, tyrosine and xanthine was tested on MA using the substrate concentrations described by Cowan & Steel (1965). Hydrolysis of aesculin, gelatin and urea and nitrate reduction were studied as described previously (Lanyi, 1987) with the modification that artificial sea water was used for preparation of media. The artificial sea water contained (per litre of distilled water) 23.6 g NaCl, 0.64 g KCl, 4.53 g $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, 5.94 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ and 1.3 g $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ (Bruns *et al.*, 2001). H_2S production was tested as described by Bruns *et al.* (2001). Presence of flexirubin pigment was investigated as described by Reichenbach (1992). Susceptibility to antibiotics was detected on MA plates by using antibiotic discs with the following concentrations: ampicillin (10 µg), benzylpenicillin (10 µg), carbenicillin (100 µg), gentamicin (10 µg), kanamycin (30 µg), lincomycin (15 µg), neomycin (30 µg), oleandomycin (15 µg), polymyxin B (300 U), streptomycin (10 µg), chloramphenicol (100 µg) and tetracycline (30 µg). Acid production from carbohydrates was determined as described by Leifson (1963). Utilization of various substrates for growth was determined as described by Yurkov *et al.* (1994).

Cell biomass for isoprenoid quinone analysis and for DNA extraction was obtained from cultivation for 3 days in MB at 30 °C. Isoprenoid quinones were analysed as described by Komagata & Suzuki (1987) using reversed-phase HPLC. Chromosomal DNA was isolated and purified according to the method described previously (Yoon *et al.*, 1996), with the exception that ribonuclease T1 was applied in combination with ribonuclease A to minimize the contamination of RNA. For fatty acid methyl ester (FAME) analysis, cell mass of the two strains was harvested from agar plates after incubation for 5 days on MA at 30 °C. The FAMES were extracted and prepared according to the standard protocol of the MIDI/Hewlett Packard Microbial Identification System (Sasser, 1990). The DNA G+C content was determined by the method of Tamaoka & Komagata (1984) with the modification that DNA was hydrolysed and the resultant nucleotides were analysed by reversed-phase HPLC.

The 16S rRNA gene was amplified by PCR using two universal primers as described previously (Yoon *et al.*, 1998). Sequencing of the amplified 16S rRNA gene was performed as described by Yoon *et al.* (2003). Alignment of sequences was carried out with CLUSTAL W software (Thompson *et al.*, 1994). Gaps at the 5' and 3' ends of the alignment were omitted from further analyses. Phylogenetic trees were inferred by using three tree-making algorithms, the neighbour-joining (Saitou & Nei, 1987), maximum-likelihood (Felsenstein, 1981) and maximum-parsimony (Kluge & Farris, 1969) methods implemented within the

PHYLP package (Felsenstein, 1993). Evolutionary distance matrices for the neighbour-joining method were calculated by the algorithm of Jukes & Cantor (1969) using the program DNADIST. The stability of relationships was assessed by a bootstrap analysis based on 1000 resamplings of the neighbour-joining dataset by using the programs SEQBOOT, DNADIST, NEIGHBOR and CONSENSE of the PHYLP package. DNA–DNA hybridization was performed fluorometrically by the method of Ezaki *et al.* (1989) using photobiotin-labelled DNA probes and microdilution wells. Hybridization was performed with five replications for each sample. The highest and lowest values obtained in each sample were excluded, and the remaining three values were used to calculate similarity values. The DNA relatedness values quoted are the mean of the three values.

Strains MSS-160^T and MSS-161 grew optimally at 25–30 °C and pH 7.0–8.0 and in the presence of 2 % (w/v) NaCl. The two strains were similar in most phenotypic characteristics except the following characteristics; gelatin hydrolysis and acid production from D-mannose and D-raffinose. Morphological, cultural, physiological and biochemical characteristics of the two strains are shown in Table 1 or given in the species description (see below).

Strains MSS-160^T and MSS-161 contained menaquinone-7 (MK-7) as the predominant isoprenoid quinone at peak area ratio of approximately 94–96 %. The two strains had cellular fatty acid profiles that contained large amounts of branched, unsaturated and hydroxy fatty acids; the major fatty acids were $\text{C}_{16:1}\omega 7\text{c}$ and/or iso- $\text{C}_{15:0}$ 2-OH and iso- $\text{C}_{15:0}$ (Table 2). The DNA G+C contents of strains MSS-160^T and MSS-161 were the same, 41 mol%. The 16S rRNA gene sequences of strains MSS-160^T and MSS-161 determined in this study comprised 1476 nucleotides, respectively, representing approximately 96 % of the *Escherichia coli* 16S rRNA gene sequence. The 16S rRNA gene sequences of strains MSS-160^T and MSS-161 were identical. The phylogenetic analyses based on 16S rRNA gene sequences showed that strains MSS-160^T and MSS-161 fall within the radiation of the cluster comprising *Algoriphagus* species (Fig. 1). The two strains exhibited highest 16S rRNA similarity value (99.3 %) to the type strain of *A. winogradskyi*, and 16S rRNA similarity levels of 96.6–98.5 % to the type strains of the other *Algoriphagus* species. The two strains exhibited 16S rRNA similarity levels of less than 95.0 % to other species included in the phylogenetic analysis. DNA–DNA hybridization was performed to determine the genetic relatedness between strains MSS-160^T and MSS-161 and between the two strains and the type strains of *Algoriphagus* species. Strains MSS-160^T and MSS-161 exhibited a mean level of DNA–DNA relatedness of 91 %, when their DNAs were used individually as labelled DNA probes for cross-hybridization. This value indicates that the two strains are members of the same genomic species. DNA–DNA relatedness levels between the two strains and the type strains of six *Algoriphagus* species were in the range 12–46 %.

Table 1. Differential phenotypic characteristics of *Algoriphagus* species

Species: 1, *A. ratkovskyi*; 2, *A. aquimarinus*; 3, *A. chordae*; 4, *A. winogradskyi*; 5, *A. halophilus*; 6, *A. antarcticus*; 7, *A. yeomjeoni* sp. nov. +, Positive; –, negative; w, weakly positive; v, variable reaction; ND, not determined. All species are: Gram-negative and rod-shaped; positive for catalase and oxidase; negative for gliding motility, flexirubin pigment production, H₂S and indole production, utilization of citrate, susceptibility to ampicillin, benzylpenicillin, gentamicin, kanamycin, neomycin, polymyxin B and streptomycin.

Characteristic	1	2	3	4	5	6	7
Nitrate reduction	–	–	–	+	–	–	–
NaCl requirement for growth	+	–	+	–	–	ND	+
Optimal growth temperature (°C)	16–19	23–25	23–25	25–28	35	20	25–30
Growth in/at:							
10 % NaCl	–	+	+	–	–	–	–
37 °C	–	–	–	+	+	–	–
Hydrolysis of:							
Agar	–	+	+	+	–	–	–
Casein	v	+	–	–	–	–	+
Gelatin	–	+	–	+	+	–	v(+)
Starch	v	–	–	+	+	–	–
Tween 80	–	+	–	–	+	ND	+
Acid production from:							
L-Arabinose	+	–	–	–	–	–	–
D-Cellobiose	+	+	+	+	–	ND	+
D-Galactose	+	–	+	+	–	ND	+
D-Glucose	+	+	+	+	+	–	+
Lactose	–	+	+	+	–	ND	+
Maltose	+	–	+	+	+	ND	+
D-Melibiose	+	+	+	+	–	–	+
L-Rhamnose	+	+	+	+	–	–	+
Sucrose	–	+	+	+	+	–	+
D-Xylose	+	+	+	+	+	ND	+
Utilization of:							
L-Arabinose	+	+	+	+	+	–	–
D-Glucose	+	+	+	+	+	–	+
D-Mannose	+	+	+	+	+	–	+
Malate	v	–	–	–	–	–	–
Susceptibility to:							
Carbenicillin	+	+	–	v	+	ND	+
Lincomycin	+	+	+	v	+	ND	+
Oleandomycin	+	+	–	+	+	ND	+
Tetracycline	+	+	–	v	+	ND	–
DNA G + C content (mol%)	35–37	41	37–40	39–42	37	40–41	41

The result of 16S rRNA gene sequence analysis revealed that strains MSS-160^T and MSS-161 have closest phylogenetic affiliations to the genus *Algoriphagus* (Fig. 1). The results obtained from chemotaxonomic analyses are consistent with the results of 16S rRNA gene sequence analysis and phylogenetic inference. The predominant menaquinone of the two strains was the same as that in other *Algoriphagus* species (Nedashkovskaya *et al.*, 2004). The fatty acid profiles of the two strains were similar to those of the type strains of *Algoriphagus* species analysed previously, although there were differences in the compositions of some fatty acids between the two strains and the type strains of *Algoriphagus* species (Table 2). The DNA G + C contents of the strains were in the range for *Algoriphagus* species (Bowman *et al.*,

2003; Nedashkovskaya *et al.*, 2004; Van Trappen *et al.*, 2004). The phenotypic, phylogenetic and genetic similarities warrant that strains MSS-160^T and MSS-161 are members of the same species. The genetic distinctiveness, together with 16S rRNA similarity data, was enough to categorize the two strains as a species distinct from previously described *Algoriphagus* species (Wayne *et al.*, 1987; Stackebrandt & Goebel, 1994). Strains MSS-160^T and MSS-161 were also distinguishable from the six *Algoriphagus* species by some differences in phenotypic properties (Table 1). Therefore, on the basis of the data presented, strains MSS-160^T and MSS-161 should be placed in the genus *Algoriphagus* as members of a novel species, for which the name *Algoriphagus yeomjeoni* sp. nov. is proposed.

Table 2. Cellular fatty acid compositions (%) of *Algoriphagus* species

Strains: 1, *A. ratkowskyi* LMG 21435^T; 2, *A. aquimarinus* KMM 3958^T; 3, *A. chordae* KMM 3957^T; 4, *A. winogradskyi* KMM 3956^T; 5, *A. halophilus* LMG 22067^T; 6, *A. yeomjeoni* MSS-160^T; 7, *A. yeomjeoni* MSS-161. Data for reference strains were taken from Nedashkovskaya *et al.* (2004). Fatty acids that represented less than 0.5% in all strains were omitted. ND, Not detected.

Fatty acid	1	2	3	4	5	6	7
Straight-chain							
C _{15:0}	1.6	1.6	2.5	1.2	1.0	0.6	0.8
C _{16:0}	2.9	1.7	1.4	1.0	—	0.8	0.9
Branched							
anteiso-C _{11:0}	2.1	0.6	1.0	1.2	1.5	—	—
iso-C _{14:0}	1.4	0.7	0.3	0.3	0.6	1.6	1.3
iso-C _{15:0}	30.5	38.9	38.1	36.6	28.4	28.6	30.1
iso-C _{15:1} G	1.1	0.3	—	2.9	—	—	0.6
anteiso-C _{15:0}	3.6	3.2	1.9	1.6	2.8	1.2	1.2
iso-C _{16:0}	3.4	2.4	5.8	3.9	7.7	4.8	4.9
iso-C _{16:1} H	1.7	2.4	1.5	2.1	3.5	1.9	1.7
iso-C _{17:1} ω9c	1.5	5.3	4.4	4.0	9.0	2.3	2.1
Unsaturated							
C _{15:1} ω6c	2.1	2.3	1.0	1.3	1.8	0.8	1.0
C _{16:1} ω5c	5.8	5.2	3.6	3.6	3.5	3.3	3.2
C _{17:1} ω6c	0.5	0.7	1.2	0.8	3.4	1.0	0.8
Hydroxy							
iso-C _{15:0} 3-OH	2.9	1.8	1.6	2.0	2.5	3.0	3.1
C _{16:0} 2-OH	—	—	—	—	—	1.3	1.0
C _{16:0} 3-OH	1.9	0.5	1.9	0.8	0.7	1.1	1.2
iso-C _{16:0} 3-OH	2.8	1.0	0.9	1.9	3.1	3.3	3.5
iso-C _{17:0} 3-OH	9.2	5.9	6.4	6.4	5.9	7.8	7.3
Summed feature*							
3	22.3	20.4	22.2	24.6	19.0	33.7	29.1
4	0.9	1.8	1.7	1.6	2.7	1.3	1.1

*Summed feature represents groups of two or three fatty acids that could not be separated by GLC with the MIDI system. Summed feature 3 contained one or more of C_{16:1} ω7c and/or iso-C_{15:0} 2-OH. Summed feature 4 contained one or more of iso-C_{17:1} I and/or anteiso-C_{17:1} B.

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the positions of strains MSS-160^T and MSS-161 and some other related taxa. The numbers on the branches indicate the bootstrap value of 1000 resamplings (greater than 50%). Bar, 0.01 substitution per nucleotide position.

Description of *Algoriphagus yeomjeoni* sp. nov.

Algoriphagus yeomjeoni (yeom.jeo'ni. N.L. gen. n. *yeomjeoni* of yeomjeon, the Korean name for a marine solar saltern).

Cells are Gram-negative and non-flagellated rods, 0.4–0.7 × 1.5–2.5 µm. Non-motile. Colonies are circular, convex, vivid orange in colour and 0.8–1.0 mm after 3 days incubation on MA at 30 °C. Growth occurs at 4 and 35 °C, with an optimum temperature of 25–30 °C; growth does not occur above 36 °C. Optimal pH for growth is between 7.0 and 8.0; growth is observed at pH 5.0, but not at pH 4.5. Optimal growth occurs in the presence of 2 % (w/v) NaCl; growth does not occur in the presence of greater than 9 % (w/v) NaCl. Growth does not occur under anaerobic conditions on MA and MA supplemented with nitrate. Flexirubin pigment is absent. Aesculin and Tweens 20, 40 and 60 are hydrolysed. Hypoxanthine, xanthine, tyrosine and urea are not hydrolysed. Susceptible to chloramphenicol. D-Cellobiose, D-fructose, D-galactose, maltose, sucrose, D-trehalose and salicin are utilized as carbon and energy sources, but D-xylose, acetate, benzoate, formate, pyruvate, succinate and L-glutamate are not. Acid is produced from D-fructose and D-trehalose. No acid is produced from D-melezitose, D-ribose, *myo*-inositol, D-mannitol or D-sorbitol. Acid production from D-mannose (negative for type strain) and D-raffinose (positive for type strain) is variable. Predominant menaquinone is MK-7. Major fatty acids are C_{16:1}ω7c and/or iso-C_{15:0} 2-OH and iso-C_{15:0}. DNA G + C content is 41 mol%. Other phenotypic properties are given in Table 1.

The type strain, MSS-160^T (= KCTC 12309^T = JCM 12598^T), was isolated from a marine solar saltern in the Yellow Sea, Korea. Reference strain is MSS-161.

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