Jannaschia seohaensis sp. nov., isolated from a tidal flat sediment

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A Gram-negative, motile and pleomorphic bacterial strain, SMK-146^T, was isolated from a tidal flat sediment of the Yellow Sea, Korea, and its taxonomic position was investigated. Strain SMK-146^T grew optimally at pH 7.0–8.0 and 30 °C. It contained Q-10 as the predominant ubiquinone and C_{18:1}ω7c and 11-methyl C_{18:1}ω7c as the major fatty acids. The major polar lipids were phosphatidylcholine, phosphatidylglycerol and phosphatidylethanolamine. The DNA G+C content was 68.4 mol%. Phylogenetic analysis based on 16S rRNA gene sequences showed that strain SMK-146^T belongs to the genus *Jannaschia*. Strain SMK-146^T exhibited 16S rRNA gene sequence similarity values of 95.3–97.0% to the type strains of the five recognized *Jannaschia* species. The mean DNA–DNA relatedness value between strain SMK-146^T and *Jannaschia seosinensis* KCCM 42114^T, the closest phylogenetic neighbour, was 17%. Differential phenotypic properties also revealed that strain SMK-146^T differs from the recognized *Jannaschia* species. On the basis of phenotypic, phylogenetic and genetic data, strain SMK-146^T represents a novel species of the genus *Jannaschia*, for which the name *Jannaschia seohaensis* sp. nov. is proposed. The type strain is SMK-146^T (=KCTC 22172^T =CCUG 55326^T).

The genus *Jannaschia* was proposed by Wagner-Döbler *et al.* (2003) and, at the time of writing, the genus comprises five species with validly published names: *Jannaschia helgolandensis* (Wagner-Döbler *et al.*, 2003), *J. rubra* (Macián *et al.*, 2005), *J. seosinensis* (Choi *et al.*, 2006), *J. donghaensis* (Yoon *et al.*, 2007) and *J. pohangensis* (Kim *et al.*, 2008). *Jannaschia cystaugens*, which had been described by Adachi *et al.* (2004), was reclassified as a synonym of *Thalassobacter stenotrophicus* by Pujalte *et al.* (2005). In this study, we report the taxonomic characterization of a *Jannaschia*-like bacterial strain, SMK-146^T, which was isolated from a tidal flat sediment of the Yellow Sea, Korea.

Strain SMK-146^T was isolated from a tidal flat sediment from Saemankum of the Yellow Sea, Korea, by means of the standard dilution-plating technique at 25 °C on marine agar 2216 (MA; Difco). The type strains of the five recognized *Jannaschia* species were used for DNA–DNA hybridization, phenotypic characterization and fatty acid analysis. *J. helgolandensis* DSM 14858^T and *J. rubra* DSM 16279^T were obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen. *J. seosinensis* KCCM 42114^T was obtained from the Korean Culture Center of

Microorganisms. *J. pohangensis* KACC 11609^T was obtained from the Korean Agricultural Culture Collection. *J. donghaensis* DSW-17^T was obtained in the study of Yoon *et al.* (2007). The morphological, physiological and biochemical characteristics of strain SMK-146^T were investigated using routine cultivation on MA at 30 °C. The cell morphology was examined by light microscopy (Nikon E600) and transmission electron microscopy. Flagellation was determined using a Philips CM-20 transmission electron microscope with cells from exponentially growing cultures: for this purpose, the cells were negatively stained with 1% (w/v) phosphotungstic acid and the grids were examined after being air-dried. Growth under anaerobic conditions was determined after incubation in a Forma anaerobic chamber on MA and MA supplemented with potassium nitrate (0.1%, w/v), both of which had been prepared anaerobically using nitrogen. Growth in the absence of NaCl was investigated using trypticase soy broth (Difco) prepared according to the manufacturer's instructions except that NaCl was omitted. Growth at various NaCl concentrations (0.5%, w/v, and 1.0–12.0%, w/v, in increments of 1.0%) was investigated in marine broth 2216 (MB; Difco) and trypticase soy broth. Growth at various temperatures (4, 10, 15, 20, 22, 25, 28 and 30–45 °C in increments of 1.0 °C) was measured on MA. The pH range and optimal pH for growth was determined in MB that was adjusted to pH 4.5–9.5 (in increments of 0.5 pH units) by the addition of HCl or

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain SMK-146^T is EU156067.

A two-dimensional TLC of polar lipids of strain SMK-146^T is available as supplementary material with the online version of this paper.

Na₂CO₃, 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 reduction of nitrate were investigated as described by Lányi (1987) with the modification that artificial seawater was used for media preparation. The artificial seawater contained [(1 distilled water)⁻¹] 23.6 g NaCl, 0.64 g KCl, 4.53 g MgCl₂·6H₂O, 5.94 g MgSO₄·7H₂O and 1.3 g CaCl₂·2H₂O (Bruns *et al.*, 2001). H₂S production was tested as described by Bruns *et al.* (2001). Susceptibility to antibiotics was investigated on MA plates by using antibiotic discs containing the following (µg unless otherwise stated): polymyxin B (100 U), streptomycin (50), penicillin G (20 U), chloramphenicol (100), ampicillin (10), cephalothin (30), gentamicin (30), novobiocin (5), tetracycline (30), kanamycin (30), lincomycin (15), oleandomycin (15), neomycin (30) and carbenicillin (100). Acid production from carbohydrates was tested as described by Leifson (1963). Utilization of various substrates for growth was tested as described by Choi *et al.* (1981) with the modification that Noble agar (Difco) was used instead of Bacto agar (Difco). Carbon sources were added at a concentration of 0.2% (w/v) after sterilization by filtration. Enzyme activities were determined by using the API ZYM system (bioMérieux). For *in vivo* pigment-absorption spectrum analysis, strain SMK-146^T was cultivated aerobically in the dark at 30 °C in MB. The pigment absorption spectrum was analysed as described by Rainey *et al.* (2003). The culture was washed twice by centrifugation with a MOPS buffer (0.01 M MOPS/NaOH, 0.1 M KCl, 0.001 M MgCl₂, pH 7.5) and disrupted by sonication with a Branson Sonifier 450. After removal of cell debris by centrifugation, the absorption spectrum of the supernatant was examined on a Beckman Coulter DU800 spectrophotometer.

Cell biomass for DNA extraction and for the analyses of isoprenoid quinones and polar lipids was obtained from cultures grown in MB at 30 °C. Chromosomal DNA was isolated and purified according to the method described by Yoon *et al.* (1996), with the exception that RNase T1 was used in combination with RNase A to minimize contamination by RNA. The 16S rRNA gene sequence was amplified by PCR using two universal primers, 5'-GAGTTTGATCCTGGCTCAG-3' and 5'-AGAAAGGAGGTGATCCAGCC-3', as described previously (Yoon *et al.*, 1998). Sequencing and phylogenetic analysis were performed as described previously (Yoon *et al.*, 2003). Isoprenoid quinones were analysed as described by Komagata & Suzuki (1987) using reversed-phase HPLC. Polar lipids were extracted according to the procedures described by Minnikin *et al.* (1984) and identified by two-dimensional TLC followed by spraying with appropriate detection reagents (Minnikin *et al.*, 1984; Komagata & Suzuki, 1987). The presence of phosphatidylcholine was

determined with Dragendorff's reagent (Sigma). For cellular fatty acid analysis, cell biomass was harvested from MA plates after cultivation for 7 days at 30 °C except for *J. donghaensis* DSW-17^T, which was harvested from MA plates after cultivation for 7 days at 25 °C. The fatty acids were extracted and fatty acid methyl esters were 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 a modification that DNA was hydrolysed using nuclease P1 (Sigma) and the resultant nucleotides were analysed by reversed-phase HPLC. DNA-DNA hybridization was performed fluorometrically by the method of Ezaki *et al.* (1989) using photobiotin-labelled DNA probes for cross-hybridization in microdilution wells. Hybridization was performed with five replications for each sample. The highest and lowest values obtained in each sample were excluded and the means of the remaining three values are quoted as DNA-DNA relatedness values.

Morphological, cultural, physiological and biochemical characteristics of strain SMK-146^T are given in the species description and in Table 1. Sonicated cell extracts of strain SMK-146^T showed absorption maxima at 406 and 864 nm. Acetone/methanol extracts had maximal absorption peaks at 414, 678 and 751–752 nm. These data indicated the presence of carotenoids and bacteriochlorophyll *a* in strain SMK-146^T. The almost-complete 16S rRNA gene sequence of strain SMK-146^T determined in this study comprised 1418 nt, which represents approximately 96% of the *Escherichia coli* 16S rRNA gene sequence. In the phylogenetic tree based on the neighbour-joining algorithm, strain SMK-146^T fell within the clade comprising *Jannaschia* species and clustered with *J. seosinensis* CL-SP26^T with a bootstrap resampling value of 80.7% (Fig. 1). The relationship between SMK-146^T and *J. seosinensis* CL-SP26^T was also recovered in trees based on the maximum-likelihood and maximum-parsimony algorithms (Fig. 1). Strain SMK-146^T exhibited 16S rRNA gene sequence similarity of 97.0% to *J. seosinensis* CL-SP26^T, 95.3–96.7% to the type strains of the other *Jannaschia* species and less than 94.6% to the other species used in the phylogenetic analysis. The predominant ubiquinone detected in strain SMK-146^T was Q-10 (peak area ratio approximately 95%). The major polar lipids found in strain SMK-146^T were phosphatidylcholine, phosphatidylglycerol and phosphatidylethanolamine; minor amounts of unidentified phospholipids and lipids were also present (Supplementary Fig. S1, available in IJSEM Online). The polar lipid profile of SMK-146^T was distinguishable from the profile of *J. helgolandensis* in that diphosphatidylglycerol and an aminolipid are absent and from the profile of *J. donghaensis* in that an unidentified glycolipid is absent (Wagner-Döbler *et al.*, 2003; Yoon *et al.*, 2007). The fatty acid profile of strain SMK-146^T and those of the type strains of the *Jannaschia* species analysed in this study are shown in Table 2. The major components (>10% of total

Table 1. Differential phenotypic characteristics of strain SMK-146^T (*Jannaschia seohaensis* sp. nov.) and *Jannaschia* species

Species: 1, *J. seohaensis* sp. nov. SMK-146^T (data from this study); 2, *J. helgolandensis* (Wagner-Döbler *et al.*, 2003; Choi *et al.*, 2006); 3, *J. rubra* (Macián *et al.*, 2005; Choi *et al.*, 2006); 4, *J. seosinensis* (Choi *et al.*, 2006); 5, *J. donghaensis* (Yoon *et al.*, 2007); 6, *J. pohangensis* (Kim *et al.*, 2008). In addition to the sources given, data for carbon source utilization, oxidase activity, antibiotic susceptibility, gelatin and starch hydrolysis, colony colour and reduction of nitrate for the type strains of all of the *Jannaschia* species were taken from this study. All species are positive for utilization of D-glucose, D-fructose, D-galactose, cellobiose, D-xylose and acetate, activity of catalase, oxidase, alkaline phosphatase, esterase (C4) and leucine arylamidase and susceptibility to carbenicillin, cephalothin, chloramphenicol, gentamicin, kanamycin, neomycin, novobiocin, penicillin G, polymyxin B and streptomycin. All species are negative for Gram-staining, hydrolysis of gelatin and starch, utilization of benzoate, formate and salicin, activity of lipase (C14), cystine arylamidase, trypsin, α -chymotrypsin, β -glucuronidase, β -glucosidase, N-acetyl- β -glucosaminidase, α -mannosidase and α -fucosidase and susceptibility to lincomycin. +, Positive; w, weakly positive; –, negative.

Characteristic	1	2	3	4	5	6
Cell characteristics						
Shape	Pleomorphic	Irregular rods	Rods	Rods	Pleomorphic	Rods
Length (μ m)	0.5–3.5	1.9–3.2	1.0–2.0	1.1–2.3	0.6–5.0	1.5–2.0
Diameter (μ m)	0.5–1.5	0.7–1.1	0.5	0.7–1.2	0.5–2.5	0.7–1.0
Motility	+	–	+	+	–	+
Colony colour (MA)	Light orange or cream*	Pale yellow	Moderate orange	Strong orange	Strong orange	Pale yellow
Temperature for growth						
Maximum ($^{\circ}$ C)	43	30	25	35	32	35
Optimum ($^{\circ}$ C)	30	25–30	20–25	30–35	25	30
Nitrate reduction to nitrite	+	–	+	–	–	–
Hydrolysis of:						
Aesculin	w	–	–	w	–	–
Tween 80	+	w	–	+	w	+
Urea	–	–	+	–	–	–
Utilization of:						
L-Arabinose	+	+	+	+	+	–
Maltose	–	–	–	–	+	–
D-Mannose	+	+	+	+	+	–
Sucrose	+	–	–	+	+	–
Trehalose	w	–	–	–	–	–
Citrate	–	+	+	–	–	–
L-Glutamate	–	+	+	+	–	w
Pyruvate	–	w	+	–	w	+
Succinate	+	+	+	+	–	+
Enzyme activity (API ZYM)†						
Esterase lipase (C8)	+	w	w	+	+	+
Acid phosphatase	–	w	+	–	+	+
Naphthol-AS-BI-phosphohydrolase	–	–	+	–	–	+
Valine arylamidase	–	–	+	+	–	–
α -Galactosidase	–	w	+	w	–	–
β -Galactosidase	–	w	w	w	–	+
α -Glucosidase	–	–	+	+	–	–
Susceptibility to:						
Ampicillin	+	+	–	+	+	+
Oleandomycin	+	–	–	+	–	w
Tetracycline	–	+	–	–	+	–
DNA G + C content (mol%)	68.4	63.0–63.1	64.6	63	65.2	63.6

*Light orange at 25 and 30 $^{\circ}$ C, but cream at 37 $^{\circ}$ C.

†Data for *J. helgolandensis* and *J. rubra* were taken from Choi *et al.* (2006).

fatty acids) were C_{18:1} ω 7c (59.3 %) and 11-methyl C_{18:1} ω 7c (23.7 %). This fatty acid profile was similar to those of *Jannaschia* species, although there were differences in the proportions of some fatty acids, particularly 11-

methyl C_{18:1} ω 7c and cyclo C_{19:0} ω 8c, between strain SMK-146^T and the type strains of *Jannaschia* species (Table 2). The DNA G + C content of strain SMK-146^T was 68.4 mol%, which is higher than those of the other

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the relative positions of strain SMK-146^T (*Jannaschia seohaensis* sp. nov.), *Jannaschia* species and some other related taxa. Bootstrap values (>50%) based on 1000 replications are shown at branch nodes. Filled circles indicate that the corresponding nodes were also recovered in maximum-likelihood and maximum-parsimony trees. Open circles indicate that the corresponding nodes were also recovered in either the maximum-likelihood or the maximum-parsimony tree. The sequence of *Stappia stellulata* IAM 12621^T was used as an outgroup. Bar, 0.01 substitutions per nucleotide position.

Jannaschia species. The results obtained from chemotaxonomic analyses supports the result of the phylogenetic analysis based on 16S rRNA gene sequences. Accordingly, it was concluded that strain SMK-146^T is a member of the genus *Jannaschia*.

The mean DNA–DNA relatedness value between strain SMK-146^T and *J. seosinensis* KCCM 42114^T was 17%, which indicates that strain SMK-146^T represents a genomic species that is distinct from *J. seosinensis* (Wayne *et al.*, 1987). Strain SMK-146^T was distinguishable from recognized *Jannaschia* species through differences in several phenotypic characteristics and in the proportions of some fatty acids, particularly 11-methyl *C*_{18:1}ω7*c* (Tables 1 and 2). The phylogenetic distinctiveness, together with the genetic distinctiveness and differential phenotypic properties, is sufficient to show that strain SMK-146^T is separate from recognized *Jannaschia* species (Wayne *et al.*, 1987; Stackebrandt & Goebel, 1994). On the basis of the data presented, strain SMK-146^T represents a novel species within the genus *Jannaschia*, for which the name *Jannaschia seohaensis* sp. nov. is proposed.

Description of *Jannaschia seohaensis* sp. nov.

Jannaschia seohaensis (seo.ha.en'sis. N.L. fem. adj. *seohaensis* of Seohae, the Korean name of the Yellow Sea of Korea, from where the type strain was isolated).

Cells are Gram-staining-negative and rod-, oval- or coccoid-shaped (0.5–1.5 × 0.5–3.5 μm). Motile by means

Table 2. Cellular fatty acid compositions of *J. seohaensis* sp. nov. SMK-146^T and the type strains of *Jannaschia* species

Strains: 1, *J. seohaensis* sp. nov. SMK-146^T; 2, *J. helgolandensis* DSM 14858^T; 3, *J. rubra* DSM 16279^T; 4, *J. seosinensis* KCCM 42114^T; 5, *J. donghaensis* DSW-17^T; 6, *J. pohangensis* KACC 11609^T. All data were taken from this study. Values are percentages of total fatty acids; fatty acids that represented <0.5% in all strains are omitted. —, Not detected.

Fatty acid	1	2	3	4	5	6
Straight-chain						
<i>C</i> _{12:0}	0.6	—	0.2	—	—	—
<i>C</i> _{16:0}	0.8	0.4	0.9	2.2	0.4	0.8
<i>C</i> _{17:0}	1.0	2.8	0.3	2.2	1.2	1.3
<i>C</i> _{18:0}	5.9	11.1	9.6	10.3	5.5	9.4
<i>C</i> _{19:0}	0.4	2.1	—	0.5	2.9	1.1
Unsaturated						
<i>C</i> _{18:1} ω7 <i>c</i>	60.7	21.2	48.8	72.6	71.4	51.8
<i>C</i> _{20:1} ω7 <i>c</i>	0.7	0.3	0.4	0.5	2.8	0.6
Hydroxy						
<i>C</i> _{10:0} 3-OH	1.4	2.9	2.7	2.7	2.0	3.9
11-Methyl <i>C</i> _{18:1} ω7 <i>c</i>	23.0	6.9	1.8	—	6.1	23.8
10-Methyl <i>C</i> _{19:0}	—	—	—	1.7	—	—
Cyclo <i>C</i> _{19:0} ω8 <i>c</i>	—	42.6	31.0	—	—	—
Summed features*						
2	0.3	2.0	0.8	2.2	—	—
3	0.5	—	0.3	1.1	0.3	—
7	1.1	2.8	—	—	3.5	2.3
Unknown ECL 11.799	3.7	4.2	3.0	3.4	2.5	5.0

*Summed features represent two or three fatty acids that cannot be separated by the Microbial Identification System. Summed feature 2 consisted of iso-*C*_{16:1} and/or *C*_{14:0} 3-OH. Summed feature 3 consisted of *C*_{16:1}ω7*c* and/or iso-*C*_{15:0} 2-OH. Summed feature 7 consisted of one or more of *C*_{19:1}ω6*c*, cyclo *C*_{19:0}ω10*c* and an unknown fatty acid (ECL 18.846).

of one or more polar flagella. Colonies on MA are circular, slightly convex, glistening, smooth and 1.5–2.0 mm in diameter after 7 days' incubation at 30 °C. Colonies are light orange in colour at 25 and 30 °C, but cream-coloured at 37 °C. Growth occurs at 4 (weak) and 43 °C (optimum 30 °C) but not at 44 °C, at pH 5.5 (optimum pH 7.0–8.0) but not at pH 5.0 and with 9% (w/v) NaCl but not with 0% or more than 10% (w/v) NaCl. Anaerobic growth does not occur on MA or on MA supplemented with nitrate. Bacteriochlorophyll *a* and carotenoids are produced. Casein, hypoxanthine, L-tyrosine and Tweens 20, 40 and 60 are hydrolysed, but xanthine is not. H₂S is not produced. L-Arabinose is utilized, but maltose, benzoate, formate, salicin and L-glutamate are not. Susceptible to ampicillin, carbenicillin, cephalothin, chloramphenicol, gentamicin, kanamycin, neomycin, novobiocin, oleandomycin, penicillin G, polymyxin B and streptomycin, but not to tetracycline or lincomycin. The predominant

ubiquinone is Q-10. The major fatty acids (>10% of total fatty acids) are C_{18:1}ω7c and 11-methyl C_{18:1}ω7c. The major polar lipids are phosphatidylcholine, phosphatidylglycerol and phosphatidylethanolamine. Other phenotypic characteristics are given in Table 1. The DNA G+C content of the type strain is 68.4 mol% (HPLC).

The type strain, SMK-146^T (=KCTC 22172^T =CCUG 55326^T), was isolated from a tidal flat sediment of the Yellow Sea, Saemankum, Korea.

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