Aeromicrobium tamlense sp. nov., isolated from dried seaweed

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A Gram-positive, non-motile, rod-shaped actinomycete strain, designated SSW1-57^T, was isolated from a dried seaweed sample from the coast of Jeju Island, Republic of Korea and subjected to a polyphasic taxonomic study. A neighbour-joining tree based on 16S rRNA gene sequences showed that the organism was related to members of the family *Nocardioidaceae* and formed a separate branch at the base of a taxon encompassing members of the genus *Aeromicrobium*, whereas it occupied an intermediate position between *Aeromicrobium alkaliterrae*–*Aeromicrobium marinum* and *Aeromicrobium erythreum*–*Aeromicrobium fastidiosum* clusters in maximum-parsimony and maximum-likelihood trees. The phylogenetic association of the isolate with the genus *Aeromicrobium* was supported by the following chemotaxonomic properties: LL-diaminopimelic acid in the peptidoglycan, MK-9(H₄) as the major menaquinone and major fatty acids *cis*-9-octadecenoic acid, hexadecanoic acid, 10-methyl octadecanoic acid and 2-hydroxy hexadecanoic acid. The polar lipid profile contained phosphatidylinositol, diphosphatidylglycerol and phosphatidylglycerol. Levels of 16S rRNA gene sequence similarity between the novel organism and the type strains of the four recognized *Aeromicrobium* species were in the range 96.5–96.7%. On the basis of phylogenetic analyses and phenotypic data, it is proposed that the organism should be classified as representing a novel species of the genus *Aeromicrobium*, with the name *Aeromicrobium tamlense* sp. nov. The type strain is SSW1-57^T (=JCM 13811^T =NRRL B-24466^T).

The genus *Aeromicrobium* was first proposed by Miller *et al.* (1991) to accommodate an aerobic, Gram-positive, motile actinomycete that showed coccoid or rod-shaped morphology; its description was emended recently by Yoon *et al.* (2005). Currently, the genus comprises four species, *Aeromicrobium alkaliterrae* (Yoon *et al.*, 2005), *Aeromicrobium erythreum* (Miller *et al.*, 1991), the type species of the genus, *Aeromicrobium fastidiosum* (Tamura & Yokota, 1994) and *Aeromicrobium marinum* (Bruns *et al.*, 2003), which were isolated from environmental samples such as soils and seawater. Comparative studies of 16S rRNA gene sequences showed that the genus *Aeromicrobium* was phylogenetically related to members of the genera *Marmoricola* and *Nocardioides* within the radiation of the family *Nocardioidaceae* (Stackebrandt *et al.*, 1997; Urzì *et al.*, 2000; Yoon *et al.*, 2005). In this paper, we describe the taxonomic status of a novel *Aeromicrobium* strain that was isolated from dried seaweed collected from a beach.

During an investigation of the genetic diversity of polysaccharide-producing marine bacteria, strain SSW1-57^T was isolated from dried seaweed collected from Samyang beach in Jeju Island, Korea, and subjected to morphological, cultural, physiological and chemotaxonomic characterization, in addition to 16S rRNA gene sequence studies. A dried seaweed sample (1 g) was placed into a sterile plastic tube containing 9 ml sterile distilled water. After mixing for 30 min using a tube rotator, aliquots (100 µl) of serial dilutions of the sample were transferred onto plates of isolation medium (SC-SW agar) containing 10 g soluble starch, 0.3 g casein, 2 g KNO₃, 2 g NaCl, 0.02 g CaCO₃, 0.05 g MgSO₄·7H₂O, 0.01 g FeSO₄·7H₂O and 18 g agar in a mixture of 600 ml natural seawater and 400 ml distilled water (pH 7.2). After incubation at 30 °C for 14 days, a colony from one plate was subcultivated on YE-SW agar (4 g yeast extract, 10 g malt extract, 4 g glucose and 18 g agar in a mixture of 600 ml natural seawater and 400 ml distilled water; pH 7.2). The pure culture was maintained at –20 and –80 °C in 20% glycerol supplemented with 60% (v/v) natural seawater.

Chromosomal DNA was extracted and purified by using a Wizard Genomic DNA Purification kit (Promega), according to the manufacturer's instructions. PCR amplification

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain SSW1-57^T is DQ411541.

A table detailing the cellular fatty acid content of strain SSW1-57^T and related species of the genus *Aeromicrobium* is available as supplementary material in IJSEM Online.

and sequencing of the 16S rRNA gene were performed as described previously (Lee *et al.*, 2000). The CLUSTAL_X program (Thompson *et al.*, 1997) was used to align an almost-complete 16S rRNA gene sequence of strain SSW1-57^T (1406 nt; determined in this study) with corresponding sequences of members of the family *Nocardioideaceae* and related genera. Phylogenetic analyses were carried out using several tree-making algorithms contained in the PHYLIP package (Felsenstein, 1993). Bootstrap analysis (Felsenstein, 1985) was performed to evaluate the reliability of the tree topology. A total of 1333 unambiguous, aligned positions present in all strains between positions 59 and 1431 (*Escherichia coli* numbering; Brosius *et al.*, 1978) were used for the final tree construction. A neighbour-joining tree based on 16S rRNA gene sequences (Fig. 1) showed that strain SSW1-57^T belongs to the family *Nocardioideaceae* and formed a peripheral branch in the *Aeromicrobium* cluster, supported by a bootstrap value of 100%. In trees

constructed using maximum-parsimony and maximum-likelihood methods, strain SSW1-57^T occupied an intermediate position between the *A. alkaliterrae*–*A. marinum* and *A. erythreum*–*A. fastidiosum* branches. The levels of 16S rRNA gene sequence similarity between strain SSW1-57^T and its phylogenetic relatives were 96.7% (with *A. erythreum*) and 96.5% (with *A. alkaliterrae*, *A. fastidiosum* and *A. marinum*). Sequence similarities between strain SSW1-57^T and other members of the family *Nocardioideaceae* were less than 94.2%.

For analysis of polar lipids, menaquinones and the cell wall, strain SSW1-57^T was cultivated in YE-SW broth for 3 days at 30 °C and analysed as described previously (Lee, 2006). The DNA G+C content of strain SSW1-57^T was 72.7 mol%. The isolate contained LL-diaminopimelic acid as the diagnostic diamino acid in the cell wall [wall chemotype I *sensu* Lechevalier & Lechevalier (1970)]. The

Fig. 1. Neighbour-joining tree based on 16S rRNA gene sequences showing the phylogenetic position of strain SSW1-57^T within the radiation encompassing members of the family *Nocardioideaceae*. The tree was reconstructed from evolutionary distances calculated using the Jukes–Cantor coefficient (Jukes & Cantor, 1969). *Streptomyces griseus* was used as an outgroup (not shown). Asterisks indicate branches that were also found in maximum-likelihood and maximum-parsimony trees. Numbers at nodes are percentage bootstrap values based on a neighbour-joining analysis of 1000 replications (only values greater than 50% are shown). Bar, 1 nucleotide substitution per 100 nucleotides.

predominant isoprenoid quinone was tetrahydrogenated menaquinone with nine isoprene units [MK-9(H₄)], indicating that strain SSW1-57^T contained a type of menaquinone that was different from that found in its phylogenetic neighbours *Marmoricola* and *Nocardioides* (Park *et al.*, 1999; Urzì *et al.*, 2000). Mycolic acids were not present. The phospholipid profile comprised phosphatidylinositol, diphosphatidylglycerol and phosphatidylglycerol [phospholipid type I pattern as defined by Lechevalier *et al.* (1981)]. Preparation and analysis of the cellular fatty acid methyl esters were performed using the Sherlock Microbial Identification System (version 6.0; MIDI), according to the instructions of the manufacturer. Strain SSW1-57^T was grown for 3 days at 30 °C on trypticase soy broth (Difco) supplemented with Bacto agar (Difco). The cellular fatty acid profile of strain SSW1-57^T was characterized by the presence (≥ 1.0 %) of C_{18:1}ω9c (47.2 %), C_{16:0} (13.8 %), C_{18:0} (13.6 %) 10-methyl C_{18:0} (8.7 %), C_{16:0} 2-OH (6.8 %), C_{17:0} (1.8 %), C_{16:1}ω9c (1.6 %), C_{12:0} (1.4 %), C_{17:0} 2-OH (1.1 %), C_{14:0} (1.0 %) and C_{15:0} (1.0 %) acids. The major fatty acids were consistent with those of members of the genus *Aeromicrobium* (Park *et al.*, 1999; Bruns *et al.*, 2003; Yoon *et al.*, 2005), as shown in Supplementary Table S1 in IJSEM Online. Thus, the chemotaxonomic markers, except for the phospholipid composition, were in agreement with the phylogenetic clustering of the novel isolate with the genus *Aeromicrobium*. The difference in polar lipids between the isolate and *A. erythreum* and *A. fastidiosum*, which contained phosphatidylethanolamine (Tamura & Yokota, 1994), suggests that the presence/absence of phospholipids with amino groups could be one phenotypic characteristic that differentiates the novel isolate from the two species. To date, the phospholipid compositions of *A. alkaliterrae* and *A. marinum* have not been reported.

Morphological and physiological characteristics were investigated using YE-SW agar at 30 °C. Cell morphology and motility were observed using phase-contrast and transmission electron microscopy with 3-day-old cultures. Colony pigmentation was observed visually and recorded using 5-day-old cultures on YE-SW agar at 30 °C. The Gram-reaction was determined by using a Gram-stain kit (bioMérieux), following the instructions of the manufacturer. Oxidation of *N,N,N',N'*-tetramethyl-*p*-phenylenediamine was used to determine oxidase activity. Catalase activity was determined using 3 % (v/v) H₂O₂ solution. Growth at a range of temperatures (4–45 °C) was tested using YE-SW agar. Growth in various NaCl concentrations was studied using ISP 2 medium (Shirling & Gottlieb, 1966). NaCl was added to the basal medium at final concentrations of 0–9, 10 and 15 % (w/v). The pH range for growth was determined on YE-SW agar adjusted to pH 4.1–12.1 (at intervals of 1.0 pH units). The pH of the medium was adjusted prior to sterilization by addition of 6 M HCl or 10 M NaOH. Decomposition of hypoxanthine, DL-tyrosine and xanthine was examined as described by Gordon *et al.* (1974). Hydrolysis of casein, gelatin and starch was tested as

described by MacFaddin (1980). The ability to utilize a variety of substrates as sole carbon and energy sources was tested using ISP 9 medium (Shirling & Gottlieb, 1966), with each filter-sterilized carbon source being used at a final concentration of 1 % (w/v) for carbohydrates and alcohols and 0.1 % (w/v) for organic acids. Strain SSW1-57^T was grown on YE-SW agar at 30 °C for 3 days and the cells were then scraped from the agar surface with a cotton swab. The cells were washed and resuspended in sterile distilled water before being used. Other physiological and biochemical properties were tested using API 20E and API ZYM systems (bioMérieux), according to the instructions of the manufacturer. Morphological, physiological and biochemical properties are given in Table 1 and the species description.

On the basis of phenotypic data and phylogenetic analyses, it is suggested that the isolate should be classified as representing a novel species of the genus *Aeromicrobium*, for which we propose the name *Aeromicrobium tamlense* sp. nov., with strain SSW1-57^T as the type strain.

Description of *Aeromicrobium tamlense* sp. nov.

Aeromicrobium tamlense (tam.len'se. N.L. neut. adj. *tamlense* of Tamla, the old name of Jeju, Republic of Korea, the site of isolation of the type strain).

Aerobic, oxidase-negative and catalase-positive. Cells are Gram-positive irregular rods ranging in size from 0.4–0.6 × 0.8–1.2 to 0.5 × 3.8–4.8 μm. Non-motile. Branching or mycelial form does not occur. Endospores are not formed. Colonies are circular, smooth, convex, yellow and 0.6–0.8 mm in diameter after incubation for 5 days on YE-SW agar at 30 °C. Temperature range for growth is 10–42 °C, with optimum growth at 30 °C. No growth occurs at 4 or 45 °C. Growth occurs at pH 5.1–10.1, with optimum growth at pH 7.1. Growth occurs in the presence of 0–5 % NaCl. Nitrate is not reduced to nitrite. Gelatin liquefaction is observed. Aesculin is hydrolysed, but not casein. H₂S and indole are not produced. Hypoxanthine is decomposed, but not DL-tyrosine or xanthine. Urease activity is not detected. In API 20E tests, the following enzymic activities are not detected: arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase and tryptophan deaminase. Voges–Proskauer reaction is weakly positive. Acetate, D-cellobiose, dextran, D-fructose, D-galactose, D-glucose, D-lactose, malate, maltose, D-mannose, methyl α-D-glucoside, L-ribose, succinate, sucrose, D-trehalose and glycerol are utilized as sole carbon and energy sources. The following carbon sources are not utilized: D-arabinose, L-arabinose, benzoate, citrate, formate, inulin, D-melezitose, methyl α-D-mannoside, D-raffinose, L-rhamnose, salicin, L-sorbose, tartrate, D-xylose, adonitol, 2,3-butanediol, D-dulcitol, *meso*-erythritol, *myo*-inositol, D-mannitol, 1,2-propanediol, D-sorbitol and D-xylitol. In API ZYM tests, alkaline phosphatase, leucine arylamidase, acid phosphatase and α-glucosidase are positive. Esterase (C4), lipase (C14), valine arylamidase, cystine arylamidase,

Table 1. Phenotypic characteristics that differentiate strain SSW1-57^T from its phylogenetic relatives

Taxa: 1, strain SSW1-57^T; 2, *A. alkaliterrae* (data from Yoon *et al.*, 2005); 3, *A. erythreum* (Miller *et al.*, 1991; Tamura & Yokota, 1994); 4, *A. fastidiosum* (Collins & Stackebrandt, 1989; Tamura & Yokota, 1994); 5, *A. marinum* (Bruns *et al.*, 2003). +, Positive; w, weakly positive; –, negative; ND, not determined.

Characteristic	1	2	3	4	5
Cell morphology	Irregular rods	Rods, cocci	Irregular rods, cocci	Rods, cocci	Rods
Motility	–	–	–	+	–
Growth at:					
42 °C	+	–	–	–	–
10 °C	+	+	–	+	+
Utilization of:					
L-Arabinose	–	+	+	+	–
Citrate	–	–	ND	+	–
D-Fructose	+	–	+	+	–
D-Glucose	+	+	+	ND	–
Maltose	+	+	–	–	–
D-Mannose	+	–	–	+	–
Salicin	–	+	–	ND	ND
Sucrose	+	+	+	ND	–
D-Xylose	–	–	+	ND	–
Hydrolysis of:					
Aesculin	+	–	+	ND	ND
Casein	–	+	+	+	–
API ZYM:					
Acid phosphatase	+	+	+	+	–
Alkaline phosphatase	+	–	–	+	–
Esterase (C4)	–	+	+	+	+
α-Glucosidase	+	+	+	+	–
Trypsin	w	–	–	–	–
Phospholipids*	DPG, PG, PI	ND	DPG, PE, PG	DPG, PE, PG	ND
DNA G + C content (mol%)	72.7	71.5	71–73	71–72	70.6

*DPG, Phosphatidylglycerol; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol.

α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, β-glucosidase, N-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase are negative. The following are weakly positive: esterase lipase (C8), trypsin and naphthol-AS-BI-phosphohydrolase. Predominant cellular fatty acids are C_{18:1}ω9c, C_{16:0}, 10-methyl C_{18:0}, C_{18:0} and C_{16:0} 2-OH. Polar lipids comprise phosphatidylinositol, diphosphatidylglycerol and phosphatidylglycerol.

The G+C content of the DNA of the type strain is 72.7 mol%. The type strain is strain SSW1-57^T (=JCM 13811^T=NRRL B-24466^T), which was isolated from dried seaweed.

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