Aeromicrobium ponti sp. nov., isolated from seawater

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A novel actinomycete strain, designated HSW-1^T, was isolated from seawater collected from Hwasun Beach on the coast of Jeju Island, Republic of Korea. The cells were aerobic, Gram-positive, oxidase-negative, catalase-positive, non-motile rods. The colonies were circular, smooth, convex and yellow in colour. The cell-wall peptidoglycan of this organism contained LL-diaminopimelic acid as the diagnostic diamino acid. The polar lipids included phosphatidylinositol, diphosphatidylglycerol, phosphatidylglycerol and three unknown phospholipids. The predominant menaquinone was MK-9(H_4). The major fatty acids were $C_{18:1}\omega9c$, $C_{16:0}$, $C_{16:0}$ 2-OH and 10-methyl $C_{18:0}$. The DNA G+C content was 74.0 mol%. A neighbour-joining tree based on 16S rRNA gene sequences showed that this organism falls within the radiation of the genus *Aeromicrobium*. The closest phylogenetic neighbours were the type strains of *Aeromicrobium erythreum* (98.2 % 16S rRNA gene sequence similarity), *Aeromicrobium alkaliterrae* (97.9 %), *Aeromicrobium marinum* (97.3 %) and *Aeromicrobium fastidiosum* (97.0 %). The DNA–DNA relatedness values between the novel isolate and its closest relative, *A. erythreum* DSM 8599^T, ranged between 32 and 36 %. On the basis of the phenotypic and DNA–DNA hybridization data, strain HSW-1^T represents a novel species of the genus *Aeromicrobium*, for which the name *Aeromicrobium ponti* sp. nov. is proposed. The type strain is HSW-1^T (=DSM 19178^T=KACC 20565^T).

The genus *Aeromicrobium* was first proposed by Miller *et al.* (1991) and, at the time of writing, comprises six recognized species: *Aeromicrobium erythreum* (Miller *et al.*, 1991), *A. fastidiosum* (Tamura & Yokota, 1994), *A. marinum* (Bruns *et al.*, 2003), *A. alkaliterrae* (Yoon *et al.*, 2005), *A. panaciterrae* (Cui *et al.*, 2007) and *A. tamlense* (Lee & Kim, 2007). *A. marinum* and *A. tamlense* were isolated from marine environments, including seawater and seaweed. Here, we describe the taxonomic characteristics of an *Aeromicrobium*-like strain isolated from seawater.

Strain HSW-1^T was isolated from a seawater sample collected from Hwasun Beach on the coast of Jeju Island, Republic of Korea. A seawater sample was spread directly onto SC-SW agar (1 % soluble starch, 0.03 % casein, 0.2 % KNO_3, 0.2 % NaCl, 0.2 % KH_2PO_4, 0.002 % $CaCO_3$, 0.005 % $MgSO_4.7H_2O$, 0.001 % $FeSO_4.7H_2O$ and 1.8 % agar in a mixture of 60 % natural seawater and 40 % distilled water) and the plate was incubated at 30 °C for 2 weeks. The single colony on SC-SW agar plate was selectively isolated and further streaked at least three times on ISP 2 medium (Shirling & Gottlieb, 1966) amended with 60 % (w/v) natural seawater. The pure culture was maintained at −20 and −80 °C as a glycerol solution including 20 % (v/v) distilled water and 60 % (v/v) natural seawater. For phenotypic and genetic comparisons, *A. alkaliterrae* KCTC 19073^T, *A. erythreum* DSM 8599^T, *A. fastidiosum* KCTC 9576^T, *A. marinum* DSM 15272^T and *A. tamlense* SSW1-57^T were grown on trypticase soy agar (TSA; Difco) for 3 days at 30 °C, while *A. panaciterrae* KCTC 19131^T was grown on R2A agar (Difco) for 7 days at 30 °C.

Cell morphology and motility were observed by using phase-contrast microscopy, with cells grown in trypticase soy broth (TSB; Difco) for 6, 15 and 24 h. For checking for the presence of flagella, cells were stained with 2 % phosphotungstic acid, placed on a gold-coated grid and observed with a JEM-1200EX II transmission electron microscope (JEOL). The colonial properties of cells grown on TSA for 5 days at 30 °C were observed and recorded. Growth was determined on TSA at 4, 10, 20, 30, 37 and 42 °C and at initial pH values of 4.1–12.1 (with increments of 1.0 pH unit). NaCl tolerance for growth was tested on

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain HSW-1^T is AM778683.

A transmission electron micrograph of a cell of strain HSW-1^T and details of the cellular fatty acid profiles of HSW-1^T and the type strains of the genus *Aeromicrobium* are available as supplementary material with the online version of this paper.

ISP 2 medium supplemented with 1–15 % (w/v) NaCl. The Gram stain, oxidase and catalase activities, degradative abilities and utilization of carbohydrates were all determined using previously described methods (Lee & Kim, 2007). Other physiological and biochemical properties were tested using the API 20E and API ZYM kits (bioMérieux) according to the manufacturer's instructions. Cells of strain HSW-1^T were aerobic, Gram-positive, non-motile rods (0.7 × 2.4 μm) (see Supplementary Fig. S1 in IJSEM Online). The cultural, physiological and biochemical characteristics of the novel strain are given in Table 1 and the species description.

Table 1. Characteristics that differentiate strain HSW-1^T from the type strains of the genus *Aeromicrobium*

Strains: 1, HSW-1^T; 2, *A. alkaliterrae* KCTC 19073^T (Yoon *et al.*, 2005); 3, *A. erythreum* DSM 8599^T (Miller *et al.*, 1991; Bruns *et al.*, 2003; Yoon *et al.*, 2005); 4, *A. fastidiosum* KCTC 9576^T (Tamura & Yokota, 1994; Bruns *et al.*, 2003; Yoon *et al.*, 2005); 5, *A. marinum* DSM 15272^T (Bruns *et al.*, 2003; Yoon *et al.*, 2005); 6, *A. panaciterrae* KCTC 19131^T (Cui *et al.*, 2007); 7, *A. tamlense* SSW1-57^T (Lee & Kim, 2007). All of the strains are positive for the Gram stain, activity of esterase lipase (C8) and utilization of trehalose, glycerol and malate. They are all negative in tests for the activities of lipase (C14), cystine arylamidase, trypsin, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, α-fucosidase, lysine decarboxylase, ornithine decarboxylase, urease and tryptophan deaminase, for citrate utilization, indole production, hydrolysis of chitin and xanthine, nitrate reduction and H_2S production and for the utilization of L-sorbose and D-xylitol. +, Positive; −, negative; W, weak; ND, not determined; DPG, diphosphatidylglycerol; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; UP, an unknown phospholipid.

Characteristic	1	2	3	4	5	6	7
Cell morphology	Rods	Rods, cocci	Irregular rods, cocci	Short rods, cocci	Rods	Rods	Irregular rods
Colony colour	Yellow	Cream	Beige to amber beige	White	Ivory	Yellowish white	Yellow
Motility	−	−	−	+	−	−	−
Temperature range (°C)	4–42	4–35	21–40	5–30	4–35	15–30	10–42
Tolerance of NaCl (%)	0–10	0–8	0–4	0–4	0.08–10.7	0–3	0–5
pH range	4.1–12.1	6.0–11.0	5.0–9.0	5.0–8.0	5.5–9.5	5.0–8.5	5.1–10.1
Oxidase activity	−	−	+	+	−	−	−
Catalase activity	+	+	+	+	+	−	+
Hydrolysis of:							
DNA	+	+	W	+	W	−	ND
Elastin	+	+	+	−	−	−	ND
Starch	−	−	+	+	−	−	ND
Casein	+	+	+	+	−	−	−
Hypoxanthine	−	−	−	−	−	−	+
Tyrosine	+	−	W	−	−	−	−
Enzyme activities:							
Alkaline phosphatase	+	−	−	+	−	+	+
Esterase (C4)	+	+	+	+	+	W	−
Leucine arylamidase	−	+	+	+	+	+	+
Valine arylamidase	W	−	−	−	−	−	−
Acid phosphatase	+	+	+	+	−	+	+
Naphthol-AS-BI-phosphohydrolase	−	+	W	W	W	+	W
α-Glucosidase	−	+	+	+	−	−	+
Voges–Proskauer reaction	+	−	+	+	+	−	W
Utilization of carbon sources							
Dextran	+	+	+	−	W	ND	+
D-Fructose	+	−	+	+	−	−	+
D-Mannose	+	−	−	+	−	+	+
L-Rhamnose	−	−	−	+	−	−	−
Sucrose	+	+	+	+	−	+	+
D-Mannitol	−	−	−	−	+	−	−
Acetate	+	−	+	+	+	+	+
Succinate	+	+	+	−	+	+	+
Polar lipids	DPG, PG, PI, UP	ND	DPG, PE, PG	DPG, PE, PG	ND	ND	DPG, PG, PI
DNA G+C content (mol%)	74.0	71.5	71–73	71–72	70.6	65.5	72.7

Genomic DNA was extracted and purified as described previously (Hopwood *et al.*, 1985). The 16S rRNA gene was amplified by using a PCR with universal primers 27f and 1492r (Lane, 1991). The thermal cycling parameters for the PCR were as follows: 5 min preheating at 95 °C, 30 cycles of 1 min denaturation at 94 °C, 1 min annealing at 55 °C and 3 min extension at 72 °C and then 20 min final extension at 72 °C. The length of the amplified fragment was checked by means of agarose gel electrophoresis. The PCR product was purified using the Wizard PCR Preps DNA Purification System (Promega) and was directly sequenced using the ABI PRISM BigDye Terminator cycle sequencing kit (Applied Biosystems) and an automatic DNA sequencer (model 3730xl; Applied Biosystems). Multiple alignments of sequences were performed using CLUSTAL_X (Thompson *et al.*, 1997). A phylogenetic tree was constructed by using the neighbour-joining method (Saitou & Nei, 1987) from evolutionary distances calculated with the coefficient of Jukes & Cantor (1969). The topology of the tree was assessed by performing bootstrap analysis with 1000 replicated datasets (Felsenstein, 1985).

The almost-complete 16S rRNA gene sequence (1350 nt) of strain HSW-1^T determined in this study was compared with the corresponding sequences of members of the genus *Aeromicrobium* and related taxa. The neighbour-joining tree (Fig. 1) based on 16S rRNA gene sequences showed that strain HSW-1^T falls within the radiation of the genus *Aeromicrobium*. The 16S rRNA gene sequence similarity values for strain HSW-1^T with respect to the type strains of the recognized *Aeromicrobium* species were as follows: *A. erythreum*, 98.2 %; *A. alkaliterrae*, 97.9 %; *A. marinum*, 97.3 %; *A. fastidiosum*, 97.0 %; *A. panaciterrae*, 96.8 %; *A. tamlense*, 96.3 %.

Cell biomass for chemotaxonomic analyses was obtained from cultures grown in TSB for 3 days at 30 °C. The type and isomer of the diaminopimelic acid in the cell-wall peptidoglycan were determined by using the method of Staneck & Roberts (1974). Respiratory quinones were extracted according to Collins (1985) and identified by HPLC (Kroppenstedt, 1985). Analysis of the polar lipids was performed using TLC as described previously (Minnikin *et al.*, 1975). For analysis of the cellular fatty acids, strain HSW-1^T and five type strains of species of the genus *Aeromicrobium* were grown on TSA for 5 days at 30 °C. Cellular fatty acid methyl esters were prepared and analysed according to the standard protocol of the Microbial Identification System (version 6; MIDI). The chemotaxonomic characteristics of HSW-1^T (with the exception of the polar lipid profile) were consistent with those of the genus *Aeromicrobium*. LL-Diaminopimelic acid was the diagnostic diamino acid in the cell-wall peptidoglycan. The predominant menaquinone was MK-9(H_4). The cellular fatty acid profile of strain HSW-1^T consisted of large amounts of saturated, unsaturated, hydroxy and 10-methyl components. The major fatty acids were $C_{18:1}\omega9c$ (34.7 %), $C_{16:0}$ (19.7 %), $C_{16:0}$ 2-OH (14.8 %) and 10-methyl $C_{18:0}$ (tuberculostearic acid, 11.0 %). The cellular fatty acid profiles of strain HSW-1^T and the type strains of the species of the genus *Aeromicrobium* are shown in Supplementary Table S1. The polar lipids included phosphatidylinositol, diphosphatidylglycerol, phosphatidylglycerol and three unknown phospholipids. Strain HSW-1^T lacked phosphatidylethanolamine, which was present in the type strains of *A. erythreum* and *A. fastidiosum*. The G+C content of the DNA of the novel strain, as determined by HPLC (Mesbah *et al.*, 1989), was 74.0 mol%.

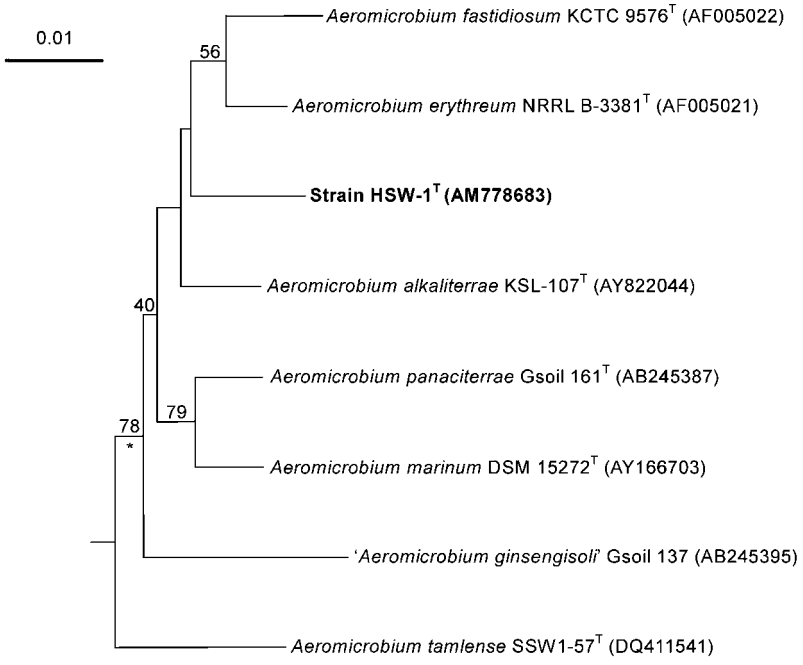

Fig. 1. Phylogenetic tree, based on 16S rRNA gene sequences, showing the position of strain HSW-1^T within the radiation of the genus *Aeromicrobium*. The tree was constructed from the evolutionary distance matrix obtained by using the neighbour-joining method (Saitou & Nei, 1987). The sequence of *Kribella sandramycini* KCTC 9609^T was used as outgroup (not shown). The asterisk identifies a branch that was also found in both the maximum-likelihood (Fitch, 1971) and maximum-parsimony (Felsenstein, 1981) trees. Bootstrap percentages (>50 %), based on 1000 replicated datasets, are shown at the nodes. Bar, 0.01 substitutions per nucleotide position.

For DNA–DNA hybridization experiments, genomic DNA was isolated using a French pressure cell (Thermo Spectroni) and purified by chromatography on hydroxyapatite (Cashion *et al.*, 1977). DNA–DNA hybridization was carried out by using the method of De Ley *et al.* (1970) with the modifications described by Huß *et al.* (1983). Since the highest level of 16S rRNA gene sequence similarity for strain HSW-1^T was found with respect to the type strain of *A. erythreum* (98.2 %), DNA–DNA hybridization experiments were performed between these strains. The DNA–DNA hybridization value was found to be between 32 and 36 %, i.e. lower than the threshold value of 70 % recommended for the definition of bacterial species (Wayne *et al.*, 1987). The characteristics that serve to differentiate the novel isolate from the type strains of the recognized species of the genus *Aeromicrobium* are summarized in Table 1.

On the basis of the phenotypic and DNA–DNA hybridization data presented in this study, strain HSW-1^T represents a novel species of the genus *Aeromicrobium*, for which the name *Aeromicrobium ponti* sp. nov. is proposed.

Description of *Aeromicrobium ponti* sp. nov.

Aeromicrobium ponti (pon′ti. L. gen. n. *ponti* of sea, belonging to the sea, referring to the isolation of the type strain from seawater).

Cells are aerobic, Gram-positive, oxidase-negative, catalase-positive, non-motile rods (0.7 × 2.4 μm). On TSA, colonies are circular, smooth, convex, yellow in colour and reach 0.8–1.2 mm in diameter after 5 days. The temperature range for growth is 4–42 °C; good growth occurs at 30–37 °C. Growth occurs at pH 4.1–12.1; good growth occurs at pH 5.1–7.1. Grows with NaCl at concentrations up to 10 %. Gelatin liquefaction and hydrolysis of carboxymethylcellulose are observed. Arginine dihydrolase is not detected. L-Arabinose, cellobiose, D-galactose, D-glucose, maltose, D-xylose and citrate are utilized as sole carbon and energy sources. Utilization of salicin is weak. The following carbon sources are not utilized: D-arabinose, inulin, D-lactose, melezitose, methyl α-D-glucoside, methyl α-D-mannoside, raffinose, dulcitol, *meso*-erythritol, *myo*-inositol, D-sorbitol, benzoate, formate and tartrate. Data for other physiological and biochemical properties are given in Table 1. LL-Diaminopimelic acid is the diagnostic diamino acid of the cell-wall peptidoglycan. The polar lipids include phosphatidylinositol, diphosphatidylglycerol, phosphatidylglycerol and three unknown phospholipids. The predominant menaquinone is MK-9(H_4). The major fatty acids are $C_{18:1}\omega9c$ (34.7 %), $C_{16:0}$ (19.7 %), $C_{16:0}$ 2-OH (14.8 %) and 10-methyl $C_{18:0}$ (tuberculostearic acid, 11.0 %). The DNA G+C content is 74.0 mol%.

The type strain, HSW-1^T (=DSM 19178^T=KACC 20565^T), was isolated from seawater from the coast of Jeju Island, Republic of Korea.

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