Aeromicrobium ponti sp. nov., isolated from seawater

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A novel actinomycete strain, designated HSW-1T, 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 L-l-L-diaminopimelic acid as the diagnostic diamino acid. The polar lipids included phosphatidylglycerol, diphasatidyglycerol, phosphatidylethanolamine and three unknown phospholipids. The predominant menaquinone was MK-9(H6). The major fatty acids were C18:1ω9c, C16:0, C16:0 2-OH and 10-methyl C18:0. 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 alkaliterre (97.9%), Aeromicrobium marinus (97.3%) and Aeromicrobium fastidiosum (97.0%). The DNA–DNA relatedness values between the novel isolate and its closest relative, A. erythreum DSM 8599T, ranged between 32 and 36%. On the basis of the phenotypic and DNA–DNA hybridization data, strain HSW-1T represents a novel species of the genus Aeromicrobium, for which the name Aeromicrobium ponti sp. nov. is proposed. The type strain is HSW-1T (=DSM 19178T=KACC 20565T).

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. marinus (Bruns et al., 2003), A. alkaliterre (Yoon et al., 2005), A. panaciterrae (Cui et al., 2007) and A. tamlense (Lee & Kim, 2007). A. marinus 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-1T 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, 2% KH2PO4, 0.002% CaCO3, 0.005% MgSO4.7H2O, 0.001% FeSO4.7H2O 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°C 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. alkaliterre KCTC 19073T, A. erythreum DSM 8599T, A. fastidiosum KCTC 9576T, A. marinus DSM 15272T and A. tamlense SSW1-57T were grown on trypticase soy agar (TSA; Difco) for 3 days at 30°C, while A. panaciterrae KCTC 19131T 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, 40 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-1T is AM778683.

A transmission electron micrograph of a cell of strain HSW-1T and details of the cellular fatty acid profiles of HSW-1T 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 deter-
mined 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-1T 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-1T from the type strains of the genus Aeromicrobium

<table>
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<tr>
<th>Characteristic</th>
<th>1</th>
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<th>5</th>
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<tr>
<td>Cell morphology</td>
<td>Rods</td>
<td>Rods, cocci</td>
<td>Irregular rods, cocci</td>
<td>Short rods, cocci</td>
<td>Rods</td>
<td>Rods</td>
<td>Irregular rods</td>
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<tr>
<td>Colony colour</td>
<td>Yellow</td>
<td>Cream</td>
<td>Beige to amber</td>
<td>White</td>
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<td>Motility</td>
<td>–</td>
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<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
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<td>Temperature range (°C)</td>
<td>4–42</td>
<td>4–35</td>
<td>21–40</td>
<td>5–30</td>
<td>4–35</td>
<td>15–30</td>
<td>10–42</td>
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<td>Tolerance of NaCl (%)</td>
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<td>0–8</td>
<td>0–4</td>
<td>0–4</td>
<td>0–4</td>
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<td>5.0–9.0</td>
<td>5.0–8.0</td>
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<td>+</td>
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<td>Catalase activity</td>
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<td>W</td>
<td>+</td>
<td>W</td>
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<td>+</td>
<td>–</td>
<td>–</td>
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<td>Starch</td>
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<td>+</td>
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<td>Casen</td>
<td>+</td>
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<td>+</td>
<td>–</td>
<td>+</td>
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<td>–</td>
<td>W</td>
<td>–</td>
<td>–</td>
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<td>+</td>
<td>+</td>
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<td>Utilization of carbon sources</td>
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<tr>
<td>Sucrose</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
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<tr>
<td>Acetate</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
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<td>Succinate</td>
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<td>Polar lipids</td>
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<td>DNA G + C content (mol%)</td>
<td>74.0</td>
<td>71.5</td>
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<td>71–72</td>
<td>70.6</td>
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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-1T 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-1T falls within the radiation of the genus Aeromicrobium. The 16S rRNA gene sequence similarity values for strain HSW-1T 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-1T 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-1T (with the exception of the polar lipid profile) were consistent with those of the genus Aeromicrobium. 1-L-Diaminopimelic acid was the diagnostic diamino acid in the cell-wall peptidoglycan. The predominant menaquinone was MK-9(H4). The cellular fatty acid profile of strain HSW-1T consisted of large amounts of saturated, unsaturated, hydroxy and 10-methyl components. The major fatty acids were C18:1ω9c (34.7%), C16:0 (19.7%), C16:0 2-OH (14.8%) and 10-methyl C18:0 (tuberculostearic acid, 11.0%). The cellular fatty acid profiles of strain HSW-1T and the type strains of the species of the genus Aeromicrobium are shown in Supplementary Table S1. 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-1T 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 9609T 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-1T 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-1T 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 (pont’i. 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-carboxymethylcellulose are observed. Arginine dihydrolase up to 10 %. Gelatin liquefaction and hydrolysis of inulin, D-lactose, melezitose, methyl D-glucoside, D-glucose, maltose, D-xylitol 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 x-d-glucoside, methyl x-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 phosphatidylglycerol, diphosphatidylglycerol, phosphatidylglycerol and three unknown phospholipids. The predominant menaquinone is MK-9(H4). The major fatty acids are C18:1ω9c (34.7 %), C16:0 (19.7 %), C16:0 2-0H (14.8 %) and 10-methyl C18:0 (tuberculostearic acid, 11.0 %). The DNA G + C content is 74.0 mol%.

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

Acknowledgements

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References


