**Agrococcus jejuensis** sp. nov., isolated from dried seaweed

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A novel marine actinomycete, designated strain SSW1-48\(^T\), was isolated from a dried seaweed sample collected from a beach in Jeju, Republic of Korea. Cells of the organism were non-motile, non-endospore-forming, Gram-positive and rod-shaped. Colonies were circular, smooth, translucent and yellow-coloured. The diagnostic diamino acid in the peptidoglycan was 2,4-diaminobutyric acid and the murein was of the acetyl type. Mycolic acids were absent. The predominant menaquinones were MK-10 and MK-9. The major fatty acids were anteiso-C\(_{15}:0\) and iso-C\(_{16}:0\). The G+C content of the DNA was 73.0 mol%. A neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showed that the isolate formed a distinct branch between *Agrococcus lahaulensis* and *Agrococcus casei* in the family *Microbacteriaceae*. The values for 16S rRNA gene sequence similarity between strain SSW1-48\(^T\) and type strains of recognized *Agrococcus* species were in the range 95.5–97.5% and those between this bacterium and other representatives of the family *Microbacteriaceae* were in the range 93.7–96.4%. On the basis of the combined data from the phenotypic and phylogenetic analyses, strain SSW1-48\(^T\) represents a novel species of the genus *Agrococcus*, for which the name *Agrococcus jejuensis* sp. nov. is proposed. The type strain is SSW1-48\(^T\) (=KCTC 19198\(^T\)=JCM 14256\(^T\)).

The genus *Agrococcus* was first proposed by Groth et al. (1996) and encompasses coryneform bacteria that possess B-type cell-wall peptidoglycan (Schleifer & Kandler, 1972) containing 2,4-diaminobutyric acid as the diagnostic diamino acid. At the time of writing, the genus contains five species with validly published names: *Agrococcus jejunensis* (Groth et al., 1996), *Agrococcus casei* (Wieser et al., 1999), *Agrococcus baldri* (Zlamala et al., 2002), *Agrococcus lahaulensis* (Mayilraj et al., 2006) and *Agrococcus casei* (Bora et al., 2007). In this study, the taxonomic status of an actinomycete isolated from seaweed was assessed by using a polyphasic approach.

A yellow-pigmented actinomycete, designated SSW1-48\(^T\), was isolated from a dried seaweed sample collected around Samyang Beach in Jeju, Republic of Korea. For the bacterial isolation, a piece of dried seaweed was transferred directly onto SC-SW agar (Lee, 2006) supplemented with 60% (v/v) natural seawater. Colonies on the plates, incubated at 30 °C for 14 days, were streaked onto ISP 2 medium (Shirling & Gottlieb, 1966) supplemented with 60% natural seawater (YE-SW agar). The pure culture was maintained at −20 and −80 °C as 20% glycerol suspensions including 60% (v/v) natural seawater and 20% (v/v) distilled water.

Cell morphology and motility were determined by using phase-contrast and transmission electron microscopy with cells grown on tryptase soy agar (TSA; Difco) for 48 h at 30 °C. Colony morphology and pigmentation were observed after 5 days growth at 30 °C on TSA. The temperature and pH ranges for growth were tested at 4, 10, 20, 30, 37 and 42 °C and at pH 4.1–12.1 (using increments of 1.0 pH unit). NaCl tolerance for growth was studied on ISP 2 medium supplemented with 0–9% (w/v) NaCl. Hydrolysis of elastin was tested on ISP 2 medium supplemented with 0.4% (w/v) elastin. Gram staining, oxidase and catalase activities and hydrolysis of casein, gelatin, starch and Tween 80 were determined according to the methods of MacFaddin (1980). Degradation tests for hypoxanthine, DL-tyrosine and xanthine were performed by using the method of Gordon et al. (1974).

Other physiological and biochemical properties were investigated using API 20NE and API ZYM strips according to the instructions of the manufacturer (bioMérieux). The inoculum sources were prepared from cells grown on ISP 2 medium at 30 °C for 3 days. Strain SSW1-48\(^T\) was found to consist of aerobic, non-endospore-forming, Gram-positive, non-motile, short rods (see Supplementary Fig. S1, available in IJSEM Online). After 5 days incubation on TSA at 30 °C, yellow-coloured, smooth and circular colonies were present that were 1–2 mm in diameter. The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain SSW1-48\(^T\) is AM396260.
Physiological and biochemical properties are given in the species description.

Genomic DNA was obtained by using a Wizard genomic DNA-purification kit (Promega). PCR amplification and sequencing of the 16S rRNA gene of strain SSW1-48T were performed as described previously (Lee et al., 2000; Lee & Jeong, 2006). Phylogenetic analyses were carried out using three tree-making algorithms, namely neighbour-joining (Saitou & Nei, 1987), maximum-likelihood (Felsenstein, 1981) and maximum-parsimony (Fitch, 1971), with a multiple alignment matrix (Thompson et al., 1997) based on 16S rRNA gene sequences. Evolutionary distances were calculated using the model of Jukes & Cantor (1969). The resultant tree topologies were evaluated by means of bootstrap analysis (Felsenstein, 1985) based on 1000 replicated datasets.

The almost-complete 16S rRNA gene sequence (1415 nt) of strain SSW1-48T was compared with those of related members of the family Microbacteriaceae. A neighbour-joining tree (Fig. 1) based on 16S rRNA gene sequences showed that the organism formed a distinct branch between A. lahaulensis and A. casei in the family Microbacteriaceae. The values for sequence similarity between this bacterium and type strains of recognized Agrococcus species were in the range 95.5–97.5 %. This relationship had a high level of bootstrap support (86 %) and was confirmed by the maximum-likelihood and maximum-parsimony data. The levels of 16S rRNA gene sequence similarity between strain SSW1-48T and other representatives of the family Microbacteriaceae were between 93.7 and 96.4 %.

For chemotaxonomic analyses, cells of strain SSW1-48T were grown in trypticase soy broth (Difco) for 3 days at 30 °C and harvested by centrifugation at 3000 r.p.m. for 20 min. The preparation and analysis of purified cell walls were performed as described previously (Lee, 2007). The molar ratios of the amino acids in purified peptidoglycan were determined using reversed-phase HPLC (2690; Waters) of fluorescent derivatives of the amino acids according to the manufacturer’s instructions. The acyl type of the murein and the presence of mycolic acids were determined as described by Uchida & Aida (1984) and Minnikin et al. (1980), respectively. The polar lipid profile was determined by using the method of Minnikin et al. (1977). The menaquinone composition was analysed using HPLC (Kroppenstedt, 1985). For analysis of the fatty acid

Fig. 1. Neighbour-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the position of strain SSW1-48T within the variation encompassing members of the family Microbacteriaceae. The tree was constructed from evolutionary distances calculated using the coefficient of Jukes & Cantor (1969). Asterisks indicate the branches of the trees that were also recovered using both the maximum-likelihood (Felsenstein, 1981) and maximum-parsimony (Fitch, 1971) tree-making algorithms. Micrococcus luteus DSM 20030T was used as an outgroup. Numbers at nodes indicate percentages of bootstrap support (>50 %), based on 1000 replicates. Bar, 0.01 substitutions per nucleotide position.
methyl esters, cells of strain SSW1-48\textsuperscript{T} were grown on TSA for 3 days at 28 °C. The fatty acid methyl esters were prepared and analysed according to the instructions of the Sherlock Microbial Identification System (version 6.0; MIDI). The G+C content of the DNA was determined using HPLC (Mesbah \textit{et al.}, 1989) with genomic DNA extracted according to the method of Hopwood \textit{et al.} (1985).

The diagnostic diaminoc acid in the cell-wall peptidoglycan was 2,4-diaminobutyric acid; alanine, glycine, glutamic acid, threonine and aspartic acid were also detected. Strain SSW1-48\textsuperscript{T} possessed MK-10 and MK-9 as the major menaquinones and can also be distinguished on the basis of some physiological properties (Table 1).

On the basis of combined data from the phenotypic and phylogenetic analyses, strain SSW1-48\textsuperscript{T} represents a novel species of the genus \textit{Agrococcus}, for which the name \textit{Agrococcus jejuensis} sp. nov. is proposed.

\textbf{Description of \textit{Agrococcus jejuensis} sp. nov.}

\textit{Agrococcus jejuensis} (je.ju.en’sis. N.L. masc. adj. \textit{jejuensis} of Jeju, Republic of Korea, where the type strain was isolated).

Cells are aerobic, Gram-positive, non-endospore-forming, oxidase-negative, catalase-positive, non-motile rods 1.3–1.5 μm in length and 0.3–0.4 μm wide. Colonies are circular, smooth, translucent, yellow-coloured and 1–2 mm in diameter. Growth occurs at 10–37 °C, with an optimum at 30 °C. No growth occurs at 4 or 42 °C. pH for growth is in the range 6.1–12.1. Growth occurs in the presence of 0–3 % NaCl, but not at 4 %. Positive for gelatin hydrolysis, but negative for indole production and glucose fermentation. Nitrate is not reduced to nitrite. Positive for β-galactosidase, but negative for arginine dihydrolase and urease. Assimilates D-glucose, malate, maltose, D-mannitol, D-mannose and N-acetyl-D-glucosamine, but not adipate.

\textbf{Table 1. Differential characteristics for strain SSW1-48\textsuperscript{T} (\textit{A. jejuensis} sp. nov.) and its phylogenetic neighbours in the family Microbacteriaceae}

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell morphology</td>
<td>Short rods</td>
<td>Irregular cocoid to rods</td>
<td>Irregular ovoid to short rods</td>
<td>Irregular spherical, ovoid or rods</td>
<td>Irregular spherical or ovoid to short rods</td>
<td>Irregular spherical rods</td>
</tr>
<tr>
<td>Cell size (μm)</td>
<td>0.3–0.4×1.3–1.5</td>
<td>0.7–1.0×1.1–1.7</td>
<td>0.3–0.4×0.8–1.0</td>
<td>0.7–1.0×1.1–1.7</td>
<td>0.7–1.0×0.7–1.7</td>
<td>0.6–1.0×1.0–1.5</td>
</tr>
<tr>
<td>Colony pigmentation</td>
<td>Yellow</td>
<td>Light yellow</td>
<td>Cream</td>
<td>Yellow</td>
<td>White and orange</td>
<td>Lemon</td>
</tr>
<tr>
<td>Optimal temperature (°C)</td>
<td>30</td>
<td>28</td>
<td>30</td>
<td>28</td>
<td>28</td>
<td>30</td>
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<tr>
<td>Aesculin hydrolysis</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Degradation of Tween 80</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>ND</td>
</tr>
<tr>
<td>Acid production from:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cellobiose</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Fructose</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Glucose</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>ND</td>
</tr>
<tr>
<td>Maltose</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Mannitol</td>
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<td>Mannose</td>
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<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Sucrose</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Trehalose</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Predominant menaquinones</td>
<td>MK-10, MK-9</td>
<td>MK-11, MK-12</td>
<td>MK-11, MK-12</td>
<td>MK-11, MK-12</td>
<td>MK-11, MK-12</td>
<td>MK-10, MK-11, MK-12</td>
</tr>
<tr>
<td>DNA G+C content (mol%)</td>
<td>73.0</td>
<td>73.8–74.9</td>
<td>65.0</td>
<td>74.0</td>
<td>74.0</td>
<td>74.1</td>
</tr>
<tr>
<td>Origin of isolation</td>
<td>Dried seaweed</td>
<td>Air</td>
<td>Cheese</td>
<td>Wall painting</td>
<td>Soil</td>
<td>Ice glacier</td>
</tr>
</tbody>
</table>

Strains: 1, SSW1-48\textsuperscript{T}; 2, \textit{A. baldri} V-108\textsuperscript{2}; 3, \textit{A. casei} LMG 22410\textsuperscript{T}; 4, \textit{A. citreus} DSM 12453\textsuperscript{T}; 5, \textit{A. jenensis} DSM 9580\textsuperscript{T}; 6, \textit{A. lahaulensis} K22-21\textsuperscript{T}. Data were taken from Bora \textit{et al.} (2007), Groth \textit{et al.} (1996), Mayilraj \textit{et al.} (2006), Wieser \textit{et al.} (1999), Zlamala \textit{et al.} (2002) and this study. +, Positive; −, negative; ND, not determined.
D-arabinose, caprate, citrate, gluconate or phenyl acetate (API 20NE). Hydrolyses casein and elastin, but not starch. Does not degrade hypoxanthine, DL-tyrosine or xanthine. Acid is produced from melibiose and salicin. Acid is not produced from adonitol, D-arabinose, L-arabinose, 2,3-butanediol, dulcitol, meso-erythritol, D-galactose, glycerol, myo-inositol, inulin, D-lactose, melezitose, methyl α-D-glucoside, methyl α-D-mannoside, 1,2-propanediol, D-raffinose, L-rhamnose, D-sorbitol, L-sorbosone, D-xylitol or D-xylene. Acid production from L-ribose is weak. Positive for alkaline phosphatase, leucine arylamidase, α-glucosidase, esterase (C4) (weak) and esterase lipase (C8) (weak), but negative for lipase (C14), valine arylamidase, cysteine arylamidase, trypsin, z-chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-galactosidase, β-glucuronidase, β-glucosidase, N-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase (API ZYM). The diagnostic cell-wall diamino acid is 2,4-diaminobutyric acid; alanine, glycine, glutamic acid, threonine and aspartic acid are also detected. The acyl type of the muramic acid is acetyl. Mycolic acids are not present. Predominant menaquinones are MK-10 and MK-9. Major fatty acids are anteiso-C15:0 (56.7%) and iso-C16:0 (13.2%). Phospholipids are diphasphatidylglycerol and phosphatidylglycerol. The DNA G+C content of the type strain is 73.0 mol%.

The type strain, SSW1-48T (=KCTC 19198T =JCM 14256T), was isolated from a dried seaweed sample collected on Samyang Beach, Jeju, Republic of Korea.

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References


