**Jannaschia donghaensis sp. nov., isolated from seawater of the East Sea, Korea**

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A Gram-negative, non-motile and rod-, oval- or coccoid-shaped strain, DSW-17\(^T\), was isolated from seawater of the East Sea, Korea, and subjected to a polyphasic taxonomic study. Strain DSW-17\(^T\) grew optimally at pH 7.0–8.0 and 25 °C. It contained Q-10 as the predominant ubiquinone and C\(_{18:1}\)ω7c as the major fatty acid. Major polar lipids were phosphatidylcholine, phosphatidylethanolamine and an unidentified glycolipid. The DNA G+C content was 65.2 mol%. Phylogenetic analysis based on 16S rRNA gene sequences showed that strain DSW-17\(^T\) was phylogenetically most closely affiliated to the genus *Jannaschia*. Strain DSW-17\(^T\) exhibited 16S rRNA gene sequence similarity values of 96.5% with the type strains of three recognized species of the genus *Jannaschia*. DNA–DNA relatedness data and differential phenotypic properties, together with the phylogenetic distinctiveness, demonstrated that strain DSW-17\(^T\) is distinguishable from the recognized species of the genus *Jannaschia*. On the basis of phenotypic, phylogenetic and genetic data, strain DSW-17\(^T\) was classified in the genus *Jannaschia* as a member of a novel species, for which the name *Jannaschia donghaensis* sp. nov. is proposed. The type strain is DSW-17\(^T\) (=KCTC 12862\(^T\)=JCM 14563\(^T\)).

KCCM 42114\(^T\) was obtained from the Korean Culture Center of Microorganisms (KCCM), Seoul, Korea. The morphological, physiological and biochemical characteristics of strain DSW-17\(^T\) were investigated using routine cultivation on MA at 25 °C.

Cell morphology was examined by light microscopy (E600; Nikon) and transmission electron microscopy (TEM). Flagellation was determined by TEM (CM-20; Philips) with cells from exponentially growing cultures. 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 nitrate, both of which had been prepared anaerobically using nitrogen. Growth in the absence of NaCl was investigated using trypticase soy broth prepared according to the formula of the Difco medium except that NaCl was excluded. Growth at various NaCl concentrations was investigated in marine broth 2216 (MB; Difco) or trypticase soy broth (Difco). Growth at various temperatures (4, 10, 15, 20, 22, 25, 28, 30, 31, 32, 33, 34, 35 and 40 °C) was measured on MA. Growth on trypticase soy agar (TSA; Difco), nutrient agar (NA; Difco) and MacConkey agar (Difco) was tested at 25 °C. Catalase and oxidase activities and hydrolysis of casein, starch and Tween 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

**Abbreviation:** TEM, transmission electron microscopy.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain DSW-17\(^T\) is EF202612.
concentrations described by Cowan & Steel (1965). Hydrolysis of aesculin, gelatin and urea and nitrate reduction were investigated as described previously (Lanyi, 1987) with the modification that artificial seawater was used for the preparation of media. The artificial seawater contained (l⁻¹ distilled water) 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 previously (Bruns et al., 2001).

Susceptibility to antibiotics was investigated on MA plates by using discs containing the following concentrations of antibiotic: 100 U polymyxin B, 50 μg streptomycin, 20 U penicillin G, 100 μg chloramphenicol, 10 μg ampicillin, 30 μg cephalothin, 30 μg gentamicin, 5 μg novobiocin, 30 μg kanamycin, 30 μg neomycin, 15 μg oleandomycin, 100 μg carbenicillin and 30 μg tetracycline. Acid production from carbohydrates was tested as described by Leifson (1963) with the modification that artificial seawater was used. Utilization of various substrates for growth was determined as described by Baumann & Baumann (1981), using supplementation with 2% (v/v) Hutner’s mineral salts solution (Cohen-Bazire et al., 1957) and 1% (v/v) vitamin solution (Staley, 1968), and by Yurkov et al. (1994). Other physiological and biochemical tests were performed with the API 20E and API ZYM systems (bioMérieux). For in vivo pigment-absorption spectrum analysis, strain DSW-18T was cultivated aerobically in the dark at 25 °C in MB. The culture was washed twice by centrifugation using a MOPS buffer (MOPS, 0.01 M NaOH, 0.1 M KCl, 0.001 M MgCl₂; PH 7.5) and disrupted by sonication (Sonifier 450; Branson). After removal of cell debris by centrifugation, the absorption spectrum of the supernatant was examined on a spectrophotometer (DU800; Beckman Coulter).

Cell biomass for DNA extraction and for isoprenoid quinone and polar lipid analyses was obtained from cultivation in MB at 25 °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 the contamination of RNA. The 16S rRNA gene was amplified by PCR using two universal primers, 5′-GAGTTTGATCCTGCTCAAG-3′ and 5′-AGAAGGAGGTATCCAGCC-3′, as described previously (Yoon et al., 1998). Sequencing of the amplified 16S rRNA gene and phylogenetic analysis were performed as described by Yoon et al. (2003).

Isoprenoid quinones were analysed according to Komagata & Suzuki (1987) using reversed-phase HPLC. Polar lipids were extracted using the procedures described by Minnikin et al. (1984) and were identified by two-dimensional TLC followed by spraying with appropriate detection reagents (Minnikin et al., 1984; Komagata & Suzuki, 1987). The presence of phosphatidylglycerol was confirmed by spraying with Dragendorff’s reagent (Sigma). For cellular fatty acid analysis, cell mass of strain DSW-17T 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 (Boehringer Mannheim) 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 and 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 were quoted as DNA–DNA relatedness values.

The morphological, cultural, physiological and biochemical characteristics of strain DSW-17T are given in the species description (see below) or are shown in Table 1. Strain DSW-17T did not produce bacteriochlorophyll a aerobically in the dark. The sonicated in vivo cell extracts of strain DSW-17T showed no absorption maximum between 400 and 1000 nm. The almost complete 16S rRNA gene sequence of strain DSW-17T determined in this study comprised 1422 nucleotides, representing approximately 96% of the Escherichia coli 16S rRNA gene sequence. Comparative 16S rRNA gene sequence analysis revealed that strain DSW-17T was most closely related to the genus Jannaschia. In the phylogenetic tree based on the neighbour-joining algorithm, strain DSW-17T joined the clade comprising species of the genus Jannaschia with a bootstrap resampling value of 95.4% (Fig. 1). The same tree topology was also found in the trees based on the maximum-likelihood and maximum-parsimony algorithms (Fig. 1). Strain DSW-17T exhibited 16S rRNA gene sequence similarity values of 96.5% to all of three recognized species of the genus Jannaschia and of less than 95.3% to other species used in the phylogenetic analysis.

The predominant isoprenoid quinone detected in strain DSW-17T was ubiquinone-10 (Q-10) at a peak area ratio of approximately 95%. Major polar lipids found in strain DSW-17T were phosphatidylcholine, phosphatidylglycerol, phosphatidylethanolamine and an unidentified glycolipid. The polar lipid profile of DSW-17T contained large amounts of unsaturated and straight-chain fatty acids; the major component (>10% of total fatty acids) was C₁₈:₁ω7c (Table 2). This fatty acid profile was similar to those of recognized species of the genus Jannaschia, although there were differences in the proportions of some fatty acids, particularly C₁₉:₀ and cyclo C₁₉:₀ω8c. These differences may result from variations in cultivation conditions and...
Table 1. Differential phenotypic characteristics of Jannaschia donghaensis sp. nov. and other species of the genus Jannaschia

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
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<tbody>
<tr>
<td>Cell shape</td>
<td>Pleomorphic</td>
<td>Irregular rods</td>
<td>Rods</td>
<td>Rods</td>
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<tr>
<td>Cell length (µm)</td>
<td>0.6–5.0</td>
<td>1.9–3.2</td>
<td>1.0–2.0</td>
<td>1.1–2.3</td>
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<tr>
<td>Cell diameter (µm)</td>
<td>0.5–2.5</td>
<td>0.7–1.1</td>
<td>0.5</td>
<td>0.7–1.2</td>
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<tr>
<td>Motility</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Colony colour</td>
<td>Strong orange</td>
<td>White</td>
<td>Red</td>
<td>Dark red</td>
</tr>
<tr>
<td>Maximum growth temperature (ºC)</td>
<td>32</td>
<td>30</td>
<td>25</td>
<td>35</td>
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<tr>
<td>Oxidase</td>
<td>+</td>
<td>W</td>
<td>+</td>
<td>–</td>
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<tr>
<td>Nitrate reduction to nitrite</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
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<tr>
<td>Hydrolysis of:</td>
<td></td>
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<tr>
<td>Aesculin</td>
<td>–</td>
<td>–</td>
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<td>+</td>
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<tr>
<td>Gelatin</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
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<tr>
<td>Starch</td>
<td>–</td>
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<td>–</td>
<td>+</td>
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<td>Tween 80</td>
<td>W</td>
<td>–</td>
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<td>–</td>
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<td>Utilization of:</td>
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<tr>
<td>Cellobose</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Citrate</td>
<td>–</td>
<td>+</td>
<td>W</td>
<td>–</td>
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<tr>
<td>D-Fructose</td>
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<td>+</td>
<td>+</td>
<td>–</td>
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<tr>
<td>Galactose</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>ND</td>
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<tr>
<td>D-Glucose</td>
<td>–</td>
<td>+</td>
<td>+</td>
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<td>Malate</td>
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<td>ND</td>
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<td>Mannose</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>–</td>
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<td>Succinate</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>D-Xylose</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>–</td>
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<td>Enzyme activity (by API ZYM)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>a-Glucosidase</td>
<td>–</td>
<td>(–)</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Acid phosphatase</td>
<td>+</td>
<td>(W)</td>
<td>+</td>
<td>–</td>
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<tr>
<td>Esterase lipase (C8)</td>
<td>+</td>
<td>(W)</td>
<td>W</td>
<td>+</td>
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<tr>
<td>Naphthol-AS-BI-phosphohydrolase</td>
<td>–</td>
<td>(–)</td>
<td>+</td>
<td>–</td>
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<tr>
<td>Valine arylamidase</td>
<td>–</td>
<td>(–)</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>DNA G+C (mol%)</td>
<td>65.2</td>
<td>63.0–63.1</td>
<td>64.6</td>
<td>63</td>
</tr>
</tbody>
</table>

Species: 1, J. donghaensis sp. nov.; 2, J. helgolandensis (data from Wagner-Döbler et al., 2003; Macián et al., 2005; Choi et al., 2006); 3, J. rubra (Macián et al., 2005; Choi et al., 2006); 4, J. seosinensis (Choi et al., 2006). +, Positive; –, negative; W, weakly positive; ND, not determined. Data in parentheses are for the type strain. All species are positive for catalase (not determined for J. rubra). All species are negative for Gram-staining and hydrolysis of urea (not determined for J. rubra).

The DNA G+C content of strain DSW-17T was 65.2 mol%.

There were no distinct phenotypic, particularly chemotaxonomic, properties which differentiated strain DSW-17T from members of the genus Jannaschia (Wagner-Döbler et al., 2003; Macián et al., 2005; Choi et al., 2006). Accordingly, it was concluded that strain DSW-17T is a member of the genus Jannaschia. Strain DSW-17T was distinguishable from recognized species of this genus by means of several phenotypic characteristics as listed in Table 1. Mean DNA–DNA relatedness values between strain DSW-17T and the type strains of the three recognized species of the genus Jannaschia were 7–13%, when their DNAs were used individually as labelled DNA probes for cross-hybridization, indicating that strain DSW-17T represents a separate species (Wayne et al., 1987). This phylogenetic distinctiveness, together with the genetic distinctiveness and differential phenotypic properties, are sufficient to designate strain DSW-17T as a representative of the genus Jannaschia (Stackebrandt & Goebel, 1994). Therefore, on the basis of the data presented, strain DSW-17T should be placed in the genus Jannaschia as representing a novel species, for which the name Jannaschia donghaensis sp. nov. is proposed.

Description of Jannaschia donghaensis sp. nov.

Jannaschia donghaensis (dong.ha.en’sis. N.L. fem. adj. donghaensis of Donghae, the Korean name for the East Sea of Korea, from where the organism was isolated).

Cells are Gram-negative and rod-, oval- or coccoid-shaped (0.5–2.5 × 0.6–5.0 µm). Colonies on MA are circular, raised, strong orange in colour and 1.0–1.5 mm in diameter after 7 days incubation at 25 ºC. Growth does not occur on TSA, NA or MacConkey agar. Growth occurs at 4 and 32 ºC, but not at 33 ºC. Optimal pH for growth is
between 7.0 and 8.0; growth occurs at pH 6.0, but not at pH 5.5. Growth occurs in the presence of 7 % (w/v) NaCl, but not in the absence of NaCl or in the presence of more than 8 % (w/v) NaCl. Anaerobic growth does not occur on MA or on MA supplemented with nitrate. Bacteriochlorophyll a is not produced. Hypoxanthine and Tween 20, 40 and 60 are hydrolysed, but casein, L-tyrosine and xanthine are not. H₂S and indole are not produced. Arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase and tryptophan deaminase are absent. In assays with the API ZYM system, alkaline phosphatase, esterase (C4) and leucine arylamidase are present, but lipase (C14), cystine arylamidase, trypsin, z-chymotrypsin, z-galactosidase, y-galactosidase, a-glucosaminidase, a-glucuronidase, N-acetyl-a-glucosaminidase, z-mannosidase and z-fucosidase are absent. L-Arabinose, maltose, sucrose, trehalose, acetate, benzoate, pyruvate, salicin, formate and L-glutamate are not utilized. Acid is not produced from L-xylose, D-mannitol, D-sorbitol or L-rhamnose, maltose, sucrose, trehalose, D-xylose, D-mannitol, D-sorbitol or myo-inositol. Susceptible to ampicillin, carbenicillin, cephalothin, chloramphenicol, gentamicin, kanamycin, neomycin, novobiocin, penicillin G, polymyxin B, streptomycin and tetracycline, but not to lincomycin or oleandomycin. The predominant ubiquinone is Q-10. The major fatty acid (≥10 % of total fatty acids) is C₁₈:1ω7c. Major polar lipids are phosphatidylcholine, phosphatidylglycerol, phosphatidylinositol and an unidentified glycolipid. The DNA G+C content is 65.2 mol% (determined by HPLC). Other phenotypic characteristics are given in Table 1.

The type strain, DSW-17ᵀ (="KCTC 12862ᵀ=JCM 14563ᵀ), was isolated from seawater off Dokdo in the East Sea, Korea.

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References


