Brevibacterium marinum sp. nov., isolated from seawater

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A novel yellow-pigmented actinobacterium was isolated from seawater collected from Hwasun Beach in Jeju, Republic of Korea. A comparative analysis of the 16S rRNA gene sequence indicated that the organism, designated HFW-26^T, was closely related to members of the genus Brevibacterium. As found for other species of the genus Brevibacterium, strain HFW-26^T possessed meso-diaminopimelic acid as the diagnostic cell-wall diamino acid, contained MK-8(H2) as the major menaquinone, contained polar lipids that included diphosphatidylglycerol, phosphatidylglycerol, phosphatidylglycerol and an unknown phospholipid, and had anteiso-C_{15:0} and anteiso-C_{17:0} as the predominant fatty acids. The G+C content of the DNA was 71.4 mol%.

The phylogenetically closest relative was Brevibacterium pictuae DSM 16132^T (99.0 % 16S rRNA gene sequence similarity). However, DNA–DNA hybridization of strain HFW-26^T showed 35.1–43.7 % relatedness with respect to B. pictuae DSM 16132^T. The novel isolate could be clearly distinguished from B. pictuae DSM 16132^T on the basis of some cultural, physiological and biochemical characteristics. A battery of phenotypic and genetic data obtained in this study suggest that strain HFW-26^T represents a novel species of the genus Brevibacterium, for which the name Brevibacterium marinum sp. nov. is proposed. The type strain is HFW-26^T (=JBRI 2001^T = KCTC 19221^T = DSM 18964^T).

The genus Brevibacterium, which was originally proposed by Breed (1953), is classified within the family Brevibacteriaceae, suborder Micrococineae (Stackebrandt et al., 1997). At the time of writing, the following 15 species with validly published names, in addition to the type species Brevibacterium linens, remain within the genus: Brevibacterium antiquum, B. aurantiacum, B. permense (Gavrish et al., 2004), B. avium (Pascual & Collins, 1999), B. casei, epidermidis (Collins et al., 1983), B. cerele (Ivanova et al., 2004), B. iodimum (Collins et al., 1980), B. luteolum (Wauters et al., 2003; Euzéby & Tindall, 2004), B. mebrellneri (McBride et al., 1993), B. otitidis (Pascual et al., 1996), B. paucivorans (Wauters et al., 2001), B. pictuae (Heyman et al., 2004), B. sanguinis (Wauters et al., 2004) and B. samyangense (Lee, 2006). While most of the species were isolated from dairy products, clinical specimens, poultry and soils, a few were recovered from marine environments, such as algae and beach sediment, as reported previously (Ivanova et al., 2004; Lee, 2006).

In the present study, an actinomycete isolated from a seawater sample collected at Hwasun Beach, Jeju, Republic of Korea, was investigated by means of a polyphasic taxonomic approach. For bacterial isolation, aliquots of a seawater sample were directly deposited on starch-casein agar (Küster & Williams, 1964) supplemented with 60 % natural seawater instead of distilled water. A colony was further subcultured on marine agar 2216 (MA; Difco). The pure culture, designated strain HFW-26^T, was maintained at −20 and −80 °C as a glycerol solution that included 20 % (v/v) distilled water and 60 % (v/v) seawater. For phenotypic comparisons and DNA–DNA hybridizations, B. pictuae DSM 16132^T was grown on tryptase soy agar (TSA; Difco) at 28 °C for 5 days.

Cell morphology and motility were observed by using phase-contrast and transmission electron microscopy, with cultures grown for 6, 15, 24, 48 and 72 h in trypticase soy broth (Difco) at 30 °C. Growth was tested on yeast extract-malt extract agar (ISP 2 medium; Shirling & Gottlieb, 1966), TSA and nutrient agar (Difco), with and without supplementation with 60 % (v/v) natural seawater, incubated for 5 days at 28 °C. The growth temperature (4–40 °C) was tested on TSA. The initial pH for growth (determined on MA) was in the range 4.1–12.1. NaCl tolerance for growth was studied using ISP 2 medium as the basal medium. The Gram stain was determined using a Color Gram 2 F kit (bioMérieux). Degradation of casein, cellulose, hypoxanthine, DL-tyrosine and xanthine was tested on MA. Hydrolysis of DNA and starch was examined on DNase test agar (Difco) and starch agar (Difco),
respectively; after incubation for 5 days, the plates were flooded with 1 M HCl and iodine solution, respectively. Catalase and oxidase activities were checked as described previously (Lee, 2006). Other physiological and biochemical properties were determined using API Coryne and API ZYM strips (bioMérieux) according to the manufacturer’s instructions. Strain HFW-26T showed good growth on all media tested; the cells were non-motile rods (0.6–0.9 µm wide and 1.5–2.4 µm long) at exponential phase. In older cultures, the cells fragmented into short rods (0.5–0.6 µm wide and 0.7–1.0 µm long) that occasionally occurred singly or, more often, in pairs or in chains. Transmission electron micrographs of cells of strain HFW-26T are shown in Supplementary Fig. S1 (available with the online version of this paper). The V-form arrangement was also observed when cells were studied using light and phase-contrast microscopy. Colonies were translucent, convex, smooth and circular with entire margins. The colony pigmentation was variable depending on the culture conditions: in the dark, colonies were white; in the light, colonies were bright yellow. Colonies of B. ptaerae DSM 16132T were white in colour, irrespective of the culture conditions. Data for other physiological and biochemical properties of strain HFW-26T are given in the species description and Table 1.

The 16S rRNA gene sequencing studies were performed as described elsewhere (Lee, 2006). An almost-complete 16S rRNA gene sequence (1455 nt) for strain HFW-26T was determined in this study and compared with those of members of the genus Brevibacterium. A phylogenetic tree (Fig. 1) based on 16S rRNA gene sequences indicated that the organism belonged to the genus Brevibacterium; it formed a coherent cluster with B. ptaerae. This relationship was supported by a high bootstrap percentage (91 %) and was also found in the maximum-likelihood and maximum-parsimony trees. The phylogenetic neighbours were B. ptaerae (99.0 % sequence similarity), B. aurantiacum (98.4 %) and B. antiquum (98.0 %). Strain HFW-26T shared 16S rRNA gene sequence similarity values of 94.7–97.6 % with respect to the type strains of the other recognized species of the genus Brevibacterium.

DNA–DNA hybridization was carried out as described by De Ley et al. (1970), as modified by Huß et al. (1983), using a Cary 100 Bio UV/Vis-spectrophotometer equipped with a Peltier-thermostatted 6 × 6 multiecell changer and a temperature controller with an in situ temperature probe (Varian). Genomic DNA was isolated using a French pressure cell (Thermo Spectronic) and was purified by chromatography on hydroxyapatite as described by Cashion et al. (1977). Several species of the genus Brevibacterium have been found to have 99 % 16S rRNA gene sequence similarity with each other, but show DNA–DNA relatedness values that are below the 70 % threshold required for delineating a genospecies (Wauters et al., 2001, 2003; Gavrish et al., 2004). Therefore, DNA–DNA hybridization for strain HFW-26T was performed only with respect to the type strain of the phylogenetically closest species. The levels of DNA–DNA relatedness for strain HFW-26T and B. ptaerae DSM 16132T were between 35.1 and 43.7 % when strain HFW-26T was used as the probe. The DNA G+C content of strain HFW-26T was 71.4 mol%, as determined by HPLC (Mesbah et al., 1989).

Chemotaxonomic characteristics of strain HFW-26T were investigated as described previously (Lee, 2006) to determine the type of diamino acid in the cell wall (Staneck & Roberts, 1974), the mycolic acids (Minnikin et al., 1980), the polar lipids (Minnikin et al., 1977) and the menaquinones (Kroppenstedt, 1985). Cells of strain HFW-26T were grown in trypticase soy broth for 3 days at 30 °C, with shaking at 170 r.p.m. After harvesting, the biomass was washed in distilled water and freeze-dried. The results

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
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</thead>
<tbody>
<tr>
<td>Colony colour</td>
<td>Yellow</td>
<td>White to orange</td>
<td>Orange</td>
<td>White</td>
</tr>
<tr>
<td>Growth at 10 °C</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Growth in the presence of 15 % (w/v) NaCl</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>–*</td>
</tr>
<tr>
<td>Hydrolysis of casein</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
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<tr>
<td>API Coryne tests</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Nitrate reduction</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Pyrazinamidase</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>β-Galactosidase</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>α-Glucosidase</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Urease</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Gelatin hydrolysis</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–*</td>
</tr>
<tr>
<td>Acid production from D-glucose</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>–*</td>
</tr>
<tr>
<td>Origin of isolation</td>
<td>Seawater</td>
<td>Permafrost sediment</td>
<td>Cheese</td>
<td>Mural painting</td>
</tr>
</tbody>
</table>

*Result differs from that reported previously (Heyrman et al., 2004).
of the chemotaxonomic analyses of strain HFW-26T are
given in the species description. All of the chemotaxonomic
characteristics were in accordance with the genus descrip-
tion (Collins et al., 1983). The cellular fatty acid methyl
esters of the novel isolate and B. picturae DSM 16132T were
prepared from cells grown on TSA for 3 days at 30°C and
analysed according to the instructions for the Sherlock
Microbial Identification System (version 6; MIDI). The
cellular fatty acid content of strain HFW-26T conformed to
the characteristic profile for the genus Brevibacterium
and consisted of saturated, anteiso- and iso-methyl branched
acids. The major components were anteiso-C15:0 (41.2 %),
anteiso-C17:0 (30.4 %), and C18:0 (12.0 %). The main
difference between strain HFW-26T and B. picturae
DSM 16132T was in relation to the proportion of C18:0 present
(Table 2).

Although strain HFW-26T shared a high level of 16S rRNA
gene sequence similarity (99.0 %) with the type strain of B.

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**Table 2.** Cellular fatty acid content (%) of strain HFW-26T and its phylogenetically closest relative

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>1</th>
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<tbody>
<tr>
<td>Straight-chain fatty acids</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C12:0</td>
<td>2.9</td>
<td>1.3</td>
</tr>
<tr>
<td>C16:0</td>
<td>5.8</td>
<td>3.4</td>
</tr>
<tr>
<td>C18:0</td>
<td>12.0</td>
<td>3.0</td>
</tr>
<tr>
<td>Branched fatty acids</td>
<td></td>
<td></td>
</tr>
<tr>
<td>iso-C15:0</td>
<td>3.2</td>
<td>5.8</td>
</tr>
<tr>
<td>iso-C16:0</td>
<td>3.0</td>
<td>3.4</td>
</tr>
<tr>
<td>iso-C17:0</td>
<td>1.2</td>
<td>1.4</td>
</tr>
<tr>
<td>anteiso-C15:0</td>
<td>41.2</td>
<td>49.7</td>
</tr>
<tr>
<td>anteiso-C17:0</td>
<td>30.4</td>
<td>29.6</td>
</tr>
</tbody>
</table>
picturae, the relatively low level of DNA–DNA hybridization (35.1–43.7 %) revealed that the novel isolate does not belong to the species B. picturae (according to the 70 % DNA–DNA similarity threshold recommended for the definition of bacterial genospecies; Wayne et al., 1987). Phenotypic features distinguishing strain HFW-26T from its phylogenetic neighbours are given in Table 1. Strain HFW-26T can also be readily differentiated from the other recognized species of the genus Brevibacterium with reference to some physiological and biochemical properties (Wauters et al., 2003; Ivanova et al., 2004; Lee, 2006).

On the basis of the phenotypic and genotypic data presented here, strain HFW-26T represents a novel species of the genus Brevibacterium, for which the name Brevibacterium marinum sp. nov. is proposed.

Description of Brevibacterium marinum sp. nov.

Brevibacterium marinum (ma.ri’num. L. neut. adj. mar- inum of the sea, marine).

Cells are Gram-positive, catalase-positive, oxidase-negative, non-motile, non-spore-forming rods that are long (0.6–0.9 µm wide and 1.5–2.4 µm long) at exponential phase. As the culture ages, cells fragment into short rods (0.5–0.6 µm wide and 0.7–1.0 µm long) that occasionally occur singly and, more often, in pairs or in chains. V-form arrangements are also observed. Colonies are translucent, convex, smooth and circular with entire margins. Colonial pigmentation is variable depending on the culture conditions: in the dark, colonies are white; in the light, colonies are bright yellow. The temperature range for growth is 10–30 °C, with an optimum of 30 °C. No growth is observed at 4 or 37 °C. Growth occurs at initial pH values in the range 5.1–12.1. Growth occurs in the presence of up to 10 % NaCl. Casein and hypoxanthine are degraded, but cellulose, DL-tyrosine and xanthine are not degraded. Hydrolysis of DNA and starch is not observed. In the API Coryne system, the results for nitrate reduction, β-galactosidase, α-glucosidase, urease and catalase are positive. Negative for the following enzyme activities: pyrazinamidase, pyrrolidonyl arylamidase, alkaline phosphatase, β-glucuronidase, N-acetyl-β-glucosaminidase and β-glucosidase (aesculin hydrolysis). Hydrolysis of gelatin is not observed. Acid production from carbohydrates (D-glucose, maltose, D-ribose, sucrose, D-xyllose, D-mannitol, D-lactose and glycogen) is negative. In the additional tests in the API ZYM system, esterase (C4) and esterase lipase (C8) are weakly positive, but alkaline phosphatase, lipase (C14), leucine arylamidase, valine arylamidase, cysteine arylamidase, trypsin, α-chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-galactosidase, β-glucosidase, α-mannosidase and α-fucosidase are negative. meso-Diaminopimelic acid is the diagnostic diamino acid in the cell wall. The polar lipids are diphosphatidylglycerol, phosphatidylglycerol and phosphatidylinositol, with an unknown phospholipid as a minor component. The major menaquinone is MK-8(H2). Mycolic acids are absent. The major cellular fatty acids are anteiso-C₁₅:₀, anteiso-C₁₇:₀ and C₁₈:₀. The DNA G+C content is 71.4 mol%.

The type strain, HFW-26T (=JBRI 2001T=KCTC 19221T=DSM 18964T), was isolated from seawater from Hwasun Beach in Jeju, Republic of Korea.

Acknowledgements

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References


