**Brevibacterium oceani** sp. nov., isolated from deep-sea sediment of the Chagos Trench, Indian Ocean

Bhaskar Bhadra, 1 Chandralata Raghukumar, 2 Pavan Kumar Pindi 1 and Sisinthy Shivaji 1

1Centre for Cellular and Molecular Biology, Uppal Road, Hyderabad 500 007, India
2National Institute of Oceanography, Dona Paula, Goa 403 004, India

Two bacterial strains, designated BBH5 and BBH7 T, were isolated from a deep-sea sediment sample collected from the Chagos Trench of the Indian Ocean (11° 06′ S 72° 31′ E). Based on their 16S rRNA gene sequence similarity (99.9 %), level of DNA–DNA relatedness (93 %) and a number of similar phenotypic characteristics, the two strains are identified as representing the same species. Their phylogenetically nearest neighbours, based on 16S rRNA gene sequence similarity values (97.9–98.4 %), were identified as *Brevibacterium iodinum*, *Brevibacterium epidermidis*, *Brevibacterium linens* and *Brevibacterium permense*. However, strains BBH5 and BBH7 T could be distinguished from the above four species by a number of phenotypic characteristics, and levels of DNA–DNA relatedness between the two new isolates and these *Brevibacterium* species were 35–42 %. Therefore, strains BBH5 and BBH7 T are considered to represent a novel species of the genus *Brevibacterium*, for which the name *Brevibacterium oceani* sp. nov. is proposed. The type strain is BBH7 T (=LMG 23457 T =IAM 15353 T).

The genus *Brevibacterium* was first described by Breed (1953) with *Brevibacterium linens* as type species; the description was emended by Collins et al. (1980). Species of the genus *Brevibacterium* exhibit a rod–coccus cell cycle, are aerobic, possess meso-diaminopimelic acid in the peptidoglycan and have MK-8(H2) as the major respiratory menaquinone, diphosphatidylglycerol, phosphatidylglycerol, dimannosidediacylglycerol and phosphatidylinositol as major polar lipids and anteiso- and iso-branched fatty acids as major cellular fatty acids (Collins et al., 1980; Jones & Keddie, 1986; Heyrman et al., 2004). *Brevibacterium* species have been isolated from diverse habitats such as milk products, clinical specimens, soil, sediment, brown algae, paintings and foot lesions of fowl (Wauters et al., 2004; Lee, 2006; Gavrish et al., 2004; Ivanova et al., 2004; Heyrman et al., 2004; Pascual & Collins, 1999). Here we describe two *Brevibacterium*-like strains, BBH5 and BBH7 T, isolated from a sediment sample collected at a water depth of 5904 m (from a 50–70-cm section of a deep sediment core of 4.6 m, approximately 50 000 years old) from the Chagos Trench in the Indian Ocean (11° 06′ S 72° 31′ E) (Raghukumar et al., 2004).

Deep-sea sediment samples were collected as described by Raghukumar et al. (2004). Approximately 1.0 g of the sediment was suspended in 10 ml 2 % NaCl and vortexed for 1 min and the suspension was then allowed to settle for 2 min. Next, 100 μl of the top aqueous layer was spread on a plate of yeast extract/peptone (YP) agar (per litre distilled water: 5 g yeast extract, 10 g peptone, 30 g NaCl, 15 g agar) and incubated at 15 °C for 15 days. The number of colony-forming units per gram of sediment ranged from 4.4 × 105 to 7.6 × 105. A total of 21 pale-orange-coloured colonies were purified and subjected to total protein analysis via SDS-PAGE (Laemmli, 1970). All 21 isolates showed similar protein banding patterns and were assumed to be representatives of a single group. Two representatives of the group, namely strains BBH5 and BBH7 T, were then selected for detailed polyphasic analysis. *Brevibacterium iodinum* JCM 2591 T, *Brevibacterium epidermidis* JCM 2593 T, *Brevibacterium permense* JCM 1327 T and *Brevibacterium oceani* sp. nov. were used as reference strains.

Nutrient agar (HiMEDIA) was used for growth and maintenance of the strains and for determination of phenotypic, including chemotaxonomic, characteristics. A Leitz Diaplan phase-contrast microscope was used to ascertain cell morphology and motility. To assess growth

---

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of BBH7 T and BBH5 are AM158906 and AM158905, respectively.

An extended phylogenetic tree based on 16S rRNA gene sequences and tables comparing the phenotypic characteristics and cellular fatty acid profiles of strain BBH7 T with those of other members of the genus *Brevibacterium* are available as supplementary material with the online version of this paper.
ata different pH, the pH of the sterile YP medium was adjusted from pH 4.0 to 10.0 by using either 0.1 M HCl or 0.1 M NaOH. Growth was also tested in YP medium containing either 50 mM acetate buffer (pH 5.2) or 50 mM Tris/HCl buffer (pH 6.8). For salt tolerance tests, 1, 3, 6, 8, 10, 12 and 15 % (w/v) NaCl was added to YP medium devoid of NaCl. Results were scored after 72 h incubation at 26 °C. Utilization of various carbon compounds as the sole carbon source was tested at 26 °C for 15 days in minimal medium [per litre distilled water: 1.2 g (NH₄)₂SO₄, 0.5 g MgSO₄·7H₂O, 0.5 g KH₂PO₄, 0.1 g KCl; pH 6.5±0.2] supplemented with 0.5 % (w/v) of the carbon source. Other physiological and biochemical characteristics were determined following the procedures given by Holding & Collee (1971) and Smibert & Krieg (1994). Strains BBH5 and BBH7T exhibited many similar phenotypic characteristics (Table 1) but were not identical. BBH7T was able to assimilate L-xylose and L-tyrosine as sole carbon sources whereas BBH5 was not. The two novel strains differed from B. iodinum JCM 2591T, B. epidermidis JCM 2593T, B. linens JCM 1327T and B. permense JCM 13318T with respect to many phenotypic characteristics (Table 1; see also Supplementary Table S1 available in IJSEM Online).

Menaquinones and polar lipids were identified from freeze-dried cells. Menaquinones were extracted as described by Collins et al. (1977) and were analysed via HPLC (Groth et al., 1997). Polar lipids were analysed by two-dimensional TLC by using pure lipids as standards following the procedure of Minnikin et al. (1975). The absence of mycolic acids was demonstrated with TLC (Minnikin et al., 1980). DNA was isolated according to the procedure of Marmur (1961) and the G+C content was determined from melting point (Tm) curves (Sly et al., 1986) obtained by using a Lambda 2 UV-Vis spectrophotometer (Perkin Elmer) equipped with the Templab 2.0 software package (Perkin Elmer). Cell walls were prepared (Komagata & Suzuki, 1987) from 500 mg (dry wt) cell mass, and amino acids and peptides in the acid hydrolysates were analysed by two-dimensional TLC by using cellulose plates instead of paper chromatography as described by Schleifer & Kandler (1972). The isomer of dianaminopimelic acid was identified according to Staneck & Roberts (1974). Results of the above experiments are reported in the species description below and in Table 1.

Fatty acids were analysed as described by Reddy et al. (2002) from cells grown in trypticase soy broth (M322; HiMEDIA) at 28 °C and harvested in the exponential phase (24 h). Fatty acids of strain BBH7T (anteiso-C₁₅:₀, 56.2 %; iso-C₁₅:₀, 3.1 %; iso-C₁₆:₀, 0.3 %; anteiso-C₁₇:₁₀ 40.4 %) were typical of the genus Brevibacterium, but the proportions differed from those reported for B. iodinum, B.

### Table 1. Phenotypic characteristics of strains BBH5 and BBH7T and other species of the genus Brevibacterium that show more than 97 % 16S rRNA gene sequence similarity

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colony colour</td>
<td>Pale orange</td>
<td>Orange</td>
<td>Yellow-orange</td>
<td>Orange</td>
<td>Whitish grey</td>
<td>Whitish yellow</td>
<td>Greyish white</td>
<td></td>
</tr>
<tr>
<td>Colony texture</td>
<td>Sticky</td>
<td>Greasy</td>
<td>Smooth</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Growth at:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 °C</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>37 °C</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>42 °C</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>W</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>NaCl tolerance (% w/v)</td>
<td>12</td>
<td>12</td>
<td>15</td>
<td>15</td>
<td>18</td>
<td>15</td>
<td>15</td>
<td>10</td>
</tr>
<tr>
<td>Caseinase</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Lipase</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Oxidase</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Pyrazinamide</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>α-Glucosidase</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>±</td>
<td>+</td>
</tr>
<tr>
<td>Nitrate reduction</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>±</td>
<td>-</td>
<td>±</td>
</tr>
<tr>
<td>Acid from phenylacetate</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>NA</td>
<td>+</td>
<td>NA</td>
<td>+</td>
</tr>
<tr>
<td>Utilization of:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D-Arabinose</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>W</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Mannitol</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>NA</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Gluconate</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>DNA G+C content (mol%)</td>
<td>59.8–60.2</td>
<td>63</td>
<td>63.5</td>
<td>62.5</td>
<td>60.1–64.3</td>
<td>66.2–67.2</td>
<td>61.4</td>
<td>69.9</td>
</tr>
</tbody>
</table>
epidermidis, B. linens and B. permense (Supplementary Table S2).

The 16S rRNA genes of strains BBH5 and BBH7 were amplified from the genomic DNA, purified and sequenced according to Reddy et al. (2000). The almost-complete 16S rRNA gene sequences of strains BBH5 and BBH7 were shown to be B. iodinum DSM 2062T, B. epidermidis NCDO 2286T, B. linens DSM 20425T and B. permense VKM Ac-2280T, with 16S rRNA gene sequence similarities of 98.4, 98.0, 97.9 and 97.9 %, respectively. To determine the phylogenetic affiliation of strains BBH5 and BBH7, the 16S rRNA gene sequence of the two strains was aligned with those of recognized species of the genus Brevibacterium by using CLUSTAL W (Thompson et al., 1997) and phylogenetic analysis was carried out as described by Bhadra et al. (2007). In the neighbour-joining phylogenetic tree constructed according to the Kimura two-parameter method (Kimura, 1980), strains BBH5 and BBH7 formed a robust clade with a bootstrap support of 96 % with the cluster comprising the four Brevibacterium type strains mentioned above, B. iodinum DSM 2062T, B. epidermidis NCDO 2286T, B. linens DSM 20425T and B. permense VKM Ac-2280T (Fig. 1; see also Supplementary Fig. S1). DNA–DNA dot-blot hybridizations were performed with the DIG DNA Labelling and Detection kit (Roche Diagnostics) as described by Bhadra et al. (2005). The two novel strains showed 93 % DNA–DNA relatedness to each other. By contrast, strains BBH5 and BBH7 respectively exhibited levels of DNA–DNA relatedness of 40 and 42 % with B. iodinum JCM 2591T, 37 and 35 % with B. epidermidis JCM 2593T, 42 and 41 % with B. linens JCM 1327T and 37 and 38 % with B. permense JCM 13318T. Reverse DNA–DNA hybridizations yielded similar results. Considering 70 % DNA–DNA relatedness as the cut-off point for species delineation (Wayne et al., 1987) and based on the differences observed in the phenotypic characteristics of strains BBH5 and BBH7 compared with those of B. epidermidis, B. linens, B. permense and B. iodinum, we consider that strains BBH5 and BBH7 represent a novel species of the genus Brevibacterium, for which the name Brevibacterium oceani sp. nov. is proposed.

Description of Brevibacterium oceani sp. nov.

Brevibacterium oceani (o.ce.a’ni. L. gen. n. oceani of the ocean).

Cells are rod-shaped (2–3 μm long and 1–1.2 μm wide), Gram-positive and non-motile. On nutrient agar after 4 days at 28 °C, colonies are sticky, pale-orange in colour with entire margins, and approximately 1.5–2 mm in diameter. Growth occurs between 10 and 35 °C, but not at 5 or 37 °C; good growth is observed at 26–28 °C. Growth occurs between pH 5.2 and 9.5, but not at pH 4 or 10; optimum growth is observed at pH 6.8. Positive for catalase, l-lysine decarboxylase and ornithine decarboxylase, but negative for oxidase, indole production, methyl red and Voges–Proskauer tests, phenylalanine deamination, ascorbin hydrolysis, H2S production, citrate utilization and β-galactosidase. Acid is produced from phenylacetate but not from D-glucose, D-galactose, fructose, mannose, rhamnose, D- or L-arabinose, D-xyllose, D-cellobiose, D-melibiose, D- raffinose, maltose, lactose, D-ribose, trehalose, sucrose, D-mannitol, dulcitol, adonitol or inositol. Utilizes malonate, L-proline, L-tyrosine, L-serine, L-arginine, L-asparagine and L-lysine, but not L-arabinose, lactose, adonitol, erythritol, d-glucononate, x-ketoglutarate or L-cystine as the sole carbon source. Resistant to polymixin B (15 μg), chloramphenicol (25 μg) and nalidixic acid (30 μg) but sensitive to kanamycin (30 μg), erythromycin (15 μg), ciprofloxacin (30 μg), roxithromycin (30 μg), streptomycin (25 μg), tetracycline (25 μg) and ampicillin (25 μg). The peptidoglycan (A1γ type) contains meso-diaminopimelic acid. Mycolic acids are absent. Phosphatidylglycerol and diphosphatidylglycerol are the major polar lipids. The major menaquinone is MK-8(H2) and the major fatty acids are anteiso-C15:0 (56.2 %) and anteiso-C17:0 (40.4 %). Other phenotypic and chemotaxonomic characteristics are given in Table 1 and Supplementary Table S1.

The type strain, BBH7T (=LMG 23457T =IAM 15353T), was isolated from a 50–70 cm section of a deep-sea sediment core of 4.6 m length obtained from the Chagos Trench, Indian Ocean, at a water depth of 5904 m. Strain BBH5, isolated from the same source, is a second strain of the species.

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the position of strains BBH5 and BBH7 and closely related species of the genus Brevibacterium. Dermabacter hominis DSM 7083T was used as an outgroup. Bootstrap values (expressed as percentages of 1000 replications) >50 % are given at nodes. Bar, 1 substitution per 100 nucleotides. An extended tree including all recognized Brevibacterium species is presented in Supplementary Fig. S1 available in IJSEM Online.
Acknowledgements

Thanks to the Ministry of Earth Sciences, Government of India, for financial assistance to S.S. through the National Centre for Antarctic and Ocean Research, Goa.

References


