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

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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, dimannosidylglycerol and phosphatidylinositol as major polar lipids and anteiso- and iso-branched fatty acids as major cellular fatty acids (Collins *et al.*, 1980; Jones & Keddle, 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 × 10³ to 7.6 × 10³. 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 linens* JCM 1327^T and *Brevibacterium permense* JCM 13318^T 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.

at 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 BBH7^T exhibited many similar phenotypic characteristics (Table 1) but were not identical. BBH7^T 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 2591^T, *B. epidermidis* JCM 2593^T, *B. linens* JCM 1327^T and *B. permense* JCM 13318^T 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 (*T*_m) 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 diaminopimelic acid was identified according to Stanek & 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 BBH7^T (anteiso-C_{15:0}, 56.2 %; iso-C_{15:0}, 3.1 %; iso-C_{16:0}, 0.3 %; anteiso-C_{17:0}, 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 BBH7^T and other species of the genus *Brevibacterium* that show more than 97 % 16S rRNA gene sequence similarity

Taxa: 1, strains BBH5 and BBH7^T; 2, *B. iodinum* JCM 2591^T; 3, *B. epidermidis* JCM 2593^T; 4, *B. linens* JCM 1327^T (data in columns 1–4 from this study); 5, *B. permense* (data from Gavriš *et al.*, 2004); 6, *B. casei* (Collins *et al.*, 1983); 7, *B. celere* (Wauters *et al.*, 2003; Ivanova *et al.*, 2004); 8, *B. sanguinis* (Wauters *et al.*, 2004). All grow at 35 °C and are gelatinase-positive and urease-negative. +, Positive; w, weakly positive; –, negative; ±, variable; NA, data not available.

Characteristic	1	2	3	4	5	6	7	8
Colony colour	Pale orange	Greyish	Pale yellow	Yellow–orange	Orange	Whitish grey	Whitish yellow	Greyish white
Colony texture	Sticky	Smooth	Smooth	Smooth	NA	Smooth	Smooth	Sticky or smooth
Growth at:								
10 °C	+	–	–	–	+	–	NA	NA
37 °C	–	+	+	+	+	+	+	+
42 °C	–	+	+	w	NA	NA	NA	NA
NaCl tolerance (% w/v)	12	12	15	15	18	15	15	10
Caseinase	+	+	+	+	+	+	–	+
Lipase	–	–	–	–	NA	NA	NA	NA
Oxidase	–	+	–	+	+	–	+	–
Pyrazinamidase	+	+	+	+	+	+	–	+
α-Glucosidase	–	–	–	–	–	±	+	+
Nitrate reduction	–	+	+	+	–	±	–	±
Acid from phenylacetate	+	+	–	+	NA	+	NA	+
Utilization of:								
D-Arabinose	–	–	–	–	w	+	–	+
Mannitol	–	–	+	–	NA	–	–	–
Gluconate	+	–	+	–	+	+	–	+
DNA G+C content (mol%)	59.8–60.2	63	63.5	62.5	60.1–64.3	66.2–67.2	61.4	69.9

epidermidis, *B. linens* and *B. permense* (Supplementary Table S2).

The 16S rRNA genes of strains BBH5 and BBH7^T 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^T (1485 and 1505 bp, respectively), following pairwise alignment, exhibited 99.9% similarity. Following BLAST analysis, the nearest phylogenetic neighbours of strain BBH7^T were shown to be *B. iodinum* DSM 2062^T, *B. epidermidis* NCDO 2286^T, *B. linens* DSM 20425^T and *B. permense* VKM Ac-2280^T, 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^T, 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^T formed a robust clade with a bootstrap support of 96% with the cluster comprising the four *Brevibacterium* type strains mentioned above, *B. iodinum* DSM 2062^T, *B. epidermidis* NCDO 2286^T, *B. linens* DSM 20425^T and *B. permense* VKM Ac-2280^T (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^T

respectively exhibited levels of DNA–DNA relatedness of 40 and 42% with *B. iodinum* JCM 2591^T, 37 and 35% with *B. epidermidis* JCM 2593^T, 42 and 41% with *B. linens* JCM 1327^T and 37 and 38% with *B. permense* JCM 13318^T. 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^T compared with those of *B. epidermidis*, *B. linens*, *B. permense* and *B. iodinum*, we consider that strains BBH5 and BBH7^T 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, lysine decarboxylase and ornithine decarboxylase, but negative for oxidase, indole production, methyl red and Voges–Proskauer tests, phenylalanine deamination, aesculin 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-xylose, 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-glucuronate, α-ketoglutarate or L-cystine as the sole carbon source. Resistant to polymyxin 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-C_{15:0} (56.2%) and anteiso-C_{17:0} (40.4%). Other phenotypic and chemotaxonomic characteristics are given in Table 1 and Supplementary Table S1.

The type strain, BBH7^T (=LMG 23457^T =IAM 15353^T), 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^T and closely related species of the genus *Brevibacterium*. *Dermabacter hominis* DSM 7083^T 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.

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