

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

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GenBank/ EMBL accession numbers for the 16S rRNA gene sequence of *Brevibacterium oceani* sp. nov. BBH7<sup>T</sup> and BBH5 are AM158906 and AM158905 respectively.

## Abstract

Two bacterial strains BBH5 and BBH87<sup>T</sup> were isolated from a deep-sea sediment sample collected from the Chagos Trench of the Indian Ocean (11 ° 06 ' S, 72 ° 31 ' E). Both the strains, based on their 16S rRNA gene sequence similarity (99.9 %), DNA-DNA relatedness (93 %) and a number of similar phenotypic characteristics are identified as strains of the same species. The phylogenetically nearest species of BBH5 and BBH7<sup>T</sup> based on 16S rRNA gene sequence similarity (97.9 – 98.4 %) were identified as *Brevibacterium iodinum*, *Brevibacterium epidermidis*, *Brevibacterium linens* and *Brevibacterium permense*. However, BBH5 and BBH7<sup>T</sup> differ from the above four nearest phylogenetic relatives by a number of phenotypic characteristics and the DNA-DNA similarity between the two isolates and the above was 35 – 42 %. Therefore, it is proposed to classify BBH5 and BBH7<sup>T</sup> as belonging to a new species of *Brevibacterium* for which the name *Brevibacterium oceani* sp. nov. is proposed with BBH7<sup>T</sup> (=LMG23457<sup>T</sup> = IAM15353<sup>T</sup>) as the type strain.

The genus *Brevibacterium* was first described by Breed (1953) with *B. linens* as type species and emended by Collins *et al.* (1980). The species of the genus *Brevibacterium* exhibit rod-coccus cycle, are aerobic, possess meso-diaminopimelic acid in the peptidoglycan, MK-8(H<sub>2</sub>) is the major respiratory menaquinone, diphosphatidylglycerol, phosphatidylglycerol, dimannosidediacylglycerol and phosphatidylinositol are the major polar lipids and possess anteiso- and iso- branched fatty acids as the major cellular fatty acids (Collins *et al.*, 1980; Jones & Keddie, 1986; Heyrman *et al.*, 2004). Species belonging to this genus have been isolated from diverse habitats such as from milk products, clinical specimens, soil, sediment, brown algae, paintings and from the foot lesion of fowl (Wauters *et al.*, 2004; Lee, 2006; Gavrish *et al.*, 2004; Ivanova *et al.*, 2004; Heyrman *et al.*, 2004; Pascual & Collins, 1999). In the present paper a novel species of the genus *Brevibacterium*, *Brevibacterium oceani* sp. nov. is proposed, which was isolated from a sediment sample collected at a 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 (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 of 2 % NaCl, vortexed for 1 minute, and the suspension was allowed to settle for 2 minutes. Then, 100 µl of the top aqueous layer was spread on a plate of yeast extract-peptone (YP) agar [(g l<sup>-1</sup>): yeast extract, 5; peptone, 10; NaCl, 30; agar, 15] plates and incubated at 15 °C for 15 days. The CFU g<sup>-1</sup> of sediment ranged from 4.4 X 10<sup>3</sup> to 7.6 X 10<sup>3</sup>. A total of 21 pale-orange colored colonies were purified and subjected to total protein analysis by SDS-PAGE (Laemmli, 1970). All the 21 isolates showed similar protein banding patterns and were assumed as representatives of a single group. Two representatives of the group, namely BBH5 and BBH7<sup>T</sup> were then selected for detailed polyphasic analysis. *Brevibacterium iodinum* JCM 2591<sup>T</sup>, *Brevibacterium epidermidis* JCM 2593<sup>T</sup>,

*Brevibacterium linens* JCM 1327<sup>T</sup> and *Brevibacterium permense* JCM 13318<sup>T</sup> were used as reference strains for the study.

Nutrient agar (HiMEDIA) was used for growth and maintenance of the strains and for determination of phenotypic, including chemotaxonomic characteristics. Leitz Diaplan phase contrast microscope was used to ascertain the morphology and motility of the strains. To assess the growth at different pH, the pH of the sterile YP medium was adjusted from pH 4.0 to pH 10.0 using either 0.1 M HCl or NaOH. Growth was also tested in YP medium containing either 50 mM acetate buffer (pH 5.2) or Tris-Cl buffer (pH 6.8). For salt tolerance test, 1, 3, 6, 8, 10, 12 and 15 % (w/v) NaCl had been 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 [(g l<sup>-1</sup>): (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 1.2; MgSO<sub>4</sub>.7H<sub>2</sub>O, 0.5; KH<sub>2</sub>PO<sub>4</sub>, 0.5; KCl, 0.1; pH 6.5 ± 0.2] supplemented with 0.5 % (w/v) of the carbon source. Other physiological and biochemical characteristics were determined following Holding & Collee (1971) and Smibert & Krieg (1994). It was found that BBH5 and BBH7<sup>T</sup> exhibit many similar phenotypic characteristics (Table 1) but are not identical. BBH7<sup>T</sup> assimilates L-xylose and L-tyrosine as sole carbon source but BBH5 does not. Both the strains, BBH7<sup>T</sup> and BBH5, differed from *B. iodinum* JCM 2591<sup>T</sup>, *B. epidermidis* JCM 2593<sup>T</sup>, *B. linens* JCM 1327<sup>T</sup> and *B. permense* JCM 13318<sup>T</sup> with respect to many phenotypic characteristics (Table 1 and Supplementary Table S1; available in IJSEM online).

Menaquinones and polar lipids were determined in freeze-dried cells. Menaquinones were extracted as described by Collins *et al.* (1977) and were analyzed by HPLC (Groth *et al.*, 1997). Polar lipids were analyzed by two-dimensional TLC using pure lipids as standards following the procedure of Minnikin *et al.* (1975). The absence of mycolic acids was demonstrated using TLC (Minnikin *et al.*, 1980). DNA was isolated according to the procedure of Marmur (1961) and G+C content (mol %) was determined

from the melting point ( $T_m$ ) curves (Sly *et al.*, 1986) obtained using a Lambda 2 UV-vis spectrophotometer (Perkin Elmer) equipped with Templab 2.0 software package (Perkin Elmer). Cells walls were prepared (Komagata & Suzuki, 1987) from 500 mg (dry wt) bacterial cells, and amino acids and peptides in the acid hydrolysates were analysed by two dimensional TLC 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 and in Table 1.

Fatty acids were analyzed as described previously (Reddy *et al.*, 2002) in cells grown in tryptic soy broth (M322 HiMEDIA) at 28 °C and harvested in the log phase (24 h). Fatty acids of strain BBH7<sup>T</sup> (anteiso-C<sub>15:0</sub> – 56.2 %, iso-C<sub>15:0</sub> – 3.1 %, iso-C<sub>16:0</sub> – 0.3 % and anteiso-C<sub>17:0</sub> – 40.4 %) were typical of the genus *Brevibacterium* and differed from *B. iodinum*, *B. epidermidis*, *B. linens* and *B. permense* (Supplementary Table S2; available in IJSEM Online).

The 16S rRNA genes of BBH5 and BBH7<sup>T</sup> were amplified from the genomic DNA, purified and sequenced according to Reddy *et al.* (2000). The almost-complete 16S rRNA gene sequence of strains BBH5 and BBH7<sup>T</sup> (1485 bp and 1505 bp respectively) following pair-wise alignment exhibited 99.9 % similarity at the rRNA gene level. Further, following BLAST analysis of the 16S rRNA gene sequence of BBH7<sup>T</sup> it is observed that *Brevibacterium iodinum* DSM 2062<sup>T</sup> (X83813), *B. epidermidis* NCDO 2286<sup>T</sup> (X76565), *B. linens* DSM 20425<sup>T</sup> (X77451) and *B. permense* VKM Ac-2280<sup>T</sup> (AY243343) are the nearest phylogenetic neighbors of BBH7<sup>T</sup> with a similarity of 98.4 %, 98 %, 97.9 % and 97.9 %, respectively. To determine the phylogenetic affiliation of BBH5 and BBH7<sup>T</sup> the 16S rRNA gene sequence of the two strains was aligned with all the species of the genus *Brevibacterium* using CLUSTAL W (Thompson *et al.*, 1997) and phylogenetic analysis was carried out as described previously (Bhadra *et al.*, 2007). In the Neighbour-Joining

phylogenetic tree constructed according to Kimura two-parameter (Kimura, 1980), BBH5 and BBH7<sup>T</sup> formed a robust clade with a bootstrap support of 96% with the cluster consisting of 4 species of *Brevibacterium* namely *B. permense* VKM Ac-2280<sup>T</sup> (AY243343), *B. epidermidis* NCDO 2286<sup>T</sup> (X76565), *B. iodinum* DSM 20626<sup>T</sup> (X83813) and *B. linens* DSM 20425<sup>T</sup> (X77451) (Fig. 1 and Supplementary Fig. S1). The DNA-DNA dot-blot hybridization was performed using 'DIG DNA Labeling and Detection kit' (Roche Diagnostics) as described by Bhadra *et al.* (2005). Strains BBH5 and BBH7<sup>T</sup> showed 93 % DNA-DNA relatedness with each other. But, the two strains BBH5 and BBH7<sup>T</sup> exhibited 40 and 42 % DNA-DNA relatedness with *B. iodinum*, 37 and 35 % with *B. epidermidis*, 42 and 41 % with *B. linens* and 37 and 38 % with *B. permense*. Reverse DNA-DNA hybridizations also 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 BBH5 and BBH7<sup>T</sup> with *Brevibacterium epidermidis*, *Brevibacterium linens*, *Brevibacterium permense* and *Brevibacterium iodinum* it is proposed to assign BBH5 and BBH7<sup>T</sup> as a new species of the genus *Brevibacterium*, for which the name *Brevibacterium oceani* sp. nov. is proposed. The type strain of *Brevibacterium oceani* sp. nov. is BBH7<sup>T</sup> (=LMG23457<sup>T</sup> =IAM15353<sup>T</sup>).

## Description of *Brevibacterium oceani* sp. nov.

*Brevibacterium oceani* (o.ce.a'ni. L. gen. n. oceani, of an ocean).

Cells are rod shaped (2 – 3  $\mu\text{m}$  long and 1 – 1.2  $\mu\text{m}$  wide), Gram-positive and non-motile. On nutrient agar after 4 days at 28 °C colonies are sticky, pale-orange in color with entire margin, and approximately 1.5 – 2 mm in diameter. Growth occurs between 10 – 35 °C, but not at 5 °C and 37 °C; good growth is observed at 26 °C to 28 °C. Growth occurs between pH 5.2 – pH 9.5, but not at pH 4 or pH 10 and optimum growth was observed about pH 6.8. Strain BBH7<sup>T</sup> is positive for catalase, lysine decarboxylase and ornithine decarboxylase; but negative for oxidase, indole production, methyl red and Voges-Proskauer tests, phenylalanine deamination, esculin hydrolysis, H<sub>2</sub>S production, citrate utilization and  $\beta$ -galactosidase. Acid is produced from phenylacetate; but not from D-glucose, D-galactose, fructose, mannose, rhamnose, D-arabinose, L-arabinose, D-xylose, D-cellobiose, D-melibiose, D-raffinose, maltose, lactose, D-ribose, trehalose, sucrose, D-mannitol, dulcitol, adonitol and 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,  $\alpha$ -keto-glutarate and L-cystine as the sole carbon source. Resistant to polymixin B (15  $\mu\text{g}$ ), chloramphenicol (25  $\mu\text{g}$ ) and nalidixic acid (30  $\mu\text{g}$ ) but sensitive to kanamycin (30  $\mu\text{g}$ ), erythromycin (15  $\mu\text{g}$ ), ciprofloxacin (30  $\mu\text{g}$ ), roxithromycin (30  $\mu\text{g}$ ), streptomycin (25  $\mu\text{g}$ ), tetracycline (25  $\mu\text{g}$ ) and ampicillin (25  $\mu\text{g}$ ). The peptidoglycan (A1 $\gamma$  type) contains *meso*-diaminopimelic acid. Mycolic acids are absent. Phosphatidylglycerol and diphosphatidylglycerol are the major polar lipids. The major menaquinone is MK-8(H<sub>2</sub>) and the major fatty acids are anteiso-C<sub>15:0</sub> (56.2 %) and anteiso-C<sub>17:0</sub> (40.4 %). Other phenotypic and chemotaxonomic characteristics are as in Table 1 and Supplementary Table S1.

The type strain BBH7<sup>T</sup> (=LMG23457<sup>T</sup> =IAM15353<sup>T</sup>) was isolated from a 50270 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.

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**Table 1. Phenotypic characteristics of *Brevibacterium oceani* sp. nov. BBH5 and BBH7<sup>T</sup> and other closely related species of *Brevibacterium* which show more than 97 % similarity at the 16S rRNA gene sequence level**

The eight species grow at 35 °C and are gelatinase positive and urease negative.

+, Positive; W, weak positive; -, negative; ±, variable; NA, data not available.

<sup>1-4</sup>, Data from Gavrish *et al.* (2004)<sup>1</sup>, Collins *et al.* (1983)<sup>2</sup>, Wauters *et al.* (2003)<sup>3</sup>, Ivanova *et al.* (2004)<sup>4</sup> and Wauters *et al.* (2004)<sup>5</sup>.

Characteristics	<i>B. oceani</i> sp. nov. BBH5 and BBH7 <sup>1</sup>	<i>B. iodinum</i>	<i>B. epidermidis</i>	<i>B. linens</i>	<i>B. permense</i> <sup>1</sup>	<i>B. casei</i> <sup>2</sup>	<i>B. celere</i> <sup>3,4</sup>	<i>B. sanguinis</i> <sup>5</sup>
Colony color	Pale orange	Grayish	Pale yellow	Yellow-orange	Orange	Whitish gray	Whitish yellow	Grayish white
Colony texture	Sticky	Smooth	Smooth	Smooth	NA	Smooth	Smooth	Sticky or smooth
<b>Growth at:</b>								
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	+
<b>Utilization of :</b>								
D-Arabinose	-	-	-	-	W	+	-	+
Mannitol	-	-	+	-	NA	-	-	-
Gluconate	+	-	+	-	+	+	-	+
Mol % G + C content of DNA	59.8 – 60.2	63	63.5	62.5	60.1 – 64.3	66.2 – 67.2	61.4	69.9

**Supplementary Table S1.** Comparison of the phenotypic characteristics of *Brevibacterium oceanii* sp. nov. BBH7<sup>T</sup>, *Brevibacterium epidermidis* JCM2593<sup>T</sup>, *Brevibacterium linens* JCM 1327<sup>T</sup> and *Brevibacterium iodinum* JCM 2591<sup>T</sup>. +, Positive; -, negative; W, weak positive; R, resistant; S, sensitive.

Phenotypic characteristics	<i>Brevibacterium oceanii</i> sp. nov. BBH7 <sup>T</sup>	<i>Brevibacterium iodinum</i> JCM 2591 <sup>T</sup>	<i>Brevibacterium epidermidis</i> JCM2593 <sup>T</sup>	<i>Brevibacterium linens</i> JCM 1327 <sup>T</sup>
<b>Carbon source utilization</b>				
D-Glucose	+	+	+	-
D-Galactose	+	-	+	-
D-Fructose	+	-	+	-
Maltose	+	-	+	+
L-Rhamnose	+	+	+	-
D-Xylose	+	-	-	-
L-Xylose	+	W	+	W
D-Ribose	+	+	+	-
D-Raffinose	+	+	W	-
D-Cellobiose	+	-	+	-
Sucrose	-	+	+	-
Melezitose	+	-	-	-
Inositol	+	-	+	-
Citrate	-	+	+	+
Acetate	+	+	+	-
L-Alanine	+	-	+	-
L-Aspartic acid	+	-	-	-
L-Isoleucine	+	-	+	-
Creatinine	+	+	+	-
L-Proline	+	-	+	-
L-Tyrosine	+	-	+	-
Chloramphenicol (25 µg)	R	S	R	S
Tetracycline (25 µg)	S	R	R	S

**Supplementary Table S2\***. Comparison of the cellular fatty acid profiles of *Brevibacterium oceanii* sp. nov. BBH7<sup>T</sup>, *Brevibacterium epidermidis* JCM2593<sup>T</sup>, *Brevibacterium linens* JCM 1327<sup>T</sup>, *Brevibacterium iodinum* JCM 2591<sup>T</sup> and *Brevibacterium permense* JCM13318<sup>T</sup>, *Brevibacterium casei* DSM 20657<sup>T</sup> and *Brevibacterium celere* KMM 3637<sup>T</sup>

\*Data in columns 1 to 5 were generated in the present study. In columns 6 and 7 data were taken from Heyrman *et al.* (2004) and Inanova *et al.* (2004) respectively.

<b>Cellular fatty acid</b>	<b><i>Brevibacterium oceanii</i> sp. nov. BBH7<sup>T</sup></b>	<b><i>Brevibacterium iodinum</i> JCM 2591<sup>T</sup></b>	<b><i>Brevibacterium epidermidis</i> JCM2593<sup>T</sup></b>	<b><i>Brevibacterium linens</i> JCM 1327<sup>T</sup></b>	<b><i>Brevibacterium permense</i> JCM13318<sup>T</sup></b>	<b><i>Brevibacterium Casei</i> DSM 20657<sup>T</sup></b>	<b><i>Brevibacterium celere</i> KMM 3637<sup>T</sup></b>
anteiso-C <sub>15:0</sub>	56.2	68.5	67.2	64.5	62.7	50.0	43.7
iso-C <sub>15:0</sub>	3.1	2.0	4.6	5.5	3.8	7.2	7.6
iso-C <sub>16:0</sub>	0.3	1.3	0.8	0.9	1.7	7.1	6.2
anteiso-C <sub>17:0</sub>	40.4	27.3	27.2	29.6	31.4	35.9	37.8

## Legends to Figures

**Fig. 1.** Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing phylogenetic position of *Brevibacterium oceani* sp. nov. BBH5 (1485 bp) and BBH7<sup>T</sup> (1505 bp) and closely related species of *Brevibacterium*. *Dermabacter hominis* DSM 7083<sup>T</sup> (X91034) was used as an out-group. Bootstrap values (expressed as percentages of 1000 replications) >50 % are given at the nodes. The bar represents 1 substitution per 100 nucleotides. The extended tree with all *Brevibacterium* species is presented in supplementary Fig. S1 available in IJSEM online.

**Supplementary Fig. S1.** Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the phylogenetic relationship between *Brevibacterium oceani* sp. nov. BBH5 and BBH7<sup>T</sup> and all the species of *Brevibacterium*. *Dermabacter hominis* DSM 7083<sup>T</sup> (X91034) was used as an out-group in the tree. Bootstrap values (expressed as percentages of 1000 replications) >50 % are given at nodes. The bar represents 5 substitutions per 1000 nucleotides.



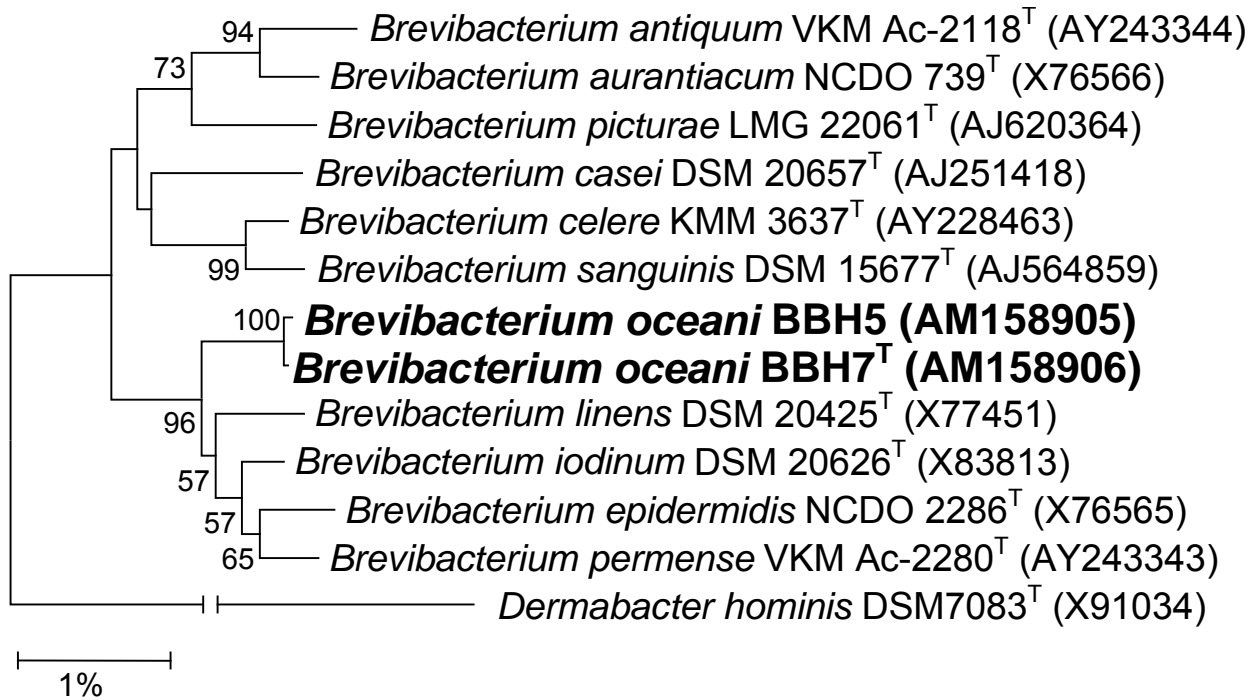
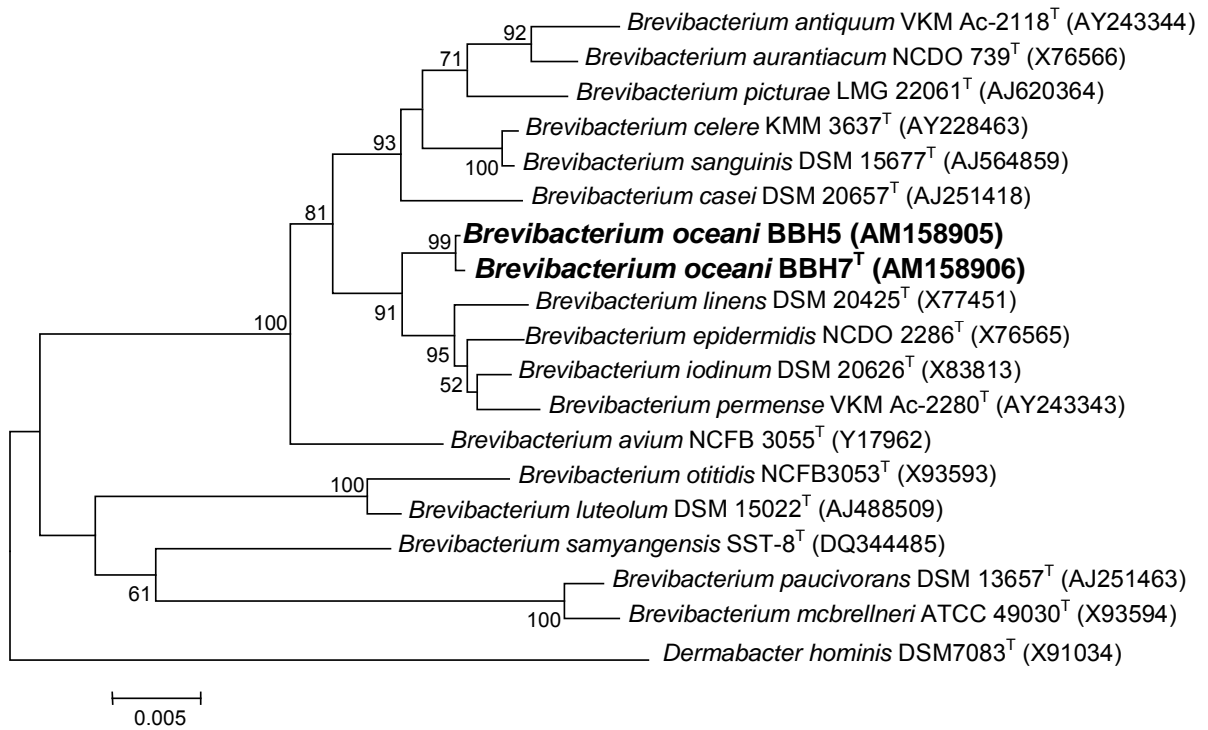


Fig. 1



Supplementary Fig. S1