Gracilibacillus bigeumensis sp. nov., a moderately halophilic bacterium from solar saltern soil

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A Gram-staining-positive, moderately halophilic bacterium, designated strain BH097^T, was isolated from solar saltern soil of Bigeum Island in south-west Korea. Cells were motile rods, producing spherical endospores at a terminal position in swollen sporangia. Strain BH097^T was strictly aerobic, grew at pH 5.5–9.5 (optimum, pH 8.0), at 10–52 °C (optimum, 37 °C) and at salinities of 1–22 % (w/v) NaCl (optimum, 7 % NaCl). On the basis of 16S rRNA gene sequence analysis, strain BH097^T was shown to belong to the genus *Gracilibacillus* within the phylum *Firmicutes*, and showed closest sequence similarity to *Gracilibacillus saliphilus* DSM 19802^T (95.8 %), *Gracilibacillus thailandensis* TP2-8^T (95.6 %), *Gracilibacillus boracitolerans* DSM 17256^T (95.5 %), '*Gracilibacillus quinghaiensis*' DSM 17858 (95.4 %) and *Gracilibacillus halophilus* DSM 17856^T (95.2 %). The DNA G+C content of this novel isolate was 37.9 mol%. The major cellular fatty acids of strain BH097^T were anteiso-C_{15:0}, iso-C_{15:0} and C_{16:0}, and its polar lipid pattern consisted of diphosphatidylglycerol, phosphatidylglycerol two unknown phospholipids and a glycolipid. The isoprenoid quinone was MK-7, and the peptidoglycan type was A1γ, with *meso*-diaminopimelic acid as the diagnostic diamino acid. On the basis of polyphasic evidence from this study, strain BH097^T represents a novel species of the genus *Gracilibacillus* for which the name *Gracilibacillus bigeumensis* sp. nov. is proposed. The type strain is BH097^T (=KCTC 13130^T=DSM 19028^T).

Moderately halophilic bacterial species have been isolated from saline or hypersaline environments such as salterns, salt lakes, soda lakes and seawater (Arahal & Ventosa, 2002; Ventosa, 2006). They are members of the family *Bacillaceae* and have similar properties and close phylogenetic relationships. Among them are members of the genus *Gracilibacillus*, which are Gram-positive, motile, endospore-forming rods or filaments containing menaquinone-7 (MK-7) as the predominant respiratory quinone. The major cellular fatty acid is anteiso-C_{15:0}, the polar lipid pattern consists of diphosphatidylglycerol and phosphatidylglycerol, and the peptidoglycan type is A1γ, with *meso*-diaminopimelic acid (*m*-DAP) as the diagnostic diamino acid. At the time of writing, this genus included ten species: *Gracilibacillus*

halotolerans, the type species of the genus and *Gracilibacillus dipsosauri* (Wainø *et al.*, 1999), *Gracilibacillus orientalis* (Carrasco *et al.*, 2006), *Gracilibacillus boracitolerans* (Ahmed *et al.*, 2007), *Gracilibacillus lacisalsi* (Jeon *et al.*, 2008), *Gracilibacillus halophilus* (Chen *et al.*, 2008a), '*Gracilibacillus quinghaiensis*' (Chen *et al.*, 2008b; name not yet validly published), *Gracilibacillus saliphilus* (Tang *et al.*, 2009), *Gracilibacillus thailandensis* (Chamroonsaksri *et al.*, 2010) and *Gracilibacillus ureilyticus* (Huo *et al.*, 2010). In the course of screening solar saltern soil in Bigeum Island, Korea to isolate halophilic bacteria, a novel endospore-forming, moderately halophilic, Gram-positive bacterium, designated strain BH097^T was isolated.

Strain BH097^T was isolated from solar saltern soil (34° 45' 42.76" N, 125° 58' 48.97" E) from Bigeum Island, which is located in south-west Korea, with an area of 44.14 km². Bigeum Island is the first and leading solar saltern in Korea, and the pH of the soil sediment of the saltern pond varies between 6.5 and 8.5. The major chemical composition of the soil and salt consists of NaCl, KCl and MgCl₂. At the time of sampling, the temperature of the soil was 12 °C and the pH was 7.8. Strain BH097^T was isolated during studies focused

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Abbreviations: MA, marine agar; MB, marine broth; *m*-DAP, *meso*-diaminopimelic acid; MH, Moderately Halophilic bacteria medium.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain BH097^T is EF520006.

Five supplementary figures are available with the online version of this paper.

on the isolation of halophilic bacteria from the solar saltern soil, and constituted a single isolate out of a total of 254 isolates from 25 different genera. It was isolated by diluting a soil sample in sterile 10 % (w/v) NaCl solution, plating on marine agar 2216 (MA) medium (Difco) with the addition of 8 % (w/v) NaCl [final concentration, 9.95 % (w/v) NaCl] followed by aerobic incubation at 32 °C. The pH of this medium was adjusted to 7.2. The strain was subsequently purified three times by plating on the same medium, and was maintained on the same medium. The strain was stored at –80 °C on this medium without agar and supplemented with 20 % (v/v) glycerol. In order to characterize strain BH097^T phenotypically, the isolate was routinely grown aerobically on MA with the addition of 5 % (w/v) NaCl (7 % MA) for 2 days at 37 °C and pH 8.0, except where indicated otherwise.

Cell morphology was examined by light microscopy (LABOPHOT-2; Nikon) using cells from exponentially growing cultures. Gram staining was performed using the bioMérieux Gram Stain kit according to the manufacturer's instruction. The presence of endospores was investigated by using the Schaeffer–Fulton staining method, and motility was analysed by the wet-mount method (Murray *et al.*, 1994). The flagellum type was examined with transmission electron microscopy using cells from the exponential growth phase. Cells were mounted on Formvar-coated copper grids, negatively stained with 1 % (w/v) potassium phosphotungstate (pH 7.0), and were examined in a transmission electron microscope (201; Phillips) operated at 80 kV. Oxidase activity was tested using Bactident Oxidase strips (Merck) and catalase activity was determined by bubble production in 3 % (v/v) hydrogen peroxide solution. Nitrate reduction, hydrolysis of aesculin, casein, DNA, gelatin, hypoxanthine, L-tyrosine, starch, Tween 80, xanthine and urea, Voges–Proskauer tests and production of indole were tested as recommended by Cowan & Steel (1965), Lányi (1987) and Smibert & Krieg (1994).

Acid production from carbohydrates was determined as described by Leifson (1963); all suspension media were supplemented with 7 % (w/v) NaCl. Additional enzymic activities and biochemical characteristics were determined using API 20E, API 20NE and API ZYM strips (bioMérieux) at 37 °C as recommended by the manufacturer. Antibiotic susceptibility was determined according to the conventional Kirby–Bauer method (Bauer *et al.*, 1966), seeding with a bacterial suspension containing 1.5×10^6 c.f.u. ml⁻¹ and using discs (ADVANTEC) impregnated with various antibiotics. Tests were carried out on medium supplemented with 7 % NaCl, the plates were incubated at 37 °C for 48 h and the inhibition zone was interpreted according to the manufacturer's manual. Growth under anaerobic conditions was determined after 7 days incubation at 37 °C on 7 % MA in an anaerobic chamber (5 % H₂, 10 % CO₂, 85 % N₂; Mart Microbiology). To determine the optimal temperature and pH for growth of the strain, broth cultures were incubated at 4, 10, 15, 18, 20, 25, 28, 30, 37, 40, 45, 50, 52, 55, 60 and 65 °C and at pH 4.0–10.5

at intervals of 0.5 pH units. pH values of <6, 6–9 and >9 were obtained by using sodium acetate/acetic acid, Tris/HCl and sodium bicarbonate/sodium carbonate buffers, respectively in 7 % marine broth (MB) media (Gomori, 1955). Growth at different NaCl concentrations (0, 0.5, 1, 2, 3, 4, 5, 7, 8, 10, 11, 12, 13, 15, 20, 22, 25 and 30 %, w/v) was tested on Moderately Halophilic bacteria medium (MH; Carrasco *et al.*, 2006) at pH 8.0. Growth was monitored by turbidity at OD₆₀₀ by using a spectroscopic method (UV-160 A; Shimadzu).

Strain BH097^T was Gram-staining-positive, motile with monotrichous flagella (Fig. S1, available in IJSEM Online), and strictly aerobic. Cells were rods with a width of 0.3–0.5 µm and a length of 2.5–4.0 µm. Spherical endospores were formed at the terminal position in swollen sporangia (Fig. S2). When grown for 2 days at 37 °C on 7 % MA medium, colonies were circular, slightly irregular, smooth, cream and had a diameter of 0.5–1.0 mm. The isolate was moderately halophilic and slightly alkaliphilic, growing in media containing 1–22 % (w/v) NaCl [optimum, 7 % (w/v) NaCl]. No growth was observed in the absence of NaCl or in the presence of more than 22 % (w/v) NaCl. Strain BH097^T grew between pH 5.5 and 9.5 (optimum, pH 8.0). Other phenotypic features are included in the species description.

Genomic DNA from strain BH097^T was prepared by using the method described by Tamaoka & Komagata (1984). The 16S rRNA gene was amplified by PCR with the forward primer Eubac 27F and the reverse primer 1492R (DeLong, 1992). Direct sequence determination of the PCR-amplified DNA was carried out using an automated DNA sequencer (ABI 3730XL; Applied Biosystems). The 16S rRNA gene sequence was aligned with the published sequences of closely related bacteria using CLUSTAL W 2.0 software (Larkin *et al.*, 2007). Gaps at the 5' and 3' ends of the alignment were omitted from further analyses. Phylogenetic trees were constructed by using three different methods: the neighbour-joining (Saitou & Nei, 1987), maximum-likelihood (Felsenstein, 1981) and maximum-parsimony (Kluge & Farris, 1969) algorithms integrated in the PHYLIP software package version 3.6 (Felsenstein, 2002) for phylogenetic inference. Evolutionary distance matrices for the neighbour-joining method were calculated using the algorithm of Kimura's two-parameter model (Kimura, 1980) with the DNADIST program. To evaluate the stability of the phylogenetic tree, a bootstrap analysis (1000 replications) was performed with the SEQBOOT, DNADIST, NEIGHBOR and CONSENSE programs in the PHYLIP package. The 16S rRNA gene sequences used for phylogenetic comparisons were obtained from GenBank and their strain designations and accession numbers are shown in Fig. 1.

The almost-complete 16S rRNA gene sequence (1452 bp) of strain BH097^T was obtained and used for initial GenBank BLAST searches and phylogenetic analysis. The identification of phylogenetic neighbours and calculation of pairwise 16S rRNA gene sequence similarity were achieved by using the

Fig. 1. Neighbour-joining tree based on 16S rRNA gene sequence comparison, showing the relationship of strain BH097^T to species of the genus *Gracilibacillus* and other related taxa. GenBank accession numbers of the sequences used in this study are shown in parentheses. Bootstrap values are shown in percentages of 1000 replicates when >50 %. *Bacillus subtilis* NCIMB 3610^T was used as an outgroup. Bar, 0.01 substitutions per nucleotide position.

EzTaxon server (<http://www.eztaxon.org/>; Chun *et al.*, 2007). Phylogenetic analysis based on the neighbour-joining algorithm revealed that strain BH097^T formed a stable clade

with *G. halophilus* DSM 17856^T (95.2 % similarity) (Fig. 1). 16S rRNA gene sequence similarity with other species of the genus *Gracilibacillus* was less than 96 %. Maximum-likelihood

Table 1. Cellular fatty acid compositions (%) of strain BH097^T and members of the genus *Gracilibacillus*

Strains: 1, BH097^T; 2, *G. halophilus* DSM 17856^T; 3, *G. lacisalsi* DSM 19029^T; 4, *G. saliphilus* DSM 19802^T; 5, *G. thailandensis* TP2-8^T; 6, *G. boracitolerans* DSM 17256^T; 7, '*G. quinghaiensis*' DSM 17858; 8, *G. halotolerans* DSM 11805^T; 9, *G. dipsosauri* DSM 11125^T; 10, *G. ureilyticus* MF38^T; 11, *G. orientalis* XH-63^T. Fatty acid data for all strains were obtained in this study under the same culture conditions. Fatty acid methyl esters were determined with cells grown on 7 % MA medium at 37 °C for 2 days. Fatty acids present at <1.0 % are not shown. —, Not detected or not reported.

Fatty acid	1	2	3	4	5	6	7	8	9	10	11
Saturated straight-chain											
C _{14:0}	1.9	1.3	1.1	—	—	2.7	3.7	3.6	—	1.5	—
C _{15:0}	2.1	2.0	7.1	2.3	1.2	—	2.4	2.7	1.7	2.1	—
C _{16:0}	18.2	14.2	12.8	10.0	9.1	10.8	18.5	24.8	10.3	14.5	4.9
C _{17:0}	1.2	1.8	4.2	2.7	1.7	—	—	—	2.5	1.5	3.7
C _{18:0}	2.8	5.9	3.6	4.3	3.7	4.9	5.2	8.7	3.2	5.8	—
Saturated branched-chain											
iso-C _{13:0} 3-OH	—	—	—	—	—	—	1.5	—	—	—	—
iso-C _{14:0}	3.8	2.1	—	—	—	—	1.4	3.7	—	2.4	2.1
anteiso-C _{14:0}	—	—	1.8	—	—	—	—	—	—	—	—
iso-C _{15:0}	20.5	22.7	15.4	16.1	17.3	15.4	14.5	8.7	16.9	12.1	9.1
anteiso-C _{15:0}	23.4	26.4	34.4	32.3	30.6	43.7	29.4	35.6	29.1	35.3	50.2
iso-C _{16:0}	8.3	—	4.4	2.1	4.0	2.4	2.8	3.9	4.2	4.8	3.9
anteiso-C _{16:0}	—	5.2	—	—	—	—	—	—	—	—	—
iso-C _{17:0}	5.5	4.3	2.5	5.0	6.2	1.7	2.4	—	7.9	3.9	2.4
anteiso-C _{17:0}	10.4	14.1	12.6	18.7	20.5	12.3	12.5	8.3	22.3	15.6	19.4
Unsaturated straight-chain											
C _{16:1} ω7c	—	—	—	—	—	—	1.2	—	—	—	—
C _{16:1} ω11c	—	—	—	1.5	1.4	1.3	1.5	—	—	—	2.1
C _{18:1} ω7c	—	—	—	—	—	—	1.4	—	—	—	—
C _{18:1} ω9c	—	—	—	—	—	2.7	1.3	—	—	—	—
Summed feature 4											
iso-C _{17:1} I/anteiso-C _{17:1} B	—	—	—	1.2	1.2	1.6	—	—	—	—	—

Table 2. Differential phenotypic characteristics of strain BH097^T and members of the genus *Gracilibacillus*

Strains: 1, BH097^T; 2, *G. halophilus* DSM 17856^T (data from Chen *et al.*, 2008a); 3, *G. laciisalsi* DSM 19029^T (Jeon *et al.*, 2008); 4, *G. saliphilus* DSM 19802^T (Tang *et al.*, 2009); 5, *G. thailandensis* TP2-8^T (Chamroensaksri *et al.*, 2010); 6, *G. boraciitolerans* DSM 17256^T (Ahmed *et al.*, 2007); 7, '*G. quinghaiensis*' DSM 17858 (Chen *et al.*, 2008b); 8, *G. halotolerans* DSM 11805^T (Wainø *et al.*, 1999); 9, *G. dipsosauri* DSM 11125^T (Lawson *et al.*, 1996); 10, *G. ureilyticus* MF38^T (Huo *et al.*, 2010); 11, *G. orientalis* XH-63^T (Carrasco *et al.*, 2006). All strains are Gram-positive, motile rods, producing endospores at the terminal position in swollen sporangia. All strains share the following characteristics: catalase-positive; hydrolyse aesculin but not casein; produce acids from glucose, arabinose and sucrose; and give a negative result in the H₂S, indole and Voges–Proskauer tests. +, Positive; w, weakly positive; –, negative; ND, no data available.

Characteristic	1	2	3	4	5	6	7	8	9	10	11
Colour of colonies*	C	C	C	C	WH	P/R	PP	C	WH	C	C
Spore shape†	S	E	S	S	S	S/E	S	E	S	S	S
Flagellation‡	Mono	Peri	Amphi	ND	Peri	Mono	ND	Peri	ND	ND	ND
Growth in NaCl (% w/v):											
Range	1–22	7–30	0.5–18	1–22	1–22	0–11	0.5–8	0–20	0–15	0–15	1–20
Optimum	7	15	5–7	10–15	5–10	0.5–3	1–3	0	3	3	10
0 % NaCl	–	–	–	–	–	+	–	+	+	+	–
5 % NaCl	+	–	+	+	+	+	+	+	+	+	+
20 % NaCl	+	+	–	+	+	–	–	+	–	–	+
Growth temperature (°C):											
Range	10–52	28–60	15–50	4–45	15–55	16–37	4–45	6–50	28–50	10–45	4–45
Optimum	37	45–50	40	28–37	37	25–28	37	47	45	35–37	37
20 °C	+	–	+	+	+	+	+	+	–	+	+
55 °C	–	+	–	–	+	–	–	–	–	–	–
pH											
Range	5.5–9.5	6.0–9.0	5.5–0.0	6.0–8.0	6.0–8.0	6.0–9.7	6.0–8.5	5–10	ND	6.5–8.5	5–9
Optimum	8.0	7.0	7.5–8.0	7.0	7.0	7.5–8.5	7.0–7.5	7.5	7.5	7.0	7.5
pH 5.5	+	–	+	–	–	–	–	+	ND	–	+
pH 9.0	+	+	+	–	–	+	–	+	ND	–	+
Nitrate reduction§	–	+	+	+	+	–	+	+	+	+	–
Oxidase activity§	+	+	+	+	+	+	+	+	+	+	–
Hydrolysis of:§											
Gelatin	w	+	w	+	+	–	–	w	w	+	+
Starch	–	+	–	+	–	–	–	+	+	+	+
Tween 80	–	+	–	–	–	–	–	+	–	–	–
Urea	–	–	–	+	–	–	+	+	–	+	–
Acid production from:§											
D-Arabinose	–	+	–	+	+	–	+	w	+	+	+
Arbutin	–	–	–	+	+	–	+	–	+	+	–
Cellobiose	–	–	+	+	+	+	+	–	+	+	–
D-Galactose	w	–	+	+	+	+	+	–	+	+	+
Gentiobiose	–	–	+	+	w	+	–	–	+	–	–
Glycerol	+	+	+	+	–	+	+	w	+	+	+
Glycogen	–	w	–	+	+	–	–	+	+	+	–
Lactose	w	–	+	+	+	+	–	–	+	+	+
D-Mannose	+	+	+	+	+	+	+	–	+	–	–
L-Rhamnose	–	w	+	+	w	+	–	–	+	+	–
L-Salicin	+	–	+	+	+	+	–	–	+	+	–
D-Sorbitol	–	w	–	–	–	w	+	+	–	–	–
Trehalose	–	+	+	+	+	+	+	w	+	+	+
Turanose	–	–	w	+	w	+	+	w	w	–	–
D-Xylose	–	+	+	+	+	w	–	+	+	+	+
API ZYM tests:§											
Cystine arylamidase	–	+	–	–	–	–	–	–	–	–	+
Leucine arylamidase	–	+	w	+	–	w	–	–	w	–	+
Lipase (C14)	w	–	–	+	–	–	–	–	w	–	–

Table 2. cont.

Characteristic	1	2	3	4	5	6	7	8	9	10	11
α -Fucosidase	+	—	—	+	—	—	—	—	—	—	+
α -Galactosidase	—	—	—	—	—	w	+	+	—	+	+
β -Galactosidase	+	+	+	—	—	+	+	—	+	+	+
α -Glucosidase	—	+	+	—	—	+	+	+	w	+	—
β -Glucosidase	+	+	+	—	—	+	+	+	+	+	+
α -Mannosidase	+	+	—	+	—	—	—	—	—	—	—
Susceptibility to ($\mu\text{g ml}^{-1}$):§											
Amoxicillin (10)	+	+	—	+	—	+	+	+	—	+	—
Ampicillin (20)	+	+	+	+	+	+	+	—	—	+	—
Bacitracin (10)	+	+	—	—	—	+	+	+	—	+	+
Chloramphenicol (30)	+	+	—	+	+	—	+	+	+	+	+
Erythromycin (15)	+	+	—	+	+	—	+	+	—	+	+
Gentamicin (10)	—	—	—	—	+	—	+	—	—	—	—
Kanamycin (30)	—	—	—	—	+	+	+	—	—	—	—
Lincomycin (15)	+	+	—	—	—	—	+	—	—	+	—
Neomycin (30)	—	—	—	+	+	+	—	—	—	—	—
Nystatin (50)	+	+	+	+	—	+	—	+	+	+	—
Penicillin G (12 IU)	+	+	—	—	—	+	+	+	—	+	—
Streptomycin (10)	—	—	—	—	—	—	+	—	—	—	+
Tetracycline (30)	+	+	—	+	+	—	+	—	+	+	—
Major polar lipids	DPG, PG, GL	DPG, PG	DPG, PG	DPG, PG	DPG, PG, GL	DPG, PG	DPG, PG	DPG, PG	DPG, PG	DPG, PG, GL	DPG, PG, PE
DNA G + C content (mol%)	37.9	42.3	38.8–39.0	40.1	37.6	35.8	40.9	38	39.4	35.3	37.1

*C, Cream; P, pink; PP, pale pink; R, red; WH, white.

†E, Ellipsoidal; S, spherical.

‡Mono, Monotrichous; Peri, peritrichous; Amphi, amphitrichous.

§Data from this study.

||DPG, Diphosphatidylglycerol; PG, phosphatidylglycerol; PE, phosphatidylethanolamine; GL, glycolipid.

and maximum-parsimony methods resulted in highly similar tree topologies (Figs S3 and S4). Based on the sequence divergence, it was evident that strain BH097^T constituted a different taxon separated from other species of the genus *Gracilibacillus*. DNA–DNA hybridization between strain BH097^T and the nearest phylogenetic neighbours was not attempted, as strains differing by >3 % of the 16S rRNA gene sequence are unlikely to exhibit >70 % DNA–DNA relatedness values at the whole-genome level (Stackebrandt & Goebel, 1994; Rosselló-Mora & Amann, 2001; Stackebrandt *et al.*, 2002; Stackebrandt & Ebers, 2006).

Fatty acid methyl esters were analysed by the GC-based (6890; Hewlett Packard) Sherlock Microbial Identification System (MIDI, Inc.) (Sasser, 1990) with the TSBA40 database according to the manufacturer's instructions. Cells were cultured on 7 % MA medium at 37 °C for 48 h. Extraction and analysis were performed conforming to the recommendations of the MIDI system. This analysis was carried out by the Identification Service of Chungnam National University, Korea. Polar lipids were separated on silica gel plates (10 × 10 cm) by TLC and were analysed according to the methods described by Minnikin *et al.*

(1984). Isoprenoid quinones were analysed as described by Komagata & Suzuki (1987) using HPLC apparatus fitted with a reversed-phase column (GROM-SIL100 ODS-2FE; Grom). Methanol : 2-propanol (2 : 1, v/v) was used as the mobile phase and quinone was detected at 270 nm. Analysis of the cell-wall peptidoglycan was carried out by the Identification Service of the DSMZ, Braunschweig, Germany. The DNA G + C content (mol%) was determined by reverse-phase HPLC using the method of Tamaoka & Komagata (1984).

The cellular fatty acid profile of strain BH097^T was characterized by anteiso-C_{15:0} (23.4 %), iso-C_{15:0} (20.5 %) and C_{16:0} (18.2 %) as the major fatty acids (Table 1). The fatty acid profiles of strain BH097^T and *G. halophilus* DSM 17856^T were similar in that they contained almost the same amount of iso-C_{15:0} as anteiso-C_{15:0}, whereas the amounts of anteiso-C_{15:0} were noticeably higher in the other members of the genus of *Gracilibacillus*. However, the presence of iso-C_{16:0} and absence of anteiso-C_{16:0} are distinct characteristics that differentiate strain BH097^T from *G. halophilus* DSM 17856^T. Strain BH097^T contained diphosphatidylglycerol, phosphatidylglycerol, two unknown phospholipids

and a glycolipid as the major polar lipids, which was a similar polar lipid pattern to *G. dipsosauri* DSM 11125^T (Fig. S5). The predominant isoprenoid quinone of strain BH097^T was menaquinone-7 (MK-7) and the cell wall contained peptidoglycan with *m*-DAP as the diagnostic diamino acid, representing cell-wall peptidoglycan type A1 γ . The DNA G+C content of strain BH097^T was 37.9 mol%. This value is within the range for members of the genus *Gracilibacillus*, but is lower than that of *G. halophilus* (Table 2). The respiratory lipoquinone, polar lipids and peptidoglycan type of the cell wall, and the DNA G+C content of strain BH097^T were consistent with the chemotaxonomic description of the genus *Gracilibacillus* (Wainø *et al.*, 1999).

The characteristics that differentiate strain BH097^T from the other species of the genus *Gracilibacillus* are summarized in Table 2. The differences in some features, such as colour of colonies, flagellation type, endospore shape, nitrate reduction, optimum growth temperature, acid production from carbohydrates, susceptibility to antibiotics, genomic DNA G+C content, fatty acid and polar lipid composition, can be used to distinguish this strain from the other species of the genus *Gracilibacillus* (Tables 1 and 2). Therefore, on the basis of these data, we propose that the strain represents a novel species of the genus *Gracilibacillus*, for which the name *Gracilibacillus bigeumensis* sp. nov. is proposed, with BH097^T as the type strain.

Description of *Gracilibacillus bigeumensis* sp. nov.

Gracilibacillus bigeumensis (bi.ge.um.en'sis. N.L. masc. adj. *bigeumensis* of Bigeum Island, Korea, the source of the soil sample from which the type strain was isolated).

Cells are Gram-staining-positive, motile with monotrichous flagella, endospore-forming rods, 0.3–0.5 $\times$ 2.5–4.0 μ m in size. Spherical endospores are produced at a terminal position in swollen sporangia. Colonies are circular, slightly irregular, smooth, cream and have a diameter of 0.5–1.0 mm on 7% MA medium after 48 h incubation at 37 °C. No diffusible pigments are produced. Strictly aerobic, slightly alkaliphilic, and moderately halophilic, capable of growth at 1–22% (w/v) NaCl [optimum, 7% (w/v) NaCl]. No growth occurs in the absence of NaCl. Grows at 10–52 °C (optimum, 37 °C) and pH 5.5–9.5 (optimum, pH 8.0). Positive result in tests for catalase, oxidase and PNPG but negative result in the Voges–Proskauer test and tests for ONPG, urease, lysine and ornithine decarboxylase, arginine dihydrolase and tryptophan decarboxylase. Hydrolyses aesculin and gelatin, but not casein, DNA, hypoxanthine, starch, urea or xanthine. Does not reduce nitrate to nitrite, or produce indole or H₂S. Utilizes L-arabinose, aesculin, D-fructose, D-galactose, D-glucose, glycerol, lactose, maltose, D-mannitol, D-mannose, melibiose, raffinose, L-salicin and sucrose for growth, but not acetate, citrate, formate, lactate, malate, adipate, D-arabinose, arbutin, cellobiose, gentiobiose, glycogen, inositol, inulin, L-rhamnose, D-ribose, D-sorbitol, trehalose or D-xylose as sole carbon and energy sources. Acid is produced from L-arabinose, erythritol,

aesculin, D-fructose, D-galactose, D-glucose, glycerol, lactose, maltose, D-mannitol, D-mannose, melibiose, potassium 5-ketogluconate, raffinose, L-salicin, sucrose and D-tagatose, but not from D-arabinose, arbutin, cellobiose, gentiobiose, glycogen, inositol, inulin, N-acetylglucosamine, L-rhamnose, D-ribose, D-sorbitol, trehalose, turanose or D-xylose. Strong enzyme activity is observed for acid phosphatase, esterase lipase (C8), naphthol-AS-BI-phosphohydrolase, α -fucosidase, α -mannosidase, β -galactosidase and β -glucosidase, whereas weak activity is observed for esterase (C4), lipase (C14) and alkaline phosphatase (API ZYM). Sensitive to (μ g per disc, unless otherwise indicated) amoxicillin (10), ampicillin (20), bacitracin (10), chloramphenicol (30), erythromycin (15), lincomycin (15), nystatin (50), penicillin G (12 U), polymyxin B (50 U), rifampicin (10), tetracycline (30) and vancomycin (30), but resistant to amikacin (30), cycloheximide (30), gentamicin (10), kanamycin (30), nalidixic acid (30), neomycin (30) and streptomycin (10). Major polar lipids are diphosphatidylglycerol, phosphatidylglycerol, two unknown phospholipids and a glycolipid. The isoprenoid quinone is MK-7. The peptidoglycan type is A1 γ , with *m*-DAP as the diagnostic diamino acid. Cellular fatty acids are anteiso-C_{15:0}, iso-C_{15:0}, C_{16:0}, anteiso-C_{17:0}, iso-C_{16:0}, iso-C_{17:0}, iso-C_{14:0}, C_{18:0}, C_{15:0}, C_{14:0} and C_{17:0}.

The type strain is BH097^T (=KCTC 13130^T=DSM 19028^T), and was isolated from solar saltern soil of Bigeum Island, south-west Korea. The DNA G+C content of the type strain is 37.9 mol% (HPLC).

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