Brevibacterium ammoniilyticum sp. nov., an ammonia-degrading bacterium isolated from sludge of a wastewater treatment plant

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A Gram-stain-positive, non-motile, chemo-organotrophic, mesophilic, aerobic bacterium, designated A1^T, was isolated from sludge of a wastewater treatment plant. Strain A1^T showed good ability to degrade ammonia and grew well on media amended with methanol and ammonia. Strain A1^T grew with 0–11 % (w/v) NaCl, at 20–42 °C, but not <15 or >45 °C and at pH 6–10 (optimum pH 8.0–9.0). The isolate was catalase-positive and oxidase-negative. The DNA G+C content was 70.7 mol%. A comparative analysis of 16S rRNA gene sequences revealed that strain A1^T formed a distinct phyletic lineage in the genus *Brevibacterium* and showed high sequence similarity with *Brevibacterium casei* NCDO 2048^T (96.9 %), *Brevibacterium celere* KMM 3637^T (96.9 %) and *Brevibacterium sanguinis* CF63^T (96.4 %). DNA–DNA hybridization revealed <43 % DNA–DNA relatedness between the isolate and its closest phylogenetic relatives. The affiliation of strain A1^T with the genus *Brevibacterium* was supported by the chemotaxonomic data: predominant quinone menaquinone MK-7(H_2); polar lipid profile containing diphosphatidylglycerol, phosphatidylglycerol and an unidentified glycolipid; characteristic cell-wall diamino acid *meso*-diaminopimelic acid; whole-cell sugars galactose, xylose and ribose; absence of mycolic acids; and major fatty acids iso-$C_{15:0}$, anteiso-$C_{15:0}$ and anteiso-$C_{17:0}$. The results of physiological and biochemical tests allowed phenotypic differentiation of strain A1^T from members of the genus *Brevibacterium*. On the basis of the results in this study, a novel species, *Brevibacterium ammoniilyticum* sp. nov., is proposed. The type strain is A1^T (=KEMC 41-098^T =JCM 17537^T =KACC 15558^T).

The genus *Brevibacterium* was first reported by Breed (1953), with *Brevibacterium linens* as the type species, which was isolated from surface-ripened cheese and described as Gram-positive, non-branching, non-spore-forming, short rods with a high G+C content. Collins (2006) stated that the genus *Brevibacterium* has *meso*-diaminopimelic acid and respiratory menaquinones MK-8(H_2) in the A1γ-type peptidoglycan. In recent years, in addition to *B. linens*, the following species in the genus *Brevibacterium sensu stricto* have been reported: (Ivanova *et al.*, 2004), (Wauters *et al.*, 2004), (Pascual & Collins, 1999), *B. marinum* (Lee, 2008), , *B. permense* (Gavrish *et al.*, 2004), (Bhadra *et al.*, 2008) and (Heyrman *et al.*, 2004). In many cases, members of the genus *Brevibacterium* have been isolated from dairy products, including cheese (Collins *et al.*, 1983), and from human skin (Collins *et al.*, 1983; Wauters *et al.*, 2004; Roux & Raoult, 2009). In some cases, *Brevibacterium* strains have been recognized in other environments such as high salinity (Bhadra *et al.*,

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain A1^T is JF937067.

A supplementary table and a supplementary figure are available with the online version of this paper.

2008; Lee 2008), poultry (Pascual & Collins 1999), a damaged mural painting (Heyrman *et al.*, 2004) and a degraded thallus of a brown alga (Ivanova *et al.*, 2004). Members of the genus have been reported not only in relation to cheese production, but also in patents from biotechnological industry, including those for production of antibiotics and wastewater treatment.

Strain A1^T was isolated from the sludge of a wastewater treatment plant near a farm area at Gwangju-si, Kyeonggi-do, South Korea (37° 21′ 31.24″ N 127° 14′ 24.50″ E; altitude 49 m). For isolation, 1 % (v/v) sludge was inoculated into ATCC medium 412 (containing l^{-1}: 5.0 g NH_4Cl, 2.0 g KH_2PO_4, 0.5 g NaCl, 0.2 g $MgSO_4$, 0.002 g $FeSO_4$, 0.002 g $MnCl_2$, 0.2 g , yeast extract 0.2 g; pH 7.0; 20 ml methanol added after autoclaving). Enrichment culture was performed aerobically with shaking (150 r.p.m.) at 28 °C for 24 h. Single colonies of strain A1^T along with other 119 strains were purified by transferring them onto new plates and the purified colonies were tentatively identified by partial 16S rRNA gene sequencing. The isolate was routinely cultured on ATCC medium 412 at 28 °C and preserved as a suspension in Luria–Bertani broth (LB; Difco) with 20 % (w/v) glycerol at −70 °C. Strain A1^T was Gram-stain-positive, non-motile and aerobic and formed white greyish colonies on ATCC medium 412. Strain A1^T was subjected to a polyphasic taxonomic investigation and the results indicated it represents a novel *Brevibacterium* species.

Strain A1^T was able to degrade ammonia nitrogen and methanol; batch culture experiments showed that 55 mg ammonia l^{-1} and 1875 mg methanol l^{-1} were degraded in 12 h (initial concentrations l^{-1}: 80 mg ammonia and 2500 mg methanol).

Total genomic DNA was extracted by a modification of the method of Marmur (1961). Cells were lysed with 10 mg lysozyme ml^{-1} and 50 U achromopeptidase ml^{-1} (Ezaki & Suzuki, 1982) to avoid the difficulty of extraction of DNA from Gram-positive bacteria. The 16S rRNA gene was

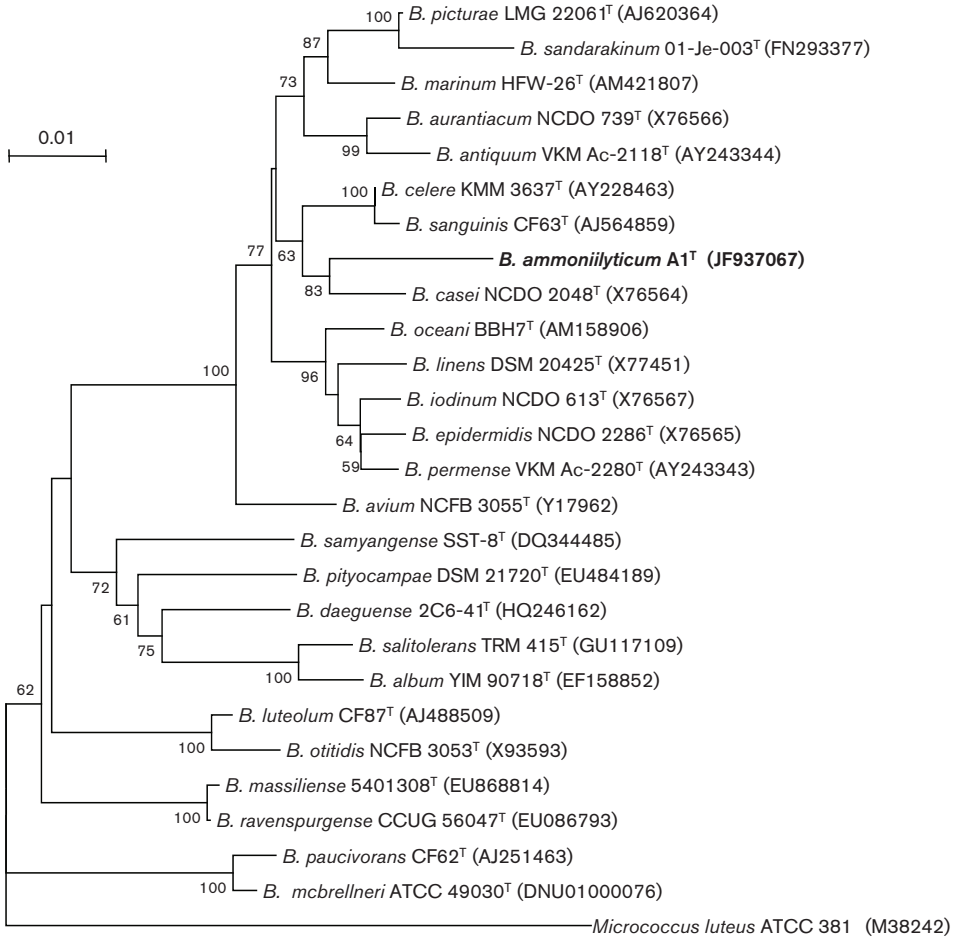

Fig. 1. Phylogenetic position of *B. ammoniilyticum* A1^T based on 16S rRNA gene sequence analysis. The tree was reconstructed using the neighbour-joining method (Saitou & Nei, 1987) in the program MEGA5 (Tamura *et al.*, 2011) with bootstrap values based on 1000 replications (Felsenstein, 1985). The evolutionary distances were calculated using the Kimura two-parameter model (Kimura, 1983). Bar, 0.01 substitutions per nucleotide position.

amplified by PCR with primers 27F and 1492R as described by Frank *et al.* (2008). Sequencing was performed by using a Big Dye Terminator cycle sequencing kit v.3.1 (Applied BioSystems). Sequencing products were resolved on an Applied Biosystems model 3730XL automated DNA sequencing system at Macrogen, Seoul, Korea. A nearly complete sequence of the 16S rRNA gene (1432 nt) was obtained and compiled with SeqMan (DNASTAR). 16S rRNA gene sequences of related taxa were obtained from GenBank and edited using BioEdit (Hall, 1999). Multiple alignments were performed with CLUSTAL X (Thompson *et al.*, 1997). The evolutionary distances were calculated using the Kimura two-parameter model (Kimura, 1983). Phylogenetic trees were constructed using the neighbour-joining (Saitou & Nei, 1987), maximum-parsimony (Fitch, 1971) and maximum- likelihood methods in MEGA5 (Tamura *et al.*, 2011) with bootstrap values based on 1000 replications (Felsenstein, 1985).

Neighbour-joining phylogenetic analysis based on 16S rRNA gene sequences revealed that strain A1^T formed a distinct lineage in the genus *Brevibacterium* (Fig. 1). The highest sequence similarities were observed with *B. casei* NCDO 2048^T (96.9 %), KMM 3637^T (96.9 %), CF63^T (96.4 %) and NCDO 739^T (96.4 %). Sequence similarity with other members of the genus *Brevibacterium* was $\leqslant$96 %. In the neighbour-joining phylogenetic tree (Fig. 1), strain A1^T clustered with *B. casei* NCDO 2048^T with a bootstrap value of 83 %, and this cluster joined the cluster comprising species of *Brevibacterium* along with *B. antiquum* VKM Ac-2118^T. These findings were supported by the maximum-parsimony analysis.

The reference strains *B. linens* KACC 14346^T, *B. casei* KCTC 3082^T, KCTC 3083^T, *B. epidermidis* KCTC 3090^T, *B. marinum* KCTC 19221^T, JCM 2590^T, JCM 11680^T, *B. permense* JCM 13318^T, JCM 13319^T, JCM 13386^T, JCM 13521^T and JCM 21798^T were obtained from the relevant culture collections. All experiments on the reference strains and strain A1^T were performed with the same methods and conditions. All strains were maintained and cultivated on LB media at pH 7.0 aerobically, unless otherwise mentioned.

The cellular morphology of strain A1^T was determined by phase-contrast microscopy and transmission electron microscopy. Gram-stained cells (Cappuccino & Sherman 2010) were examined with a BX 51 phase-contrast microscope (Olympus, Japan) at magnification $\times$1000. Strain A1^T was grown on LB agar for 1, 2 and 3 days and single colonies at each time interval were suspended and stained with 1 % (w/v) phosphotungstic acid (Hayat & Miller 1990; Roux & Raoult 2009). Cell morphology was examined on a JEM 1010 transmission electron microscope (Jeol, Japan) at an operating voltage of 60–80 kV (Fig. 2). Motility testing was performed by stabbing the centre of tube filled with 0.4 % LB agar (Brown, 2008). Growth and colony morphology were examined on complex agars such as trypticase soy agar (TSA; Fgvo), nutrient agar (NA; Difco), LB agar and Columbia blood agar base (Scharlau)

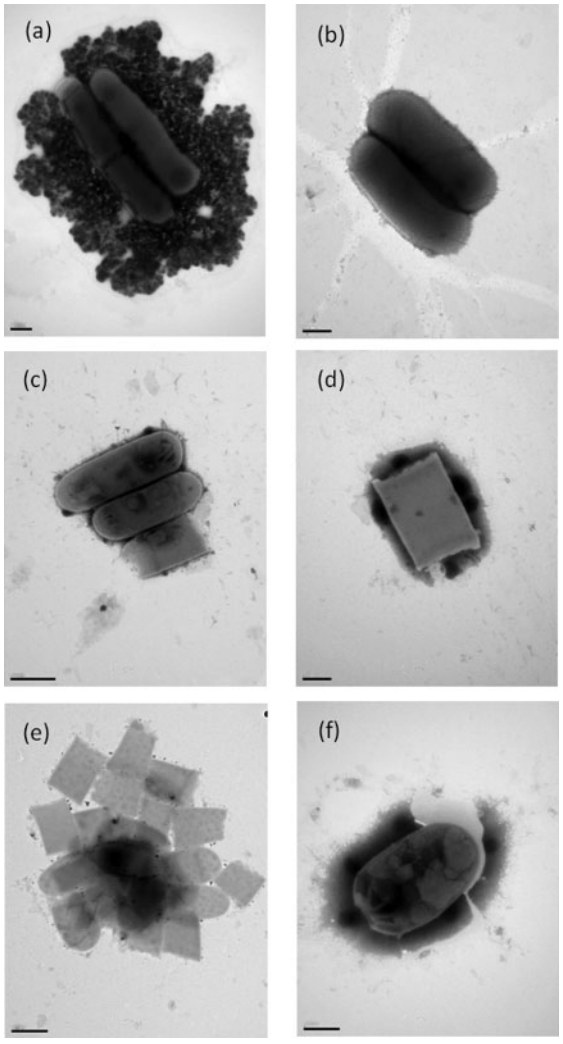

Fig. 2. Transmission electron micrographs of strain A1^T showing the rod–coccus cycle at 1 day (a, b), 2 days (c, d) and 3 days (e, f). Cells were grown on Luria–Bertani agar, suspended and stained with 1 % (w/v) phosphotungstic acid. Bars: a, b, d, f, 200 nm; c, e, 0.5 μm.

with 5 % sheep blood. Growth at pH 3–12 (in increments of one pH unit), at 5, 10, 15, 20, 25, 37 and 42 °C and with 0–15 % (w/v) NaCl (in increments of 1 %) was determined at 28 °C in LB medium (1 % inoculum, OD_{600}=1.0) for 72 h aerobically. Results were evaluated as positive if OD_{600} increased by more than 0.300. Biochemical characteristics were determined using the API CORYNE, API ZYM, API 50 CH/E, API 20 E and ID 32 GN systems (bioMérieux) as described in the manufacturer's instructions. Catalase was determined with 3 % H_2O_2 and oxidase was assayed by applying cells to moistened discs impregnated with dimethyl-*p*-phenylenediamine (bioMérieux). Hydrolysis of casein, starch and Tween 80 (Atlas, 1993) was also investigated; reactions were read after 5 days. Fermentation and hydrogen sulfide production were examining using TSI slants (Cappuccino & Sherman 2010). The phenotypic

Table 1. Differential characteristics of strain A1^T and closely related members of the genus *Brevibacterium*

Strains: 1, *Brevibacterium ammoniilyticum* sp. nov. A1^T; 2, *B. linens* KACC 14346^T; 3, *B. casei* KCTC 3082^T; 4, KCTC 3083^T; 5, *B. epidermidis* KCTC 3090^T; 6, *B. marinum* KCTC 19221^T; 7, JCM 2590^T; 8, *B. avium* JCM 11680^T; 9, *B. permense* JCM 13318^T; 10, JCM 13319^T; 11, *B. sanguinis* JCM 13386^T; 12, *B. celere* JCM 13521^T; 13, *B. oceani* JCM 21798^T. All data were taken from this study. All strains are Gram-positive, non-motile, aerobic, non-spore-forming, catalase-positive rods. +, Positive; W, weakly positive; −, negative.

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13
Colony colour on:*													
Complex media	WC	YO	WC	WC (with iodine crystals)	WC	BI	YI	WC	YO	I	I	WC	PO
Columbia blood media	GW	GW	GW	GW	GW	GW	GW	GW	YO	GW	GW	GW	PO
Oxidase	−	−	−	+	−	−	−	+	−	−	−	−	−
Growth at (°C):													
15	−	+	+	+	+	+	+	+	−	−	+	+	+
42	+	−	+	−	+	+	−	−	−	−	−	−	+
Optimum	30	20	25	20	25	30	25	30	30	30	37	20	30
Range	20–42	15–30	15–42	15–37	15–42	15–42	15–37	15–37	25–37	25–37	15–37	15–37	15–42
pH for growth													
Optimum	8–9	8–9	7–9	8–9	7–8	8–9	8–9	7–9	8–9	7–8	8–9	8–9	7–8
Range	6–10	6–10	6–10	6–10	6–10	5–12	6–10	6–10	6–11	6–9	6–12	5–10	5–9
NaCl for growth (%)	0–11	0–15	0–11	0–11	0–10	0–10	0–15	0–15	0–15	0–15	0–10	0–15	0–12
Nitrate reduction	+	+	−	+	+	−	+	+	−	−	−	−	−
Enzyme activity (API ZYM, API CORYNE)													
Acid phosphatase	+	−	+	−	−	−	+	+	W	+	+	+	−
Alkaline phosphatase	+	−	+	+	+	+	+	+	+	+	+	+	−
Cystine arylamidase	+	−	−	−	W	−	−	+	+	+	W	W	W
α-Galactosidase	+	−	−	−	−	−	−	−	−	−	−	−	−
β-Galactosidase (ONPG)	+	−	−	−	−	−	+	−	−	−	−	−	−
α-Glucosidase (starch)	+	−	+	−	−	−	−	−	−	+	+	+	−
β-Glucosidase (aesculin)	+	−	−	−	−	+	−	−	−	−	−	−	−
β-Glucosidase	−	−	−	−	−	+	−	−	−	−	−	−	−
β-Glucuronidase	−	−	−	−	−	−	+	+	−	−	−	−	−
Leucine arylamidase	−	−	+	+	−	−	−	+	+	+	+	+	+
α-Mannosidase	W	−	−	−	−	−	−	−	−	−	−	−	−
Protease (gelatin)	+	+	+	+	+	+	+	+	+	+	−	+	+
Pyrazinamidase	−	+	−	+	+	+	−	+	+	−	−	−	+
Pyrrolidonyl arylamidase	+	−	+	−	−	+	−	+	−	+	+	+	−
Urease	−	−	−	−	−	−	+	+	W	−	+	+	−
Valine arylamidase	+	−	−	−	−	−	−	−	−	−	−	−	−
Utilization (API CORYNE)													
Glucose	+	−	+	−	−	−	+	−	−	−	+	−	−
Lactose	−	−	−	−	−	−	+	−	−	−	−	−	−
Maltose	+	−	−	−	−	−	+	−	−	−	−	−	−
Ribose	+	W	−	−	−	−	−	−	−	−	+	−	−
Sucrose	−	−	−	−	−	−	+	−	−	−	−	−	−
Xylose	−	−	+	−	−	−	−	−	−	−	−	−	−
Mannitol	+	−	−	−	−	−	−	−	−	−	−	−	−
Glycogen	+	−	−	−	−	−	−	−	−	−	−	−	−

Table 1. cont.

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13
Assimilation (API ID 32GN)													
4-Hydroxybenzoate	w	w	−	+	+	−	−	+	−	+	+	+	+
DL-3-Hydroxybutyrate	+	w	+	−	+	+	+	−	−	+	+	+	+
2-Ketogluconate (α)	−	+	w	−	−	−	−	−	−	−	+	−	−
Acetate	+	−	+	+	+	+	+	−	−	+	+	+	+
Citrate	+	−	+	+	+	+	+	w	−	+	+	+	+
Itaconate	−	−	−	−	−	−	−	−	−	−	−	−	+
DL-Lactate	+	−	+	−	+	+	+	+	−	+	+	+	+
Malonate	+	−	+	−	+	+	+	−	+	−	+	w	−
Propionate	+	−	+	+	+	+	−	−	−	+	+	+	+
Suberate	+	−	+	+	+	+	−	−	−	−	+	w	+
n-Valerate	+	−	+	+	+	+	−	−	−	+	+	+	+
L-Fucose	+	−	+	−	+	+	−	−	−	+	+	+	−
D-Glucose	+	−	−	+	+	w	+	+	−	+	+	+	w
Maltose	w	−	−	−	−	−	+	−	−	+	+	+	−
L-Rhamnose	+	−	+	w	w	w	w	−	−	−	w	w	+
D-Ribose	+	−	−	+	−	−	−	−	−	−	−	−	−
Sucrose	−	−	−	+	−	−	−	−	−	+	+	+	−
myo-Inositol	−	−	+	+	+	−	−	−	−	+	+	+	−
D-Mannitol	−	+	−	w	+	w	+	+	−	−	−	−	−
L-Alanine	+	−	w	+	+	+	−	−	−	+	+	+	+
L-Histidine	w	−	−	+	+	+	−	−	−	+	+	+	+
L-Proline	+	−	+	+	+	+	−	+	−	+	+	+	−
L-Serine	+	−	w	+	+	+	+	+	−	+	+	+	w
N-Acetyl-D-glucosamine	+	−	+	+	+	−	−	+	−	+	+	+	−
Salicin	−	−	+	−	−	−	−	−	−	−	−	−	−
Glycogen	+	w	w	−	−	−	+	+	+	−	w	−	−
DNA G+C content (mol%)	70.7	62.1	68.6	64.8	67.0	62.3	68.4	67.8	67.3	70.4	70.2	71.7	68.5

*BI, Beige ivory; GW, greyish white; I, ivory; PO, pale orange; WC, white creamy; YI, yellowish ivory; YO, yellow orange.

characteristics of strain A1^T are given in the species description and those that are differentiated strain A1^T from its closest phylogenetic relatives are listed in Table 1.

For cellular fatty acid analysis, strain A1^T and the reference strains were grown on TSA for 48 h at 30 °C. Two loopfuls of bacterial mass were collected from fourth quadrant of the plates (according to the MIDI technical note) and subjected to saponification, methylation and extraction using the methods of Kuykendall *et al.* (1988). The fatty acid methyl esters were separated using the Sherlock Microbial Identification System (TSBA version 6.0; MIDI), analysed by GC (model 6890; Hewlett Packard) and identified using the Microbial Identification software package (Sasser, 1990). The fatty acid composition of strain A1^T was characterized by predominant amounts of anteiso-$C_{15:0}$ (46.8 %), anteiso-$C_{17:0}$ (28.1 %) and iso-$C_{15:0}$ (17.5 %), which have been also found previously in its close relatives. The fatty acid composition of strain A1^T is compared with the reference strains in Table 2. Gruner *et al.* (1993) proved that anteiso-$C_{15:0}$ and anteiso-$C_{17:0}$ were the major fatty acids in *B. casei* NCDO 2048^T, *B. linens* DSM 20425^T and *B. epidermidis* NCDO 2286^T.

For chemotaxonomic characterization, cells were grown in LB medium for 3 days and freeze dried. Polar lipids were extracted and examined by two-dimensional TLC (Minnikin *et al.*, 1977). The polar lipid profile of strain A1^T contained predominant amounts of diphosphatidylglycerol, followed by phosphatidylglycerol and a glycolipid, smaller amounts of two aminolipids and minor amounts of three unknown phospholipids (Fig. S1, available in IJSEM Online). Collins (2006) and Jones & Keddie (1986) described diphosphatidylglycerol, phosphatidylglycerol and dimannosyldiacylglycerol as the major polar lipids of the genus *Brevibacterium*. Kämpfer *et al.* (2010) proved that diphosphatidylglycerol, phosphatidylglycerol and glycolipid were major polar lipids of *B. sandarakinum* 01-Je-003^T.

Isoprenoid quinones were extracted from freeze-dried cells of strain A1^T, *B. linens* KACC 14346^T and *B. casei* KCTC

Table 2. Fatty acid compositions of strain A1^T and closely related members of the genus *Brevibacterium*

Strains: 1, *Brevibacterium ammoniilyticum* sp. nov. A1^T; 2, *B. linens* KACC 14346^T; 3, *B. casei* KCTC 3082^T; 4, KCTC 3083^T; 5, *B. epidermidis* KCTC 3090^T; 6, *B. marinum* KCTC 19221^T; 7, *B. aurantiacum* JCM 2590^T; 8, *B. avium* JCM 11680^T; 9, *B. permense* JCM 13318^T; 10, *B. picturae* JCM 13319^T; 11, *B. sanguinis* JCM 13386^T; 12, *B. celere* JCM 13521^T; 13, *B. oceani* JCM 21798^T. All data were taken from this study using cells grown on TSA for 2 days at 30 °C. Fatty acids amounting to <1 % of the total in all strains are not shown. tr, Trace (<1 %); −, not detected.

Fatty acid	1	2	3	4	5	6	7	8	9	10	11	12	13
$C_{16:0}$	tr	tr	tr	tr	1.1	tr	1.3	tr	tr	tr	tr	tr	tr
iso-$C_{14:0}$	tr	−	−	−	−	−	1.5	−	tr	tr	tr	tr	−
iso-$C_{15:0}$	17.5	9.2	14.4	8.2	9.7	17.0	8.3	11.0	16.4	8.4	6.1	8.9	10.0
iso-$C_{16:0}$	3.4	4.5	3.6	2.2	3.3	6.0	tr	4.2	3.7	4.9	4.6	5.0	2.5
iso-$C_{17:0}$	3.1	3.5	5.0	1.7	2.7	6.5	6.3	3.6	4.0	2.9	2.5	3.8	3.4
anteiso-$C_{15:0}$	46.8	45.3	37.4	57.7	44.3	31.9	37.4	41.0	42.8	40.5	40.5	35.5	43.0
anteiso-$C_{17:0}$	28.1	36.1	38.7	27.5	38.8	38.1	13.7	39.4	31.0	42.3	44.7	46.2	38.9
anteiso-$C_{17:1}$ A	−	tr	−	1.6	−	−	−	−	−	−	−	−	tr

3082^T with chloroform/methanol (2 : 1, v/v), evaporated under vacuum and extracted in n-hexane/water (1 : 1, v/v). The crude n-hexane quinone solution was then purified using silica Sep-Pak Vac cartridges (Waters) and subsequently analysed by HPLC (MD-2015 plus; JASCO, Japan), as described elsewhere (Hiraishi *et al.*, 1996). Strain A1^T contained MK-7(H_2) as the predominant menaquinone, which is similar to *B. linens* KACC 14346^T and *B. casei* KCTC 3082^T (Collins *et al.*, 1979, 1980, 1983). Strain A1^T also contained a minor amount of MK-8(H_2), and Gavrish *et al.* (2004) described that MK-7 and MK-8(H_2) are the predominant menaquinones of the genus *Brevibacterium*.

The cell-wall peptidoglycan was analysed as described by Schleifer & Kandler (1972) using TLC on cellulose sheets instead of paper chromatography. Absence of mycolic acids was demonstrated according to Minnikin *et al.* (1975) using silica gel TLC. The whole-cell sugars contained galactose, xylose and ribose (Collins, 2006) and the characteristic cell-wall diamino acid was *meso*-diaminopimelic acid (Ivanova *et al.* 2004), both of which are common to all members of the genus *Brevibacterium*. Cai & Collins (1994) and DSMZ (2001) have described that the peptidoglycan type A1γ (*meso*-diaminopimelic acid direct, type A31) is characteristic for the genus *Brevibacterium*. We confirm that the cell wall of strain A1^T contained *meso*-diaminopimelic acid, L-glutamic acid, L-alanine and minor amounts of L-aspartic acid.

For analysis of DNA G+C content, genomic DNA was extracted as mentioned above and enzymically degraded into nucleosides. The nucleosides were analysed using HPLC as described elsewhere (Tamaoka & Komagata, 1984; Mesbah *et al.*, 1989). The DNA G+C content of strain A1^T was 70.7 mol%.

DNA–DNA hybridization experiments were performed fluorometrically by the method of Ezaki *et al.* (1989), using photobiotin-labelled DNA probes of strain A1^T, KCTC 3082^T and JCM 13521^T) with micro-dilution wells. Hybridization was performed with five replications for each sample. The highest and lowest values obtained for each sample were excluded and the means of the remaining three values were quoted as DNA–DNA relatedness. As a probe, strain A1^T showed relatively low DNA–DNA relatedness with *B. casei* KCTC 3082^T(43±4 %), JCM 13521^T(42±4 %), *B. picturae* JCM 13319^T (46±3 %), *B. sanguinis* JCM 13386^T (42±6 %), *B. aurantiacum* JCM 2590^T(13±1 %), *B. linens* KACC 14346^T (10±1 %), *B. oceani* JCM 21798^T (8±0 %), KCTC 3083^T (8±1 %), *B. avium* JCM 11680^T (8±1 %), *B. epidermidis* KCTC 3090^T (5±0 %), *B. marinum* KCTC 19221^T (3±1 %) and *B. permense* JCM 13318^T (3±0 %). The results of the reciprocal reactions, using the probes of *B. casei* KCTC 3082^T and JCM 13521^T, also exhibited low relatedness with the isolate (Table S1). These results indicate that strain A1^T is not related at the species level to any recognized member of the genus *Brevibacterium* (Wayne *et al.*, 1987).

Strain A1^T was a Gram-positive, non-motile, chemoorganotrophic, mesophilic, aerobic bacterium. Menaquinone MK-7(H_2) was the major quinone. The polar lipid profile contained major amounts of diphosphatidylglycerol, phosphatidylglycerol and a glycolipid. The cell-wall peptidoglycan contained *meso*-diaminopimelic acid as the main diamino acid and was of the A1γ type. The major fatty acids iso-$C_{15:0}$, anteiso-$C_{15:0}$ and anteiso-$C_{17:0}$ supported the affiliation of strain A1^T with the genus *Brevibacterium*. Strain A1^T could be distinguished from members of the genus by the production of α-galactosidase, α-mannosidase and valine arylamidase and the utilization of mannitol and glycogen. The DNA–DNA relatedness results also support the phenotypic, chemotaxonomic and phylogenetic findings that strain A1^T represents a novel species within the genus *Brevibacterium*, for which the name *Brevibacterium ammoniilyticum* sp. nov. is proposed.

Description of *Brevibacterium ammoniilyticum* sp. nov.

Brevibacterium ammoniilyticum [am.mo.ni.i.ly′ti.cum. N.L. n. *ammonium* ammonia; N.L. neut. adj. *lyticum* (from Gr.

neut. adj. *lutikon*) able to loose, able to dissolve; N.L. neut. adj. *ammoniilyticum* ammonia-degrading, to reflect the activity of the bacterium].

Cells are Gram-stain-positive, non-motile, coryneform, aerobic and non-spore-forming. A rod–coccus cycle was observed: rods (0.45–0.50 μm wide and 0.8–1.2 μm long) in exponential phase (1–2 days); cocci (0.45–0.50 μm wide and 0.55–0.65 μm long) in stationary phase (>3 days). On complex medium, colonies are snowball-like, circular, convex with entire margins and creamy white (0.8–1.2 mm in diameter). On Colombia blood agar, colonies are circular, convex with entire margins and greyish white (0.8–1.2 mm in diameter). Grows with 0–11 % (w/v) NaCl, at 20–42 °C, but <15 or >45 °C, and at pH 6–10 (optimum pH 8.0–9.0). Catalase-positive and oxidase-negative. Grows on ATCC medium 412. With API CORYNE, nitrate is reduced to nitrite, gelatin and aesculin are hydrolysed, alkaline phosphatase, *β*-galactosidase, α-glucosidase and pyrrolidonyl arylamidase are positive and amygdalin, fructose, galactose, glucose, glycogen, mannitol, mannose, maltose, rhamnose, ribose and sorbitol are utilized, but *N*-acetyl-*β*-glucosaminidase, *β*-glucuronidase, pyrazinamidase and urease are negative and lactose, sucrose and xylose are not utilized. With API ZYM, acid phosphatase, cystine arylamidase, esterase (C4), esterase lipase (C8), α-galactosidase, phosphoamidase and valine arylamidase are positive and *β*-galactosidase and α-glucosidase are weakly positive, but alkaline phosphatase, chymotrypsin, α-fucosidase, *β*-glucosidase, leucine arylamidase, lipase (C14), trypsin and *N*-acetyl-*β*-glucosaminidase are negative. With ID 32GN, *N*-acetyl-glucosamine, L-alanine, L-fucose, D-glucose, glycogen, 3-hydroxybutyric acid, lactic acid, L-proline, propionic acid, L-rhamnose, D-ribose, L-serine, sodium acetate, sodium malonate, suberic acid, trisodium citrate, 3-hydroxybenzoic acid and valeric acid were assimilated and L-histidine, 4-hydroxybenzoic acid and maltose were weakly assimilated, but L-arabinose, capric acid, inositol, itaconic acid, D-mannitol, melibiose, sucrose, salicin, D-sorbitol, potassium 2-ketogluconate and potassium 5-ketogluconate are not assimilated. With API 50 CH/E after 24 h, *N*-acetylglucosamine, amygdalin, arbutin, cellobiose, aesculin, fructose, glucose, D-glucoside, glycerol, glycogen, inulin, lactose, mannose, mannitol, maltose, α-methyl ribose, salicin, starch, trehalose and D-turanose are positive and after 48 h D-arabitol, D-fucose, galactose, inulin, lactose, melibiose, methyl *β*-D-xylopyranoside, rhamnose and D-xylose are positive, but adonitol, D-arabinose, L-arabinose, dulcitol, erythritol, gentiobiose, inositol, methyl α-D-mannopyranoside, sorbose, sorbitol, trehalose, xylitol, L-xylose, L-arabitol, L-fucose, 2-ketogluconate, 5-ketogluconate, D-lyxose, D-tagatose and turanose are negative. With API 20 E, acetoin production (Voges–Proskauer), arginine dihydrolase, citrate utilization and H_2S production are positive, but *ortho*-nitrophenyl *β*-D-galactopyranosidase, ornithine decarboxylase, lysine decarboxylase and nitrite and nitrogen production are negative. The major quinone is menaquinone MK-7(H_2) and a minor amount of MK-8(H_2) is also detected. The polar lipid profile consists of major amounts of diphosphatidylglycerol, phosphatidylglycerol and glycolipid, moderate amounts of two unknown aminophospholipids and minor amounts of three unknown phospholipids. Mycolic acids are absent. Cell-wall diamino acid is *meso*-diaminopimelic acid and the whole-cell sugars are galactose, xylose and ribose. The major fatty acids are anteiso-$C_{15:0}$, anteiso-$C_{17:0}$ and iso-$C_{15:0}$.

The type strain, A1^T (=KEMC 41-098^T =JCM 17537^T =KACC 15558^T), was isolated from sludge of a wastewater treatment plant near a farm area at Gwangju-si, Kyeonggi-do, South Korea. The DNA G+C content of the type strain is 70.7 mol%.

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