Tumebacillus soli sp. nov., isolated from non-rhizosphere soil

Jong-Hwa Kim and Wonyong Kim

Department of Microbiology, Chung-Ang University College of Medicine, Seoul, Republic of Korea

Correspondence
Wonyong Kim
kimwy@cau.ac.kr

A Gram-stain-positive, strictly aerobic, motile, spore-forming, rod-shaped bacterial strain, CAU 11108^T, was isolated from soil in Danghangpo, Republic of Korea, and its taxonomic position was investigated using a polyphasic approach. The bacterium grew optimally at 37 °C, at pH 8, and in the presence of 1 % (w/v) NaCl. Phylogenetic analysis based on 16S rRNA gene sequence similarity revealed that strain CAU 11108^T formed a distinct lineage within the genus *Tumebacillus* and was most closely related to *Tumebacillus luteolus* U13^T (98.2 %). The strain contained menaquinone-7 (MK-7) as the major respiratory quinone and iso-$C_{15:0}$ and anteiso-$C_{15:0}$ as the major fatty acids. The DNA G+C content was 54.6 mol%. On the basis of phenotypic differentiation, phylogenetic and chemotaxonomic data, strain CAU 11108^T represents a novel species of the genus *Tumebacillus*, for which the name *Tumebacillus soli* sp. nov. is proposed. The type strain is CAU 11108^T (=KCTC 33141^T=CECT 8918^T).

The genus *Tumebacillus*, a member of the family *Alicyclobacillaceae*, was first created by Steven *et al.* (2008) with the description of *Tumebacillus permanentifrigoris* as the type species of the genus. The genus *Tumebacillus* comprises Gram-stain-positive, motile, rod-shaped bacteria that are characterized chemotaxonomically by the presence of MK-7 as the major isoprenoid quinone and iso-$C_{15:0}$ as the predominant cellular fatty acid. At the time of writing, this genus consists of four species with validly published names, *T. permanentifrigoris* (Steven *et al.*, 2008), *T. ginsengisoli* (Baek *et al.*, 2011), *T. flagellatus* (Wang *et al.*, 2013) and *T. algifaecis* (Wu *et al.*, 2015). They have been isolated from diverse environments such as Arctic permafrost, soil, wastewater and decomposing algal scum. In the course of screening bacteria from environmental samples, a novel bacterial strain, designated CAU 11108^T, was isolated from a non-rhizosphere soil sample (pH 8.0 and salinity 1.5 %) taken at a depth 10 cm in Danghangpo (35° 03′ 17.28″ N, 128° 23′ 42.82″ E) in the Republic of Korea. The purpose of present study was to establish the taxonomic position of this bacterial strain using a polyphasic characterization that included chemotaxonomic, phenotypic and genotypic properties.

Isolation was performed using the dilution plating technique. The crushed soil sample was diluted with sterilized saline solution. Sample dilutions were plated on R2A agar (BD Difco) supplemented with cycloheximide (50 mg l^{-1}) and nalidixic acid (20 mg l^{-1}). One hundred microlitres of the diluted samples (10^{-2} and 10^{-6}) were spread on R2A agar plates and incubated at 30 °C for 7 days under aerobic conditions. Colonies were randomly selected through subculture and strain CAU 11108^T was purified from a single colony from an R2A agar plate. Strain CAU 11108^T has been deposited in the Korean Collection for Type Cultures (KCTC; Taejon, Korea) and Colección Española de Cultivos Tipo (CECT; Paterna, Spain).

The type strains of four species of the genus *Tumebacillus* were used as reference strains in fatty acid analyses. *T. permanentifrigoris* JCM 14557^T, *T. ginsengisoli* KCTC 13942^T, *Tumebacillus. flagellatus* DSM 25748^T, *T. algifaecis* NBRC 108765^T, *Tumebacillus luteolus* U13^T (JCM 19866^T) and *Tumebacillus luteolus* U14 (JCM 19867) were obtained from the Japan Culture Collection of Microorganisms (JCM; Osaka, Japan), the KCTC, the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ; Braunschweig, Germany) and the Biological Resource Center, NITE (NBRC; Chiba, Japan).

Genomic DNA of strain CAU 11108^T was extracted by the method of Marmur (1961). PCR amplification of 16S rRNA genes was accomplished using 27F and 1525R primers (Lane, 1991) with conditions according to Nam *et al.* (2004). The amplified 16S rRNA gene was sequenced directly using a BigDye Terminator Cycle Sequencing kit (Applied Biosystems) and a 3730 automatic DNA sequencer (Applied Biosystems). The 16S rRNA gene sequence of the strain was determined and compared with available

Abbreviation: TEM, transmission electron microscopy.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain CAU 11108^T is JX233496.

One supplementary figure is available with the online Supplementary Material.

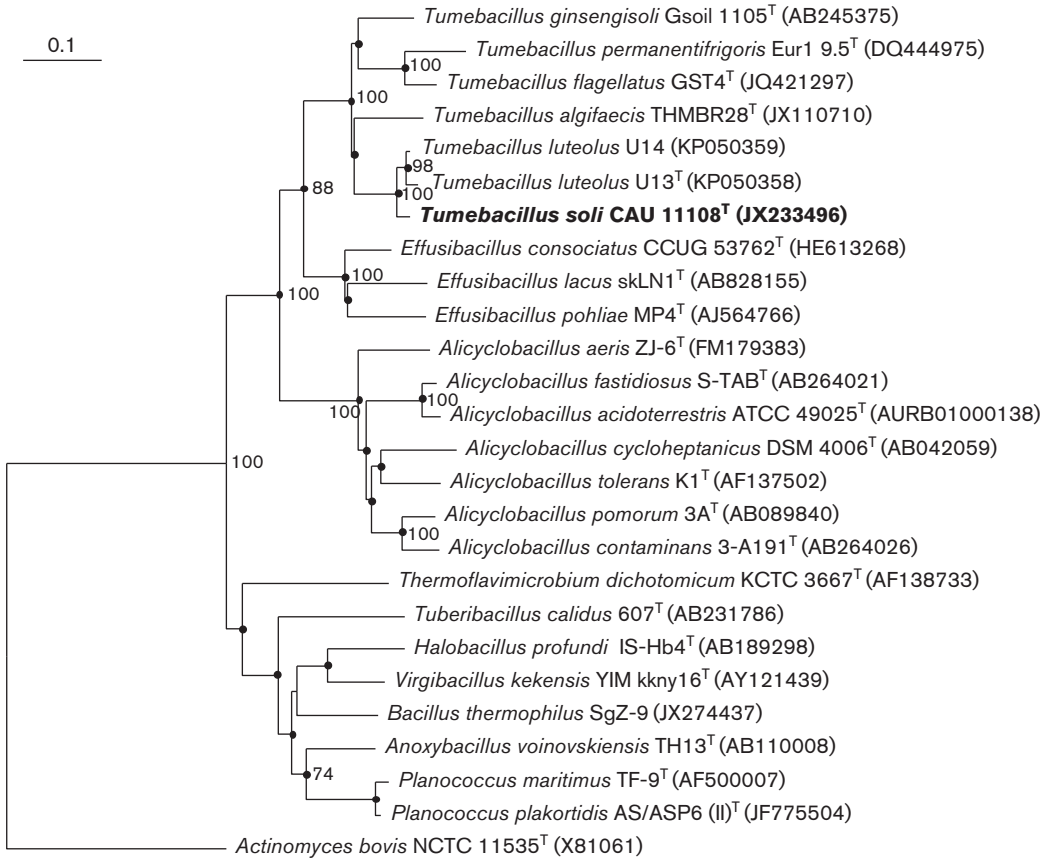

Fig. 1. Neighbour-joining phylogenetic tree based on nearly complete 16S rRNA gene sequences showing the relationships between strain CAU 11108^T and representative strains of recognized species of the genus *Tumebacillus*. Filled circles indicate that the corresponding nodes were also recovered in the trees generated with the maximum-likelihood and least-squares algorithms. Numbers at nodes indicate levels of bootstrap support based on a neighbour-joining analysis of 1000 resampled datasets; only values >70 % are given. Bar, 0.1 substitutions per nucleotide position. *Actinomyces bovis* NCTC 11535^T (X81061) is used as an outgroup organism.

reference sequences in the GenBank database (Benson *et al.*, 2013), accessed June 2015. Multiple sequence alignments with sequences of a broad selection of members of the genus *Tumebacillus* and calculation of sequence similarity levels were carried out by using EzTaxon-e (Kim *et al*, 2012; http://www.ezbiocloud.net/eztaxon) and CLUSTAL -X 2.1 (Larkin *et al.,* 2007). Evolutionary distance matrices were generated by the neighbour-joining method described by Jukes & Cantor (1969). Phylogenetic trees were reconstructed using the neighbour-joining (Saitou & Nei, 1987), least-squares (Fitch & Margoliash, 1967) and maximum-likelihood (Felsenstein, 1981) algorithms in the PHYLIP package (Felsenstein, 1989). Tree topology was evaluated by the bootstrap resampling method (Felsenstein, 1985) with 1000 replicates of the neighbour-joining dataset with the SEQBOOT and CONSENSE programs from the PHYLIP package. The extent of DNA–DNA relatedness between CAU 11108^T and the most closely related phylogenetic neighbours, *T. luteolus* U13^T and *T. luteolus* U14, was determined using the fluorometric microplate method (Ezaki *et al*., 1989), as modified by Goris *et al*. (1998). The DNA G+C content (mol%) of the genomic DNA was determined using HPLC by the method of Tamaoka & Komagata, 1984.

The nearly complete 16S rRNA gene sequence of strain CAU 11108^T (1522 bp) was sequenced and compared with four reference sequences of type strains of the genus *Tumebacillus* in the GenBank database (accessed August 2015). Phylogenetic analysis indicated that strain CAU 11108^T fell into the genus *Tumebacillus* (Fig. 1). Strain CAU 11108^T exhibited the highest similarity of 16S rRNA gene sequence to *T. luteolus* U13^T (98.2 %), *T. luteolus* U14 (97.9 %), *T. ginsengisoli* Gsoil 1105^T (95.3 %), *T. flagellatus* GST4^T (95.0 %), *T. algifaecis* THMBR28^T (95.0 %) and *T. permanentifrigoris* Eur1 9.5^T (93.1 %). The DNA–DNA relatedness between CAU 11108^T and the most closely related strains, *T. luteolus* U13^T and *T. luteolus* U14, was 23 % and 25 %, respectively. These values are well below the 70 % cut-off point recommended by Moore

Table 1. Differential properties of strain CAU 11108^T and the type strains of the most closely related species of the genus *Tumebacillus* species

Strains: 1, CAU 11108^T; 2, *T. permanentifrigoris* JCM 14557^T; 3, *T. ginsengisoli* KCTC 13942^T; 4, *T. flagellatus* DSM 25748^T; 5, *T. algifaecis* NBRC 108765^T. +, Positive; −, negative; w, weakly positive. All strains were positive for hydrolysis of casein, and assimilation of cellobiose and lactose. All strains were sensitive to ampicillin, chloramphenicol, kanamycin and streptomycin. Data were obtained in this study unless indicated.

Characteristic	1	2	3	4	5
Colony colour	Cream	Yellow*	White*	Light yellow*	White†
Motility	+	−*	−*	+*	+†
Flagella	+	−*	−*	+*	+†
Temperature range for growth (°C)	25–45	5–37*	20–42*	20–42*	20-45†
Optimum temperature for growth	37	25–30*	30*	37*	30†
pH range for growth	5.5–8.5	5.5–9.0*	5.0–8.5*	4.5–8.5*	5.0–9.5†
NaCl tolerance (%)	0–2	0–0.5*	0–1*	0–1*	0–1†
Optimum	1	0*	0*	0.5*	0.5†
Oxidase	+	−*	+*	+*	+†
Hydrolysis of:					
Gelatin	+	−*	+*	+*	+†
Starch	−	+*	+*	+*	−†
Citrate	+	w*	−*	−*	−†
Response to antibiotics					
Erythromycin (15 µg)	S	S*	R*	S*	S
Penicillin (10 U)	S	R*	S*	S*	S†
Acid production from:					
D-Fructose	−	+	−	+	−
L-Arabinose	−	−	+	−	+
D-Xylose	−	−	+	+	−
D-Galactose	−	+	+	+	+
D-Glucose	−	+	+	+	+
D-Mannitol	−	+	+	+	−
D-Sorbitol	−	−	+	+	−
D-Maltose	−	+	−	+	+
D-Trehalose	−	+	+	+	−
DNA G+C content (mol%)	54.6	53.1*	55.6*	53.7*	57.6†

*Data from Wang *et al.* (2013).
†Data from Wu *et al.* (2015).

et al., 1987 for the delineation of genomic species, supporting the proposal that strain CAU 11108^T represents a separate species. The genomic DNA of strain CAU 11108^T had a G+C content of 54.6 mol%, a value in the range (53.1–57.6 mol%) reported for species of the genus *Tumebacillus* (Steven *et al.*, 2008; Baek *et al.*, 2011; Wang *et al.*, 2013; Wu *et al.*, 2015).

Strain CAU 11108^T was cultivated on R2A medium to investigate all morphological, physiological and biochemical characteristics. Cell morphology was examined by light (DM 1000; Leica) and transmission electron (TEM; JEM 1010; JEOL) microscopy. The presence of flagella was determined using TEM using cells from an exponentially growing culture after negatively staining cells with 1 % (w/v) phosphotungstic acid. Gram staining was carried out using a Gram staining kit (bioMérieux) according to the manufacturer's instructions. Motility was assessed in a half strength R2A liquid medium for 72 h using the hanging-drop method (Bowman *et al.*, 2000). Growth in R2A medium at 4, 10, 15, 20, 25, 30, 37, 40, 45 and 50 °C in a MIR-253 aerobic incubator (Sanyo) and in a Bactron anaerobic chamber (Sheldon) containing a humidified atmosphere of 90 % nitrogen, 5 % carbon dioxide and 5 % hydrogen was evaluated by measuring the turbidity of the broth after 72 h. Growth was tested at 37 °C in R2A medium adjusted to pH 4.0–11.5 at increments of 0.5 pH intervals by using sodium acetate/acetic acid and Na_2CO_3 buffers. Growth in the absence of NaCl and in the presence of 0–15.0 % (w/v) NaCl at 1 % intervals was investigated at 37 °C in R2A medium prepared according to the formula of the Difco medium except that NaCl was excluded and that 0.45 % (w/v) $MgCl_2.6H_2O$ and 0.06 % (w/v) KCl were added. Catalase and oxidase tests were performed as

Table 2. Cellular fatty acid contents (percentages) of strain CAU 11108^T and the type strains of the most closely related species of the genus *Tumebacillus*

Strains: 1, CAU 11108^T; 2, *T. permanentifrigoris* JCM 14557^T; 3, *T. ginsengisoli* KCTC 13942^T; 4, *T. flagellatus* DSM 25748^T; 5, *T. algifaecis* NBRC 108765^T. Data are from this study. All strains were cultured under identical conditions (R2A agar for 3 days at 37 °C) and analysed using the Microbial Identification System. Fatty acids amounting to >0.5 % of the total fatty acids in all strains are shown.

Fatty acids	1	2	3	4	5
$C_{9:0}$	1.01	–	–	1.1	2.9
$C_{14:0}$	–	1.3	1.6	–	–
$C_{16:0}$	6.7	4.5	2.9	2.3	11.2
$C_{18:0}$	4.9	–	–	–	3.3
$C_{13:1}$ at 12–13	1.0	–	–	–	–
$C_{18:1}\omega 9c$	3.1	–	–	0.6	2.5
anteiso-$C_{15:0}$	13.2	27.6	24.1	4.1	–
anteiso-$C_{17:0}$	0.8	–	2.4	–	–
anteiso-$C_{17:1}\omega 9c$	1.1	–	–	–	–
iso-$C_{11:0}$	–	–	–	–-	1.7
iso-$C_{13:0}$	–	0.8	0.6	1.3	–
iso-$C_{14:0}$	1.1	8.3	2.8	9.1	–
iso-$C_{15:0}$	46.9	50.7	58.2	66.9	58.9
iso-$C_{16:0}$	3.4	1.3	5.6	2.1	–
iso-$C_{17:0}$	1.4	0.5	1.0	0.6	2.9
iso-$C_{16:1}$ H	–	–	0.8	–	–
iso-$C_{13:0}$ 3-OH	3.3	–	–	0.7	2.4
iso-$C_{15:1}$ F	3.5	–	–	0.6	2.5
Summed feature 1	–	2.7	0.9	9.2	2.8
Summed feature 3	1.3	0.7	–	–	–
Summed feature 4	–	1.6	–	0.8	8.8
Summed feature 8	7.2	–	–	–	–

Summed features represent groups of two or three fatty acids that could not be separated by GLC with the MIDI system. Summed feature 1 contained iso-C13 : 0 3-OH/iso-$C_{15:1}$H, summed feature 3 contained $C_{16:1}\omega 7c/\omega 6c$, summed feature 4 contained anteiso-$C_{17:1}$ B/iso-$C_{17:1}$ I and summed feature 8 contained $C_{18:1}\omega 7c/\omega 6c$.

described by Cappuccino & Sherman (2002). Hydrolysis of gelatin, casein, starch, aesculin and citrate was determined according to the methods of Lányi (1987) and Smibert & Krieg (1994). Biochemical characterizations were performed using the API 20E and API 50CH systems (bioMérieux). API 20E strips were read after 24 h and API 50CH strips after 24 h and 48 h, respectively. Antibiotic susceptibility was examined on R2A at 37 °C by the disc diffusion method by using Sensi-Disc susceptibility test discs (BD BBL). The following antibiotics were tested: ampicillin (10 μg), chloramphenicol (30 μg), erythromycin (15 μg), kanamycin (30 μg), penicillin (10 U), streptomycin (10 μg) and nalidixic acid (30 μg). Inhibition zone >10 mm in diameter indicated susceptibility and the absence of inhibition zones indicated resistance.

Cells of strain CAU 11108^T were Gram-stain-positive and rod-shaped (0.7–1.25 μm in diameter and 0.7–2.7 μm in length). Strain CAU 11108^T was observed to be motile and TEM demonstrated the presence of peritrichous flagella and swollen terminal endospores (Fig. S1, available in the online Supplementary Material). The phenotypic properties of strain CAU 11108^T are given in Table 1 and in the species description. Strain CAU 11108^T hydrolysed casein, gelatin and citrate and it was positive for assimilation of cellobiose and lactose. However, strain CAU 11108^T differed from its closest relatives, *T. permanentifrigoris* JCM 14557^T, *T. ginsengisoli* KCTC 13942^T and *T. flagellatus* DSM 25748^T by its inability to hydrolyse of starch or produce acid from D-galactose, D-glucose, D-mannitol and D-trehalose. Overall, the results obtained in this study are in agreement with previously published data for the three species of the genus *Tumebacillus* (Steven *et al.,* 2008; Baek *et al.,* 2011; Wang *et al.*, 2013).

Chemotaxonomic properties including cellular fatty acids, respiratory quinone and cell-wall peptidoglycan of strain CAU 11108^T were compared under the same culture conditions used in previous studies of members of the genus *Tumebacillus* (Steven *et al.,* 2008; Baek *et al.,* 2011; Wang *et al.*, 2013). The cell mass of strain CAU 11108^T was harvested from R2A agar (BD Difco) after cultivation for 3

days at 37 °C. The physiological age of the biomass harvested for fatty acid analysis was standardized by observing growth development during incubation of the cultures and choosing the moment of harvesting according to the standard MIDI protocol (Sherlock Microbial Identification System version 6.1). Cellular fatty acid methyl esters (FAMEs) were obtained according to the method of Minnikin *et al.* (1980) and separated by a 6890N automated gas chromatography system (Agilent Technologies). Peaks were identified by using the Microbial Identification software package (MOORE library v. 5.0; MIDI database TSBA6). Respiratory quinones were extracted according to the method of Komagata & Suzuki (1987) and analysed by HPLC. The peptidoglycan structure was determined as described by Schleifer & Kandler (1972). Strain CAU 11108^T contained menaquinone-7 (MK-7) as the predominant isoprenoid quinone and *meso*-diaminopimelic acid, suggesting peptidoglycan type A1γ. These characteristics are in agreement with those reported for *T. ginsengisoli* Gsoil 1105^T (Baek *et al.,* 2011), *T. flagellatus* GST4^T (Wang *et al.*, 2013) and *T. permanentifrigoris* Eur1 9.5^T (Steven *et al.,* 2008), the type species of the genus *Tumebacillus*.

The fatty acid profile of strain CAU 11108^T is shown Table 2. The fatty acids (>10 % of the total) were iso-$C_{15:0}$ (46.9 %) and anteiso-$C_{15:0}$ (13.2 %). The following fatty acids were present to at least 0.5 %: $C_{9:0}$, $C_{16:0}$, $C_{18:0}$, $C_{13:1}$ at 12–13, $C_{13:1}\omega 9c$, iso-$C_{14:0}$, iso-$C_{16:0}$, iso-$C_{17:0}$, iso-$C_{13:0}$ 3-OH, iso-$C_{15:1}$ F, anteiso-$C_{17:0}$ and anteiso-$C_{17:1}\omega 9c$. This fatty acid profile is similar to those of the closely related members of the genus *Tumebacillus* in that iso-$C_{15:0}$ and anteiso-$C_{15:0}$ were predominant fatty acids, although there were differences between strain CAU 11108^T and other species of the genus *Tumebacillus* in the percentages of other fatty acids.

Therefore, these data together provide sufficient evidence to support the proposal to recognize strain CAU 11108^T as a representative of a novel species of the genus *Tumebacillus*, for which the name *Tumebacillus soli* sp. nov. is proposed.

Description of *Tumebacillus soli* sp. nov.

Tumebacillus soli sp. nov. (so′li. L. gen. n. *soli* of the soil).

Cells are Gram-stain-positive, motile, strictly aerobic rods approximately 0.7–1.25 μm in diameter and 0.7–2.7 μm in length, with peritrichous flagella and terminal endospores in swollen sporangia. Colonies on R2A agar plates are cream-coloured, circular and convex with entire margins after 3 days of incubation at 37 °C. Growth occurs at 20 °C–45 °C (optimum, 37 °C) and at pH 5.5–8.5 (optimum, pH 8.0). Growth occurs with 0–2 % (w/v) NaCl (optimum, 1 %). Oxidase production is positive but catalase reduction is negative. Hydrolyses casein, gelatin and citrate, and assimilates cellobiose and lactose. The cell-wall peptidoglycan contains *meso*-diaminopimelic acid. train CAU 11108^T is susceptible to ampicillin, chloramphenicol, erythromycin, kanamycin, penicillin, streptomycin and nalidixic acid. The major isoprenoid quinone is menaquinone-7. The predominant cellular fatty acids are iso-$C_{15:0}$ and anteiso-$C_{15:0}$.

The type strain, CAU 11108^T (=KCTC 33141^T=CECT 8918^T), was isolated from soil collected from Danghangpo in the Republic of Korea. The DNA G+C content of the type strain is 54.6 mol%.

Acknowledgements

This work was supported by the project on survey of indigenous species of Korea of the National Institute of Biological Resources (NIBR) under the Ministry of Environment (MOE).

References

Baek, S. H., Cui, Y., Kim, S. C., Cui, C. H., Yin, C., Lee, S. T. & Im, W. T. (2011). *Tumebacillus ginsengisoli* sp. nov., isolated from soil of a ginseng field. *Int J Syst Evol Microbiol* **61**, 1715–1719 .

Benson, D. A., Cavanaugh, M., Clark, K., Karsch-Mizrachi, I., Lipman, D. J., Ostell, J. & Sayers, E. W. (2013). Genbank. *Nucleic Acids Res* **41**, D36–D42.

Bowman, J. P. (2000). Description of *cellulophaga algicola* sp. nov., isolated from the surfaces of antarctic algae, and reclassification of *cytophaga uliginosa* (zobell and upham 1944) reichenbach 1989 as *cellulophaga uliginosa* comb. nov. *Int J Syst Evol Microbiol* **50** , 1861–1868.

Cappuccino, J. G. & Sherman, N. (2002). *Microbiology: a Laboratory Manual. Menlo Park*, 6th edn, CA: Benjamin/Cummings.

Ezaki, T., Hashimoto, Y. & Yabuuchi, E. (1989). Fluorometric deoxyribonucleic acid-deoxyribonucleic acid hybridization in microdilution wells as an alternative to membrane filter hybridization in which radioisotopes are used to determine genetic relatedness among bacterial strains. *Int J Syst Bacteriol* **39**, 224–229.

Felsenstein, J. (1981). Evolutionary trees from DNA sequences: a maximum likelihood approach. *J Mol Evol* **17**, 368–376.

Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**, 783–791.

Felsenstein, J. (1989). PHYLIP – phylogeny inference package (version 3.2). *Cladistics* **5**, 164–166.

Fitch, W. M. & Margoliash, E. (1967). Construction of phylogenetic trees. *Science* **155**, 279–284.

Fitch, W. M. (1971). Toward defining the course of evolution: minimum change for a specific tree topology. *Syst Zool* **20**, 406–416.

Goris, J., Suzuki, Ken-ichiro., Vos, P. D., Nakase, T. & Kersters, K. (1998). Evaluation of a microplate DNA - DNA hybridization method compared with the initial renaturation method. *Can J Microbiol* **44**, 1148–1153.

Jukes, T. H. & Cantor, C. R. (1969). Evolution of protein molecules. In *Mammalian Protein Metabolism*, pp. 21–132. Edited by H. H. Munro. New York: Academic Press.

Kim, O. S., Cho, Y. J., Lee, K., Yoon, S. H., Kim, M., Na, H., Park, S. C., Jeon, Y. S., Lee, J. H. & other authors (2012). Introducing eztaxon-e: a prokaryotic 16S rrna gene sequence database with phylotypes that represent uncultured species. *Int J Syst Evol Microbiol* **62**, 716–721.

Komagata, K. & Suzuki, K. (1988). Lipid and cell-wall analysis in bacterial systematics. *Methods Microbiol* **19**, 161–207.

Lane, D. J. (1991). 16S/23S RNA sequencing. In *Nucleic Acid Techniques in Bacterial Systematics*, pp. 115–175. Edited by E. Stackebrandt & M. Goodfellow. London: John Wiley & Sons Ltd.

Lanyi, B. (1988). Classical and rapid identification methods for medically important bacteria. *Methods Microbiol* **19**, 1–67.

Larkin, M. A., Blackshields, G., Brown, N. P., Chenna, R., McGettigan, P. A., McWilliam, H., Valentin, F., Wallace, I. M., Wilm, A. (2007). Clustal W and Clustal X version 2.0. *Bioinformatics* **23**, 2947–2948.

Marmur, J. (1961). A procedure for the isolation of deoxyribonucleic acid from micro-organisms. *J Mol Biol* **3**, 208–218.

Minnikin, D. E., Hutchinson, I. G., Caldicott, A. B. & Goodfellow, M. (1980). Thin-layer chromatography of methanolysates of mycolic acid-containing bacteria. *J Chromatogr A* **188**, 221–233.

Moore, W. E. C., Stackebrandt, E., Kandler, O., Colwell, R. R., Krichevsky, M. I., Truper, H. G., Murray, R. G. E., Wayne, L. G., Grimont, P. A. D. & other authors (1987). Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. *Int J Syst Evol Microbiol* **37**, 463–464.

Nam, S. W., Kim, W., Chun, J. & Goodfellow, M. (2004). *Tsukamurella pseudospumae* sp. nov., a novel actinomycete isolated from activated sludge foam. *Int J Syst Evol Microbiol* **54**, 1209–1212.

Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.

Schleifer, K. H. & Kandler, O. (1972). Peptidoglycan types of bacterial cell walls and their taxonomic implications. *Bacteriol Rev* **36**, 407–477.

Smibert, R. M. & Krieg, N. R. (1994). Phenotypic characterization. In *Methods for General and Molecular Bacteriology*, pp. 607–654. Edited by P. Gerhardt, R. G. E. Murray, W. A. Wood & N. R. Krieg. Washington, DC: American Society for Microbiology.

Steven, B., Chen, M. Q., Greer, C. W., Whyte, L. G. & Niederberger, T. D. (2008). *Tumebacillus permanentifrigoris* gen. nov., sp. nov., an aerobic, spore-forming bacterium isolated from canadian high arctic permafrost. *Int J Syst Evol Microbiol* **58**, 1497–1501.

Tamaoka, J. & Komagata, K. (1984). Determination of DNA base composition by reversed-phase high-performance liquid chromatography. *FEMS Microbiol Lett* **25**, 125–128.

Wang, Q., Xie, N., Qin, Y., Shen, N., Zhu, J., Mi, H. & Huang, R. (2013). *Tumebacillus flagellatus* sp. nov., an α-amylase/pullulanase-producing bacterium isolated from cassava wastewater. *Int J Syst Evol Microbiol* **63**, 3138–3142.

Wu, Y. F., Zhang, B., Xing, P., Wu, Q. L. & Liu, S. J. (2015).*Tumebacillus algifaecis* sp. nov., isolated from decomposing algal scum. *Int J Syst Evol Microbiol* **65**, 2194–2198.