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ORIGINAL PAPER

Cellulosimicrobium marinum sp. nov., an actinobacterium isolated from sea sediment

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Abstract A novel Gram stain positive actinobacterium, designated RS-7-4^T, was isolated from a sea sediment sample collected in Indonesia, and its taxonomic position was investigated using a polyphasic approach. Strain RS-7-4^T was observed to form vegetative hyphae in the early phase of growth, but the hyphae eventually fragmented into short rods to coccoid cells. Growth occurred at 15–37 °C, pH 6.0–11.0 and in the presence of 0–7 % (w/v) NaCl. Phylogenetic analysis based on 16S rRNA gene sequence comparisons revealed that strain RS-7-4^T was closely related to the members of the genus *Cellulosimicrobium*, with a similarity range of 98.08–99.10 %. The peptidoglycan type of strain RS-7-4^T was found to be A4α L-Lys–L-Thr–D-Asp. The predominant menaquinone was MK-9(H_4), and the major fatty acids were anteiso-$C_{15:0}$, iso-$C_{15:0}$ and anteiso-$C_{17:0}$. The DNA G+C content was 75.6 mol%. These chemotaxonomic features corresponded to those of the genus *Cellulosimicrobium*. Meanwhile, the results of DNA–DNA hybridization, and physiological and biochemical tests revealed that strain RS-7-4^T was different from the recognized species of the genus *Cellulosimicrobium*. Therefore, strain RS-7-4^T represents a novel species of the genus *Cellulosimicrobium*, for which the name *Cellulosimicrobium marinum* sp. nov. is proposed. The type strain is RS-7-4^T (=NBRC 110994^T =InaCC A726^T).

Keywords Actinobacteria · Sea sediment · *Micrococcineae* · *Cellulosimicrobium marinum* · Polyphasic taxonomy

Introduction

The genus *Cellulosimicrobium* within the family *Promicromonosporaceae* was proposed by the reclassification of *Cellulomonas cellulans* based on its phylogenetic and chemotaxonomic evidences (Schumann et al. 2001). To date, three more species, *Cellulosimicrobium variabile* (Bakalidou et al. 2002), *Cellulosimicrobium funkei* (Brown et al. 2006) and *Cellulosimicrobium terreum* (Yoon et al. 2007), have been described within the genus. However, *C. variabile* was later reclassified to the novel genus *Isoptericola* within the family *Promicromonosporaceae* as *Isoptericola variabilis* based on distinct phylogenetic position and the amino acid composition of the peptidoglycan (Stackebrandt et al. 2004). The members of the genus *Cellulosimicrobium* have peptidoglycan type of A4α, with lysine as the diagnostic diamino acid and the predominant menaquinone is MK-9(H_4). The type strains of *C. cellulans* and *C. terreum* were isolated from soils, while that of *C. funkei* was isolated from human blood.

Marine environments have attracted attention as an isolation source of actinobacteria, and it has been reported that marine-derived actinobacteria are useful for screening for novel bioactive substances (Blunt et al. 2007; Lam 2006). During the course of a study on the actinobacterial

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diversity in seashore environments, a novel actinobacterium was isolated from a sea sediment sample collected in Indonesia. Comparative 16S rRNA gene sequence analysis revealed that the isolate was phylogenetically related to the members of the genus *Cellulosimicrobium*. In this study, we determined the taxonomic position of the isolate using a polyphasic approach.

Materials and methods

Bacterial strain and isolation

Strain RS-7-4^T was isolated from a sea sediment sample that had been collected from the foreshore of Rambut Island (5°58′634″S 106°41′596″E), DKI Jakarta, Indonesia. Approximately 1 g of the sample was diluted 10-, 100- and 1000-fold with artificial seawater (Wako) before 0.2 ml of each dilution was spread on plates of 1/5 NBRC medium 802 [0.2 % (w/v) polypeptone, 0.04 % (w/v) yeast extract, 0.02 % (w/v) $MgSO_4 \cdot 7H_2O$ and 1.5 % (w/v) agar; at pH 7.0] supplemented with 5.0 % (w/v) NaCl, 0.005 % (w/v) cycloheximide and 0.002 % (w/v) nalidixic acid. The plates were incubated at 30 °C for 4 days, and then visible colonies were transferred to new plates. After single-colony isolation, strain RS-7-4^T was obtained. As the strain did not require NaCl for growth, full-strength NBRC medium 802 [1.0 % (w/v) polypeptone, 0.2 % (w/v) yeast extract, 0.1 % (w/v) $MgSO_4 \cdot 7H_2O$ and, if required, 1.5 % (w/v) agar, at pH 7.0] was used as the basal medium unless otherwise stated for this study. *C. cellulans* NBRC 15516^T, *C. funkei* NBRC 104118^T and *C. terreum* KCTC 19206^T were used as the reference strains in this study.

Morphological, physiological and biochemical tests

Colony appearance was examined after cultivation at 28 °C for 2 days on an agar plate of NBRC medium 802. Morphological features were observed depending on the age (up to 5 days) under a light microscope (BX-51; Olympus) and a scanning electron microscope (JSM-6060; JEOL). The temperature range and optimum temperature for growth were determined by cultivating cultures at 5, 10, 15, 20, 25, 28, 37, 45 and 60 °C on agar plates of NBRC medium 802 for 4 days (15–60 °C) or 14 days (5 and 10 °C). The pH range and optimum pH for initial growth were established by using liquid NBRC medium 802 adjusted to pH 4–12 (in 1.0 pH unit intervals) with either 4 M HCl or 5 M KOH after 4 days incubation at 28 °C. Tolerance to NaCl was determined using liquid NBRC medium 802 adjusted to NaCl concentrations of 1, 3, 5, 7, 10 and 15 % (w/v) after 4-day incubation at 28 °C. Cell motility, oxidase and catalase activities, anaerobic growth and Gram staining were determined using the methods described by Hamada et al. (2012). Other physiological and biochemical tests were performed using API ZYM, API Coryne and API 50 CH systems (bioMérieux) according to the manufacturer's instructions.

16S rRNA gene sequence determination and phylogenetic analysis

DNA was isolated using PrepMan Ultra Reagent (Applied Biosystems) according to the manufacturer's instructions. The 16S rRNA gene was amplified by PCR using KOD FX (Toyobo) with the following primer pair: 9F (5′-GAGTTTGATCCTGGCTCAG) and 1541R (5′-AAGGAGGTGATCCAGCC). The amplified 16S rRNA gene was subjected to cycle sequencing using a BigDye Terminator version 3.1 cycle sequencing kit (Applied Biosystems) with the following primers: 9F, 785F (5′-GGATTAGATACCCTGGTAGTC), 802R (5′-TACCAGGGTATCTAATCC) and 1541R. The products were analyzed using an automated DNA sequencer (ABI PRISM 3730 Genetic Analyzer; Applied Biosystems). Phylogenetic neighbors were identified, and pairwise 16S rRNA gene sequence similarities were calculated using the EzTaxon-e server (Kim et al. 2012). The CLUSTAL_X program (Thompson et al. 1997) was used to align the almost-complete 16S rRNA gene sequence of strain RS-7-4^T (1476 nt) with corresponding sequences of related taxa. Phylogenetic trees were reconstructed by the neighbor-joining (NJ) (Saitou and Nei 1987) and maximum likelihood (ML) (Felsenstein 1981) algorithms by using the MEGA 6.0 program (Tamura et al. 2013). The resultant tree topologies were evaluated by bootstrap analysis (Felsenstein 1985) based on 1000 replicates.

G+C content of genomic DNA and DNA–DNA hybridization

Genomic DNA was obtained using the method of Saito and Miura (1963). The DNA G+C content was determined by the method of Tamaoka and Komagata (1984). The microplate hybridization method (Ezaki et al. 1989) was used to determine DNA–DNA relatedness. DNA–DNA hybridizations were performed using five replications. After the highest and lowest values for each sample were excluded, the mean of the remaining three values was reported as the DNA–DNA relatedness value.

Chemotaxonomic tests

Biomass for chemotaxonomic studies, except for fatty acid analysis, was obtained by cultivating the strain in shake

flasks at 28 °C and 100 r.p.m. for 48 h. Amino acids and their isomers in cell wall hydrolysate, cell wall sugars, isoprenoid quinones and DNA G+C content were determined according to the methods described by Hamada et al. (2012). Polar lipids were extracted using the method described by Minnikin et al. (1975) and analyzed by TLC with chloroform/methanol/water (65:25:4, by vol.) in the first direction and chloroform/acetic acid/methanol/water (80:18:12:5, by vol.) in the second. Lipids were visualized by spraying the TLC plate with 10 % molybdophosphoric acid. Anisaldehyde (sugar), ninhydrin (amino groups), Schiff's reagent (glycol) and Dittmer–Lester reagent (phosphorous; Dittmer and Lester 1964) were also used as specific spray reagents for polar lipids. For fatty acid methyl ester analysis, strain RS-7-4^T and the reference strains were cultivated on tryptic soy agar (Difco) for 24 h at 28 °C. Cellular fatty acid methyl esters were analyzed by GC (6890 N; Agilent Technologies) according to the standard protocol of the Sherlock Microbial Identification System (Sasser 1990) with Sherlock MIDI software (version 6.2) and the TSBA6 database (version 6.2).

Nucleotide sequence accession numbers

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain RS-7-4^T is LC042213.

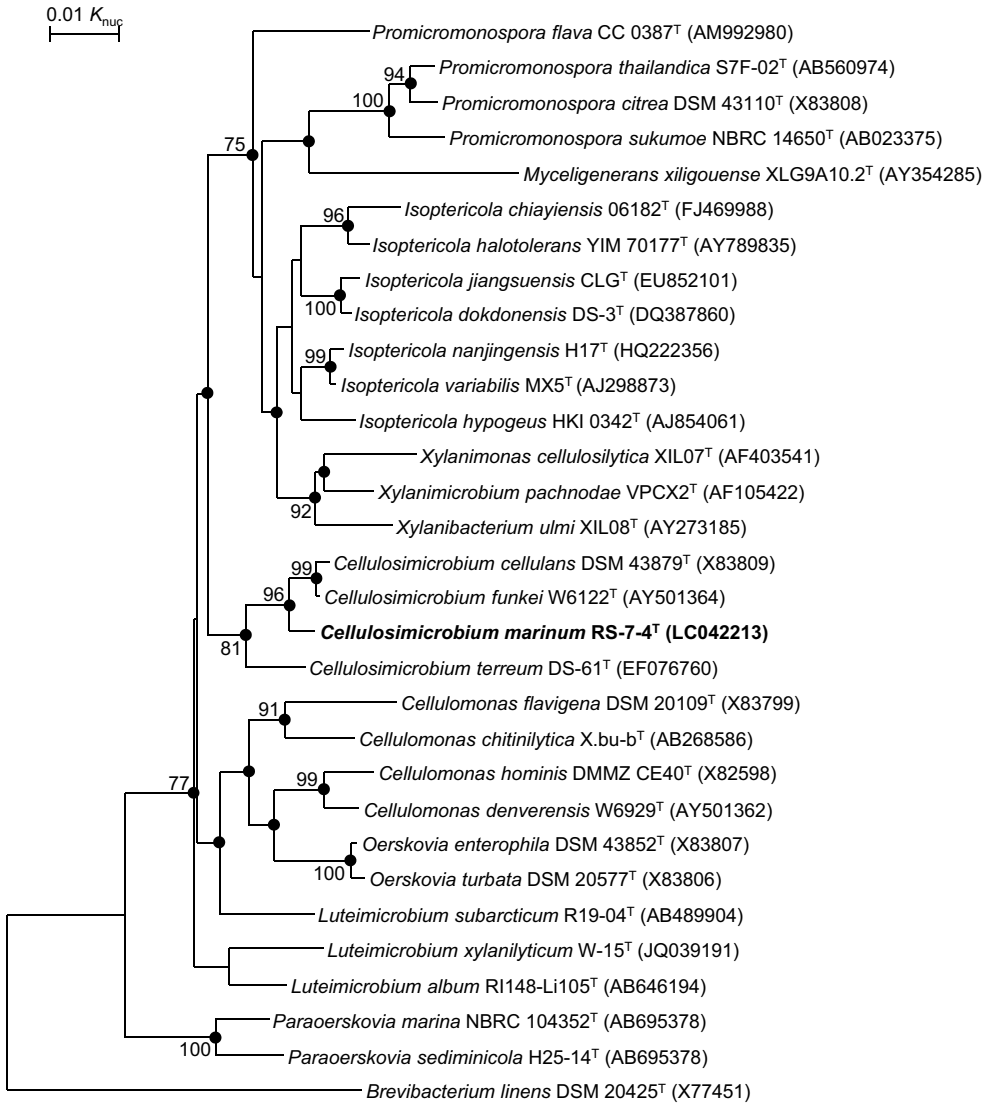

Fig. 1 Phylogenetic tree derived from the 16S rRNA gene sequences of strain RS-7-4^T and their taxonomic neighbors, reconstructed with the neighbor-joining algorithm. The 16S rRNA gene sequence of *Brevibacterium linens* DSM 20425^T (X77451) was used as the outgroup. Bootstrap values (>70 %) based on 1000 replicates are shown at branch nodes. *Filled circles* indicate that the corresponding nodes were also recovered in the tree generated with the maximum likelihood algorithm. Bar, 0.01 K_{nuc} substitutions per nucleotide position

Results and discussion

Strain RS-7-4^T was observed to form yellow, circular and smooth colonies that were approximately 0.5–1.0 mm in diameter after 2-day cultivation. The cells were Gram stain positive, non-motile and non-endospore forming. Strain RS-7-4^T was found to develop branched vegetative hyphae (0.4–0.5 × 3–10 μm) in the early phase of growth, but these hyphae eventually fragmented into short rods to coccoid cells (0.4–0.5 × 0.4–1.0 μm) (Supplementary Fig. S1). The strain was catalase positive but oxidase negative. The strain grew at 15–37 °C but not at 5, 10, 45 or 60 °C. The pH range for growth was 6.0–11.0. Optimal growth was noted at 28 °C and pH 7.0–8.0. The strain exhibited good growth with NaCl concentrations of 0–5 % (w/v) and moderate growth with 7 % (w/v) NaCl; no growth was observed with 10 or 15 % (w/v) NaCl. Growth under anaerobic conditions was not observed. The results of other physiological and biochemical tests are summarized in the species description below.

Phylogenetic analysis based on the 16S rRNA gene sequences revealed that strain RS-7-4^T was closely related to the members of the genus *Cellulosimicrobium*. In the NJ tree, strain RS-7-4^T and the type strains of *C. cellulans* and *C. funkei* formed a monophyletic cluster with a bootstrap resampling value of 96 % (Fig. 1). This cluster was also recovered in the tree generated by the ML algorithm. The highest 16S rRNA gene sequence similarity was observed with *C. funkei* ATCC BAA-886^T (99.10 %), followed by *C. cellulans* LMG 16121^T (99.04 %), *C. terreum* DS-61^T (98.08 %) and *Luteimicrobium xylanilyticum* W-15^T (97.04 %). Strain RS-7-4^T showed low DNA–DNA relatedness values with *C. funkei* NBRC 104118^T (19–20 %), *C. cellulans* NBRC 15516^T (20–30 %) and *C. terreum* KCTC 19206^T (15–28 %) (Table 1). These values are well below the 70 % cutoff point of DNA–DNA relatedness, which is a criterion for the assignment of bacterial strains to the same genomic species (Wayne et al. 1987).

The peptidoglycan sample of strain RS-7-4^T was found to contain alanine (Ala), aspartic acid (Asp), glutamic acid (Glu), lysine (Lys) and threonine (Thr) in a molar ratio of 2.2:1.0:1.0:0.9:0.7. Enantiomeric analysis of the peptidoglycan amino acids revealed the presence of D-Ala, L-Ala, D-Asp, D-Glu, L-Lys and L-Thr. These data strongly suggested that the peptidoglycan type of strain RS-7-4^T is A4α L-Lys–L-Thr–D-Asp, A11.45 (Schleifer and Kandler 1972; Schumann 2011). This interpeptide bridge was the same as that of *C. terreum* but differed from those of *C. cellulans* and *C. funkei* (Yoon et al. 2007; Schumann et al. 2001; Schumann, personal communication). A small amount of glucose was detected as the only cell wall sugar. The predominant menaquinone was identified as MK-9(H_4), with MK-8(H_4) and MK-9(H_6) present as minor components (92:5:3). The major cellular fatty acids of strain RS-7-4^T were anteiso-$C_{15:0}$ (41.4 %), iso-$C_{15:0}$ (18.9 %) and anteiso-$C_{17:0}$ (15.8 %) (Table 2). The profile was found to be qualitatively similar to those of the recognized species of the genus *Cellulosimicrobium* although quantitative differences were found. The polar lipids were found to be phosphatidylglycerol, diphosphatidylglycerol, phosphatidylinositol and phosphatidylinositol mannosides

Table 1 DNA–DNA relatedness values (%) among strain RS-7-4^T and *Cellulosimicrobium* species

Strain	Relatedness value (%) with labeled DNA from			
	1	2	3	4
1. RS-7-4^T	100	30	20	28
2. *C. funkei* NBRC 104118^T	20	100	36	14
3. *C. cellulans* NBRC 15556^T	19	37	100	16
4. *C. terreum* KCTC 19206^T	15	27	26	100

Table 2 Cellular fatty acid compositions (%) of strain RS-7-4^T and *Cellulosimicrobium* species

Fatty acid	1	2	3	4
$C_{14:0}$	2.4	tr	1.4	1.6
$C_{16:0}$	9.8	4.3	7.3	9.5
$C_{17:0}$	tr	tr	tr	tr
$C_{18:0}$	tr	–	tr	1.9
iso-$C_{14:0}$	tr	tr	tr	tr
iso-$C_{15:0}$	**18.9**	**18.2**	**19.4**	8.4
iso-$C_{16:0}$	5.4	7.6	9.8	7.0
iso-$C_{17:0}$	3.7	2.4	2.6	2.0
iso-$C_{18:0}$	–	–	–	tr
anteiso-$C_{15:0}$	**41.4**	**48.6**	**42.6**	**40.9**
anteiso-$C_{17:0}$	**15.8**	**16.0**	**14.4**	**26.0**
anteiso-$C_{19:0}$	–	–	–	tr
$C_{17:1}$ ω5c	tr	tr	tr	–
$C_{18:3}$ ω6c (6,9,12)	tr	tr	tr	tr
3-OH $C_{17:0}$	–	tr	tr	–

Bold type indicates major components (>10 %). All strains were cultivated on tryptic soy agar at 28 °C for 24 h and were analyzed in parallel in this study

Strains: *1* RS-7-4^T, *2 C. funkei* NBRC 104118^T, *3 C. cellulans* NBRC 15556^T, *4 C. terreum* KCTC 19206^T

–, Not detected; *tr*, trace component (<1 %)

Table 3 Differential phenotypic characteristics of strain RS-7-4^T and *Cellulosimicrobium* species

Characteristic	1	2	3	4
Motility	−	+[b]	−[b]	−[d]
Growth at 37 °C	+	+	+	−
Enzyme activity (API ZYM)				
N-Acetyl-β-glucosaminidase	+	+	+	−
Acid phosphatase	−	+	+	+
Esterase (C4)	+	w	+	−
β-Galactosidase	−	+	+	−
β-Glucosidase	−	+	+	+
Phosphohydrolase	−	+	+	+
Trypsin	+	w	+	−
Enzyme activity (API Coryne)				
Nitrate reduction	−	+	+	+
Pyrazinamidase	−	−	+	+
Pyrrolidonyl arylamidase	+	−	−	−
Acid production from (API 50CH)				
L-Arabinose	−	+	+	+
Gluconate	+	−	−	−
Methyl-β-D-xylopyranoside	−	+	+	−
Interpeptide bridge of the peptidoglycan	L-Lys–L-Thr–D-Asp	L-Lys–D-Ser–D-Asp[c]	L-Lys–D-Ser–D-Asp[d]	L-Lys–L-Thr–D-Asp[e]
Polar lipids[a]	PG, DPG, PI, PIMs	ND	ND	PG, DPG, PI, PL[e]
DNA G+C content (mol%)	75.6	74.5 (T_m)[b]	74 (T_m)[d]	72.9[e]

Data are from this study unless indicated

Strains: *1* RS-7-4^T, *2 C. funkei* NBRC 104118^T, *3 C. cellulans* NBRC 15556^T, *4 C. terreum* KCTC 19206^T

[a] *PG* phosphatidylglycerol; *DPG* diphosphatidylglycerol; *PI* phosphatidylinositol

PIMs phosphatidylinositol mannosides; *PL* unidentified phospholipid

[b] Data are from Brown et al. (2006)

[c] Data are from Schumann (personal communication)

[d] Data are from Schumann et al. (2001)

[e] Data are from Yoon et al. (2007)

+ Positive, − negative, *w* weakly positive, *nd* no data available

(Supplementary Fig. S2). The DNA G+C content of strain RS-7-4^T was determined to be 75.6 mol%.

The result of the phylogenetic analysis based on the 16S rRNA gene sequences suggested that strain RS-7-4^T belonged to the genus *Cellulosimicrobium*, and the major chemotaxonomic characteristics of the strain also corresponded to those of the genus. Therefore, it is appropriate to regard the strain RS-7-4^T as a member of the genus *Cellulosimicrobium*. Meanwhile, the results of physiological and biochemical tests enabled strain RS-7-4^T to be distinguished from the type strains of the recognized species: *C. funkei*, *C. cellulans* and *C. terreum* (Table 3), and the DNA–DNA relatedness values between strain RS-7-4^T and these type strains were also low (Table 1). Therefore, it is proposed that strain RS-7-4^T should be classified as a representative of a novel species of the genus *Cellulosimicrobium*, with the name *Cellulosimicrobium marinum* sp. nov.

Description of *Cellulosimicrobium marinum* sp. nov

Cellulosimicrobium marinum (ma.ri′num. L. neut. adj. *marinum* belonging to the sea, marine)

Cells are Gram stain positive, aerobic, non-motile and non-endospore forming, and develop branched vegetative hyphae (0.4–0.5 × 3–10 μm) in the early phase of growth, but eventually fragment into short rods to coccoid cells (0.4–0.5 × 0.4–1.0 μm). Colonies are yellow, circular and smooth. Catalase positive and oxidase negative. The temperature range for growth is 15–37 °C (optimum 28 °C), and the pH range for growth is 6.0–11.0 (optimum pH 7.0–8.0). Growth occurs in NaCl concentrations of 0–7 % (w/v). *N*-Acetyl-β-glucosaminidase, alkaline phosphatase, esterase (C4), esterase lipase (C8), α-glucosidase, leucine

arylamidase, pyrrolidonyl arylamidase and trypsin are present, but acid phosphatase, chymotrypsin, cystine arylamidase, α-fucosidase, α- and β-galactosidases, β-glucosidase, β-glucuronidase, lipase (C14), α-mannosidase, phosphohydrolase, pyrazinamidase, urease and valine arylamidase are not. In API 50 CH assays, acid is produced from *N*-acetyl-glucosamine, amygdalin, D-cellobiose, D-fructose, D-galactose, gentiobiose, gluconate, D-glucose, glycerol, glycogen, D-lactose, D-maltose, D-mannose, methyl α-D-glucopyranoside, salicin, starch, D-sucrose, trehalose, turanose and D-xylose. Aesculin and gelatin are hydrolyzed. Nitrate is not reduced. The cell wall peptidoglycan is of the A4α L-Lys–L-Thr–D-Asp. The predominant menaquinone is MK-9(H_4); MK-8(H_4) and MK-9(H_6) are present as the minor components. The major cellular fatty acids are anteiso-$C_{15:0}$, iso-$C_{15:0}$ and anteiso-$C_{17:0}$, while $C_{16:0}$, iso-$C_{16:0}$, iso-$C_{17:0}$ and $C_{14:0}$ are found in minor amounts. The polar lipids are phosphatidylglycerol, diphosphatidylglycerol, phosphatidylinositol and phosphatidylinositol mannosides.

The type strain, RS-7-4^T (=NBRC 110994^T = InaCC A726^T), was isolated from a sea sediment sample collected from the foreshore of Rambut Island, Indonesia. The DNA G+C content of the type strain is 75.6 mol%.

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References

Bakalidou A, Kämpfer P, Berchtold M, Kuhnigk T, Wenzel M, König H (2002) *Cellulosimicrobium variabile* sp. nov., a cellulolytic bacterium from the hindgut of the termite *Mastotermes darwiniensis*. Int J Syst Evol Microbiol 52:1185–1192

Blunt JW, Copp BR, Hu WP, Munro MH, Northcote PT, Prinsep MR (2007) Marine natural products. Nat Prod Rep 24:31–86

Brown JM, Steigerwalt AG, Morey RE, Daneshvar MI, Romero LJ, McNeil MM (2006) Characterization of clinical isolates previously identified as *Oerskovia turbata*: proposal of *Cellulosimicrobium funkei* sp. nov. and emended description of the genus C*ellulosimicrobium*. Int J Syst Evol Microbiol 56:801–804

Dittmer JC, Lester RL (1964) A simple, specific spray for the detection of phospholipids on thin-layer chromatograms. J Lipid Res 5:126–127

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 39:738–791

Hamada M, Yamamura H, Komukai C, Tamura T, Suzuki K, Hayakawa M (2012) *Luteimicrobium album* sp. nov., a novel actinobacterium isolated from lichen collected in Japan, and emended description of the genus *Luteimicrobium*. J Antibiot 65:427–431

Kim OS, Cho YJ, Lee K, Yoon SH, Kim M, Na H, Park SC, Jeon YS, Lee JH, Yi H, Won S, Chun J (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

Lam KS (2006) Discovery of novel metabolites from marine actinomycetes. Curr Opin Microbiol 9:245–251

Minnikin DEM, Collins D, Goodfellow M (1975) Fatty acid and polar lipid composition in the classification of *Cellulomonas*, *Oerskovia* and related taxa. J Appl Bacteriol 47:87–95

Saito H, Miura K (1963) Preparation of transforming deoxyribonucleic acid by phenol treatment. Biochim Biophys Acta 72:619–629

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

Sasser M (1990) Identification of bacteria by gas chromatography of cellular fatty acids, MIDI Technical note 101. MIDI Inc, Newark, DE

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

Schumann P (2011) Peptidoglycan structure. In: Rainey F, Oren A (eds) Taxonomy of prokaryotes, methods in microbiology, vol 38. Academic Press, London, pp 101–129

Schumann P, Weiss N, Stackebrandt E (2001) Reclassification of *Cellulomonas cellulans* (Stackebrandt and Keddie 1986) as *Cellulosimicrobium cellulans* gen. nov., comb. nov. Int J Syst Evol Microbiol 51:1007–1010

Stackebrandt E, Schumann P, Cui XL et al (2004) Reclassification of Cellulosimicrobium variabile Bakalidou, 2002 as *Isoptericola variabilis* gen. nov., comb. nov. Int J Syst Evol Microbiol 54:685–688

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

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6: molecular evolutionary genetics analysis version 6.0. Mol Biol Evol 30:2725–2729

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG (1997) The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res 25:4876–4882

Wayne LG, Brenner DJ, Colwell RR, Grimont PAD, Kandler O, Krichevsky MI, Moore LH, Moore WEC, Murray RGE, Stackebrandt E, Starr MP, Truper HG (1987) Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. Int J Syst Bacteriol 37:463–464

Yoon JH, Kang SJ, Schumann P, Oh TK (2007) *Cellulosimicrobium terreum* sp. nov., isolated from soil. Int J Syst Evol Microbiol 57:2493–2497