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Mucilaginibacter terrae sp. nov., isolated from Antarctic soil

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Abstract

A bacterial strain designated CCM 8645^T was isolated from a soil sample collected nearby a mummified seal carcass in the northern part of James Ross Island, Antarctica. The cells were short rods, Gram-stain-negative, non-motile, catalase and oxidase positive, and produced a red-pink pigment on R2A agar. A polyphasic taxonomic approach based on 16S rRNA gene sequencing, extensive biotyping using conventional tests and commercial identification kits and chemotaxonomic analyses were applied to clarify its taxonomic position. Phylogenetic analysis based on the 16S rRNA gene placed strain CCM 8645^T in the genus *Mucilaginibacter* with the closest relative being *Mucilaginibacter daejeonensis* Jip 10^T, exhibiting 96.5 % 16S rRNA pairwise similarity which was clearly below the 97 % threshold value recommended for species demarcation. The major components in fatty acid profiles were Summed feature 3 ($C_{16:1}\omega7c/C_{16:1}\omega6c$), $C_{15:0}$ iso and $C_{17:0}$ iso 3OH. The cellular quinone content was exclusively menaquinone MK-7. The major polyamine was *sym*-homospermidine and predominant polar lipids were phosphatidylethanolamine and phosphatidylserine. Based on presented results, we propose a novel species for which the name *Mucilaginibacter terrae* sp. nov. is suggested, with the type strain CCM 8645^T (=LMG 29437^T).

The genus *Mucilaginibacter* is a member of the family *Sphingobacteriaceae* [1, 2] within the phylum *Bacteroidetes*. The genus *Mucilaginibacter* was proposed by Pankratov *et al.* [2] and subsequently the genus description was emended by Urai *et al.* [3], Baik *et al.* [4], and Chen *et al.* [5]. In recent years numerous novel *Mucilaginibacter* spp. have been discovered worldwide among environmental bacteria and described as novel species [5–13]. Members of the genus *Mucilaginibacter* are known to hydrolyse organic matter such as xylan, pectin and laminarin, and produce large amounts of extracellular polymeric substances [2, 14, 15]. Representatives of *Mucilaginibacter* spp. have been isolated from various habitats of environment, e.g. from rhizosphere of plants [14–16], fresh water [4, 6, 10], soil [8, 17, 18], marine sand [19] or moss [5]. The majority of mucilaginibacters are psychrotolerant organisms revealing good growth at 4 °C. However, they have not been reported to be found in polar regions, except for *Mucilaginibacter soli* isolated from Arctic tundra soil [17]. In the present taxonomic study we report classification of strain CCM 8645^T representing a novel species of the genus *Mucilaginibacter*.

Strain CCM 8645^T was isolated from a soil sample collected nearby a mummified seal carcass on James Ross Island, Antarctica (63°49′52″ S, 57°49′55″ W) in 2012. The seal carcass was located at the bottom of V-shaped valley well supplied by thawing water [20]. Thanks to local microclimate and availability of nutrients, a small-in-area vegetation spot (about 16 m^2) rich in terrestrial algae, lichens and mosses had developed in the neighbourhood of the carcass. Sampling was carried out by dispersing 1 g of soil sample in 5 ml of sterile saline solution and 100 µl of the suspension was spread on R2A agar (Oxoid) plate and cultivated at 15 °C for 4 days. Afterwards, individual red-pink pigmented colonies were purified by repeated streaking on R2A plates and incubation at 15 °C, and the final pure strain CCM 8645^T was stored in R2A broth supplemented with 15 % glycerol (v/v) and maintained at −70 °C until analysed.

Extraction of DNA for molecular analyses by FastPrep Lysing Matrix type B and FastPrep Homogenizer (MP Biomedicals), 16S rRNA gene amplification by PCR and partial 16S rRNA gene sequencing (1472 bp) was performed as described previously [21]. The 16S rRNA gene

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Abbreviation: FAME, fatty acid methyl ester.
The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of *Mucilaginibacter terrae* CCM 8645^T is KY171988.
Four supplementary figures are available with the online Supplementary Material.

sequences with the highest scores were identified by the calculation of pairwise sequence similarity with strain CCM 8645^T using a global alignment algorithm, which was implemented at the EzBioCloud (http://www.ezbiocloud.net/; [22]). Resulting 16S rRNA gene sequence similarities demonstrated that strain CCM 8645^T is a member of the genus *Mucilaginibacter* and identified *Mucilaginibacter daejeonensis* Jip 10^T as its closest relative (96.5 %). Other *Mucilaginibacter* species did not show higher sequence similarities than 95.5 %. Since the nearest neighbours exhibited similarity scores of <97 %, strain CCM 8645^T was considered a candidate for a new species. Phylogenetic analysis was performed using MEGA version 6 software [23]. Genetic distances were calculated using Tamura and Nei [24] model and the evolutionary history was inferred using the maximum-likelihood and neighbour-joining methods. Maximum-likelihood analysis (Fig. 1) matched the tree topology obtained by the neighbour-joining clustering (Fig. S1, available in the online Supplementary Material) except the position of *Mucilaginibacter koreensis* which was placed in different clusters. Phylogenetically, strain CCM 8645^T formed a common branch with *M. daejeonensis* which is in accordance with the highest value of 16S rRNA gene sequence similarity.

The cell morphology of strain CCM 8645^T was observed by Gram-staining and by transmission electron microscopy (Morgagni 268D Philips, FEI Company) using a sample stained with 2 % ammonium molybdate (Fig. S2). The presence of flexirubin-type pigments was investigated using a 20 % (w/v) KOH solution [25]. Spectrophotometric characterization of carotenoid pigments was done from the cells grown on R2A agar for 72 h at 20 °C [21]. The basic phenotypic classification was performed using key tests relevant for Gram-negative rods. Catalase (ID colour Catalase, bioMérieux) and oxidase (OXItest, Erba-Lachema) activity was tested according to manufacturers' instructions. Further tests were done as follows: oxidation-fermentation (OF) test [26], arginine dihydrolase, ornithine and lysine decarboxylase [27], urease [28], hydrolysis of aesculin, starch [29], gelatin, Tween 80 [30], casein, tyrosine [31], and DNA (CM321, Oxoid), egg-yolk reaction [32], ONPG [33], nitrate and nitrite reduction, growth on Simmon's citrate agar [29], utilization of acetamide [34] and sodium malonate [35]. Motility was observed in a glucose oxidation tube. Cells grown at 20 °C for 48–72 h on R2A agar were used to inoculate all tests during all experiments. Liquid culture was not used because of the poor growth of the isolate CCM 8645^T in broth media. Growth on several media such as Plate

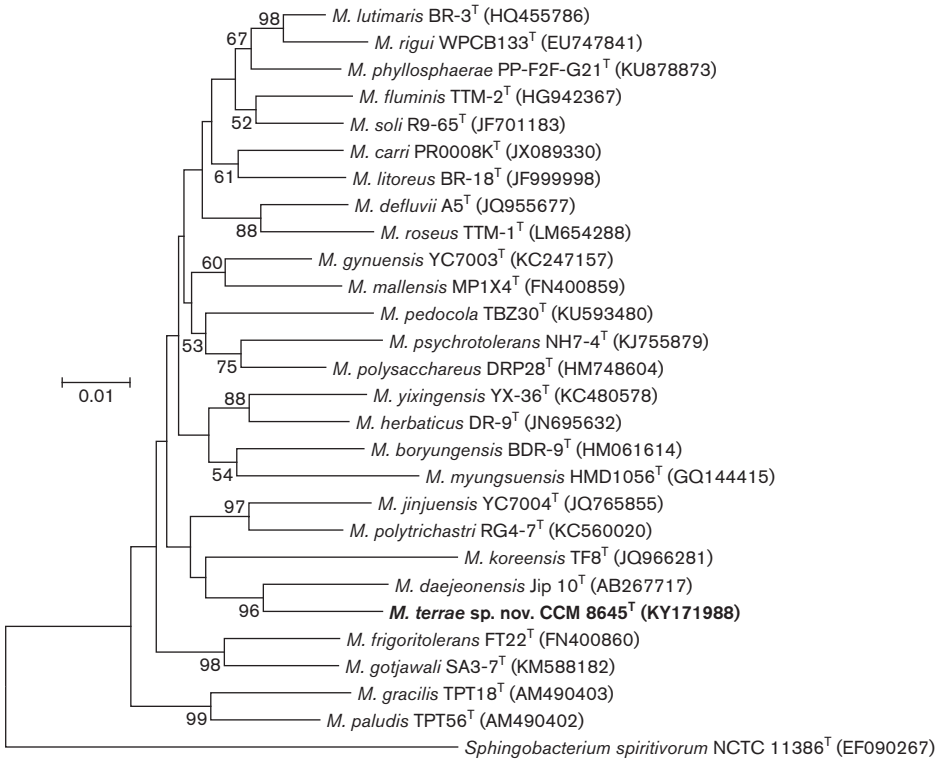

Fig. 1. Phylogenetic tree based on 16S rRNA gene sequence comparison showing the position of strain CCM 8645^T within the genus *Mucilaginibacter*. The evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura-Nei model. The tree with the highest log likelihood is shown. Bootstrap probability values (percentages of 500 tree replications) greater than 50 % are indicated at branch points. There were a total of 1465 positions in the final dataset. Bar indicates number of substitutions per nucleotide position. *Sphingobacterium spiritivorum* was used as an outgroup.

count agar (Oxoid), Tryptone soya agar (Oxoid), Nutrient agar CM03 (Oxoid), MacConkey agar (Becton Dickinson) and Brain heart infusion agar (Oxoid) at 20 °C was evaluated. Anaerobic growth on R2A agar was tested using the Anaerocult A system (Merck) at 20 °C for 72 h and compared with those cultivated in the ambient atmosphere. Growth at different temperatures (1, 5, 10, 15, 20, 25, 30 and 35 °C) and tolerance to various NaCl concentrations (0.5, 1, 2, 3, 4 and 5 % w/v) were determined based on cultivation on R2A agar plates for up to 4 days. The pH range for growth was tested on R2A agar plates adjusted to pH 5.0–10.0 by using the buffer system (pH 5.0–8.0, 0.1M KH_2PO_4/0.1M NaOH; pH 9.0–10.0, 0.1M $NaHCO_3$/0.1M Na_2CO_3; at interval of 1 pH unit) for one week at 20 °C [36]. Further extended phenotyping using identification test kits API ZYM (bioMérieux) and GN2 MicroPlate (Biolog) was performed according to the manufacturers' instructions and enabled detailed characterization of the isolate. Inoculated kits were incubated at 20 °C, and the results were read after 18 h (API ZYM) or 24 and 48 h (GN2 MicroPlate). Antibiotic resistance pattern was assessed by the disc diffusion method on R2A agar for 2 days at 20 °C. Sixteen antibiotic discs generally used for Gram-negative rods [37, 38] were chosen: ampicillin (10 µg), aztreonam (30 µg), carbenicillin (100 µg), cefixim (5 µg), ceftazidime (10 µg), cephalothin (30 µg), ciprofloxacin (5 µg), gentamicin (10 µg), chloramphenicol (30 µg), imipenem (10 µg), kanamycin (30 µg), co-trimoxazole (25 µg), piperacillin (30 µg), polymyxin B (300 U), streptomycin (10 µg) and tetracycline (30 µg). EUCAST/CLSI standards were strictly followed for cultivation and inhibition zone diameter reading.

Strain CCM 8645^T represented psychrotolerant (growing in the temperature range 5–25 °C), Gram-stain-negative, aerobic, catalase and oxidase positive non-fermenting short rods and formed slimy colonies with a pink-red colour. The extracted pigments were separated by HPLC, which revealed at least five different compounds with absorbance properties typical for carotenoids (Fig. S3). The red-shifted spectrum of the most abundant pigment in CCM 8645^T resembled major carotenoid species of the recently described *Rufibacter ruber* CCM 8646^T strain [21] with maxima at 482 and 506 nm and a shoulder at 450 nm. Nonetheless, HPLC mobility and absorbance spectra of these pigments were slightly shifted, which suggests that these carotenoids are similar but not identical (Fig. S3). Strain CCM 8645^T was susceptible to ciprofloxacin, imipenem, co-trimoxazole, streptomycin and tetracycline, resistant to ampicillin, aztreonam, carbenicillin, cefixim, ceftazidime, gentamicin, chloramphenicol, kanamycin, piperacillin and polymyxin B and intermediate result was revealed for cephalothin.

Comprehensive morphological, biochemical and physiological traits of strain CCM 8645^T are summarized in the species description given below. The tests distinguishing strain CCM 8645^T from the phylogenetically closest *Mucilaginibacter* spp. are shown in Table 1.

Fatty acid methyl esters (FAME) analysis was performed using cells grown on R2A agar (Difco) incubated at 20±1 °C for 72 h, where the bacterial communities reached the late exponential stage of growth according to the four quadrants streak method [39]. The extraction of fatty acids methyl esters was performed according to a standard protocol of Sherlock Microbial Identification System [39]. Cellular fatty acid extract was analysed by GC (model 7890B, Agilent) by using the rapid Sherlock Identification system (MIS, version 6.2B, MIDI database: RTSBA6, MIDI Inc.). The predominant fatty acids of CCM 8645^T were Summed feature 3 ($C_{16:1}\omega7c$/$C_{16:1}\omega6c$) (37.0 %), $C_{15:0}$ iso (22.6 %) and $C_{17:0}$ iso 3OH (13.4 %). Cellular contents of remaining fatty acids detected in strain CCM 8645^T were as follows: $C_{16:1}\omega5c$ (8.3 %), Summed Feature 9 ($C_{17:1}$ 10-methyl/iso $\omega9c$) (5.2 %), $C_{15:0}$ iso 3OH (2.2 %), $C_{18:1}\omega9c$ (2.0 %), Summed Feature 4 ($C_{17:1}$ anteiso B/iso I) (1.7 %), $C_{17:0}$ iso (1.6 %), and $C_{16:0}$ (1.3 %). The FAME profile of CCM 8645^T was similar to profiles of other *Mucilaginibacter* species [2, 4].

Quinones and polar lipids were extracted from freeze-dried biomass grown on R2A medium and analysed as described previously [40–43]. The major respiratory quinone was menaquinone MK-7, which is in agreement with the description of the genus *Mucilaginibacter* [44]. The predominant polar lipids were phosphatidylethanolamine, phosphatidylserine and an unidentified lipid (L4) lacking a detectable functional group. Strain CCM 8645^T also contained moderate to minor amounts of nine unidentified lipids L1-L3, L5-L10 lacking a functional group, four unidentified aminolipids AL1-AL4, unidentified phospholipid PL1 and unidentified glycolipid GL1 (Fig. S4). These chemotaxonomic data were in agreement with the emended description of the genus *Mucilaginibacter* [5]. The presence of sphingolipids in strain CCM 8645^T was investigated by applying the method of Kato *et al.* [44]. After mild alkaline hydrolysis, one-dimensional thin layer chromatography and detection with ninhydrin one positive lipid spot was detected which was negative after detection with molybdenum blue. These results demonstrate that this alkaline-stable lipid is not a sphingophospholipid as known to be present in members of the genus *Sphingobacterium*. However, this observation is in agreement with findings in *Mucilaginibacter litoreus*, *Mucilaginibacter sabulilitorius* and *Mucilaginibacter gallii* [19, 45, 46]. The biomass subjected to polyamine analysis was grown on R2A agar, scrapped off the surface, freeze dried and then extracted according to Busse and Auling [47] and analysed by HPLC as reported by Busse *et al.* [48]. The HPLC equipment applied was described by Stolz *et al.* [41]. Strain CCM 8645^T contained *sym*-homospermidine [26.5 µmol (g dry weight)$^{-1}$] as the main polyamine. Moreover, spermine [0.4 µmol (g dry weight)$^{-1}$], spermidine [1.7 µmol (g dry weight)$^{-1}$] and putrescine [0.1 µmol (g dry weight)$^{-1}$] were present in smaller amounts. Similar polyamine patterns with the major compound *sym*-homospermidine have also been detected in

Table 1. Phenotypic characteristics that differentiate *Mucilaginibacter terrae* sp. nov. from closely related *Mucilaginibacter* spp.

Strains: 1, *M. terrae* sp. nov. CCM 8645^T; 2, *M. daejeonensis* Jip 10^T; 3, *M. boryungensis* BDR-9^T; 4, *M. lutimaris* BR-3^T; 5, *M. jinjuensis* YC7004^T; 6, *M. polytrichastri* RG4-7^T. +, positive; w, weakly positive; −, negative; data for reference type strains were obtained from An *et al.* [49]; Kang *et al.* [18]; Kim *et al.* [50]; Khan *et al.* [51] and Chen *et al.* [5].

Characteristics	1	2	3	4	5	6
Pigment	Red-pink	Orange	Light yellow	Light pink	Pale orange	Orange
Growth at 5 °C	+	−	+	+	+	+
Growth at 30 °C	−	+	+	+	+	+
Growth in >1 % NaCl	−	+	−	−	−	+
α−galactosidase	−	+	+	+	w	+
Cystine arylamidase	−	+	−	−	−	−
Esterase (C4)	+	+	−	−	−	+
N-acetyl-β-glucosaminidase	−	+	+	+	+	+
α−mannosidase	−	−	−	+	−	−

Mucilaginibacter phyllosphaerae, *Mucilaginibacter auburnensis* and *M. gallii* [7, 46].

All phenotypic, genotypic and chemotaxonomic results obtained in the present taxonomic study demonstrated that strain CCM 8645^T isolated from soil in Antarctica represents a novel *Mucilaginibacter* species, for which the name *Mucilaginibacter terrae* sp. nov. is proposed.

DESCRIPTION OF *MUCILAGINIBACTER TERRAE* SP. NOV. (TER′RAE. L. GEN. N. *TERRAE*, OF SOIL)

Cells are Gram-stain-negative short rod-shaped to coccoid cells, occurring predominantly in pairs or in irregular clusters and non-spore forming. Red-pink pigmented colonies on R2A agar are circular, with whole margins, slightly mucous, smooth, glistening, and 2 mm in diameter. Aerobic, non-haemolytic on sheep blood agar. Grows on Plate count agar, but not on Tryptone soya agar, Brain heart infusion agar, MacConkey agar and CM03 agar. No anaerobic growth on R2A agar. Grows in the range from 5 to 25 °C but not at 1 or 30 °C. Grows in the presence of up to 1 % NaCl and at pH range from 5 to 8. No fluorescein pigment on King B medium. Hugh and Leifson OF fermentation test negative. Catalase, oxidase (weak), DNase (weak), esterase (C 4), esterase lipase (C 8) (weak), leucine arylamidase, valine arylamidase, naphthol-AS-Bi-phosphohydrolase, β-galactosidase, α-glucosidase (weak), alkaline and acid phosphatase positive. Aesculin, ONPG, casein (weak) and starch hydrolysis positive. Negative Simmons citrate, sodium malonate, acetamide and hydrolysis of Tween 80, gelatine, tyrosine and lecithin (egg-yolk reaction). Urease, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, lipase (C 14), cystine arylamidase, trypsin, chymotrypsin, α-galactosidase, β-glucuronidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, α-fucosidase, nitrate reduction and nitrite reduction negative. Acid is produced from glucose, fructose, xylose and maltose. Acid is not produced from mannitol.

Strain CCM 8645^T has the ability to use the following carbon sources via respiration: α-cyclodextrin, dextrin, Tween 40, cellobiose, D-galactose, α-D-glucose, α-lactose, lactulose, maltose, D-mannose, trehalose, D-glucuronic acid and L-glutamic acid. Negative utilization tests were revealed for glycogen, Tween 80, *N*-acetyl-D-galactosamine, *N*-acetyl-D-glucosamine, adonitol, L-arabinose D-arabitol, erythritol, m-inositol, D-mannitol, melibiose, β-methyl-D-glucoside, D-psicose, raffinose, L-rhamnose, D-sorbitol, sucrose, succinic acid monomethyl ester, acetic acid, cis-aconitic acid, citric acid, formic acid, D-galactonic acid lactone, D-gluconic acid, D-glucosaminic acid, α-hydroxybutyric acid, β-hydroxybutyric acid, γ-hydroxybutyric acid, p-hydroxy phenyl acetic acid, itaconic acid, α-keto butyric acid, α-keto glutaric acid, α-keto valeric acid, D,L-lactic acid, malonic acid, propionic acid, quinic acid, D-saccharic acid, sebacic acid, succinic acid, bromosuccinic acid, succinamic acid, glucuronamide, L-alaninamide, D-alanine, L-alanine, L-alanyl-glycine, L-asparagine, L-aspartic acid, glycyl-L-aspartic acid, L-histidine, hydroxy-L-proline, L-leucine, L-ornithine, L-phenylalanine, L-pyroglutamic acid, D-serine, D,L-carnitine, γ-aminobutyric acid, urocanic acid, inosine, uridine, thymidine, phenyl ethylamine, putrescine, 2-aminoethanol, 2,3-butanediol, glycerol, D,L-α-glycerol phosphate, α-D-glucose-1-phosphate and D-glucose-6-phosphate. The borderline results were obtained for utilization of D-fructose, L-fucose, gentiobiose, turanose, xylitol, pyruvic acid methyl ester, D-galacturonic acid, glycyl-L-glutamic acid, L-proline, L-serine and L-threonine.

The quinone system consists exclusively of menaquinone MK-7 and the major compound in the polyamine pattern is *sym*-homospermidine. In the polar lipid profile phosphatidylethanolamine, phosphatidylserine and an unidentified lipid (L4) are predominant. In addition moderate to minor amounts of nine unidentified lipids (L1-L3, L5-L10) lacking a functional group, four unidentified aminolipids (AL1-AL4), unidentified phospholipid PL1 and unidentified glycolipid GL1 are present. One alkali-stable aminolipid is present. The fatty acid profile contains as major components Summed feature 3 ($C_{16:1}$ *ω7c*/$C_{16:1}$ω6c), $C_{15:0}$ iso and $C_{17:0}$ iso 3OH.

The type strain CCM 8645^T (=LMG 29437^T) was isolated on James Ross Island, Antarctica, from a soil sample collected nearby a mummified seal carcass.

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Conflicts of interest
The authors declare that there are no confilcts of interest.

References

1. **Lambiase A.** The family *Sphingobacteriaceae*. In: Rosenberg E, DeLong EF, Lory S, Stackebrandt E, Thompson F *et al.* (editors). *The Prokaryotes*, 4 ed. Heidelberg, Germany: Springer; 2014. pp. 907–9014.
2. **Pankratov TA, Tindall BJ, Liesack W, Dedysh SN.** *Mucilaginibacter paludis* gen. nov., sp. nov. and *Mucilaginibacter gracilis* sp. nov., pectin-, xylan- and laminarin-degrading members of the family *Sphingobacteriaceae* from acidic *Sphagnum* peat bog. *Int J Syst Evol Microbiol* 2007;57:2349–2354.
3. **Urai M, Aizawa T, Nakagawa Y, Nakajima M, Sunairi M.** *Mucilaginibacter kameinonensis* sp., nov., isolated from garden soil. *Int J Syst Evol Microbiol* 2008;58:2046–2050.
4. **Baik KS, Park SC, Kim EM, Lim CH, Seong CN.** *Mucilaginibacter rigui* sp. nov., isolated from wetland freshwater, and emended description of the genus *Mucilaginibacter*. *Int J Syst Evol Microbiol* 2010;60:134–139.
5. **Chen XY, Zhao R, Tian Y, Kong BH, Li XD *et al.*** *Mucilaginibacter polytrichastri* sp. nov., isolated from a moss (*Polytrichastrum formosum*), and emended description of the genus *Mucilaginibacter*. *Int J Syst Evol Microbiol* 2014;64:1395–1400.
6. **Joung Y, Kang H, Lee BI, Kim H, Joh K *et al.*** *Mucilaginibacter aquaedulcis* sp. nov., isolated from fresh water. *Int J Syst Evol Microbiol* 2015;65:698–703.
7. **Kämpfer P, Busse HJ, McInroy JA, Glaeser SP.** *Mucilaginibacter auburnensis* sp. nov., isolated from a plant stem. *Int J Syst Evol Microbiol* 2014;64:1736–1742.
8. **Lee KC, Kim KK, Eom MK, Kim JS, Kim DS *et al.*** *Mucilaginibacter gotjawali* sp. nov., isolated from soil of a lava forest. *Int J Syst Evol Microbiol* 2015;65:952–958.
9. **Hwang YM, Baik KS, Seong CN.** *Mucilaginibacter defluvii* sp. nov., isolated from a dye wastewater treatment facility. *Int J Syst Evol Microbiol* 2014;64:565–571.
10. **Joung Y, Kim H, Kang H, Lee BI, Ahn TS *et al.*** *Mucilaginibacter soyangensis* sp. nov., isolated from a lake. *Int J Syst Evol Microbiol* 2014;64:413–419.
11. **Joung Y, Kim H, Lee BI, Kang H, Kim TS *et al.*** *Mucilaginibacter flavus* sp. nov., isolated from wetland. *Int J Syst Evol Microbiol* 2014;64:1304–1309.
12. **Park CS, Han K, Ahn TY.** *Mucilaginibacter koreensis* sp. nov., isolated from leaf mould. *Int J Syst Evol Microbiol* 2014;64:2274–2279.
13. **Paiva G, Abreu P, Proença DN, Santos S, Nobre MF *et al.*** *Mucilaginibacter pineti* sp. nov., isolated from *Pinus pinaster* wood from a mixed grove of pines trees. *Int J Syst Evol Microbiol* 2014;64:2223–2228.
14. **Han SI, Lee HJ, Lee HR, Kim KK, Whang KS.** *Mucilaginibacter polysaccharieus* sp. nov., an exopolysaccharide-producing bacterial species isolated from the rhizoplane of the herb *Angelica sinensis*. *Int J Syst Evol Microbiol* 2012;62:632–637.
15. **Madhaiyan M, Poonguzhali S, Lee JS, Senthilkumar M, Lee KC *et al.*** *Mucilaginibacter gossypii* sp. nov. and *Mucilaginibacter gossypiicola* sp. nov., plant-growth-promoting bacteria isolated from cotton rhizosphere soils. *Int J Syst Evol Microbiol* 2010;60:2451–2457.
16. **Kim BC, Poo H, Lee KH, Kim MN, Kwon OY *et al.*** *Mucilaginibacter angelicae* sp. nov., isolated from the rhizosphere of *Angelica polymorpha* Maxim. *Int J Syst Evol Microbiol* 2012;62:55–60.
17. **Jiang F, Dai J, Wang Y, Xue X, Xu M *et al.*** *Mucilaginibacter soli* sp. nov., isolated from Arctic tundra soil. *Int J Syst Evol Microbiol* 2012;62:1630–1635.
18. **Kang SJ, Jung YT, Oh KH, Oh TK, Yoon JH.** *Mucilaginibacter boryungensis* sp. nov., isolated from soil. *Int J Syst Evol Microbiol* 2011;61:1549–1553.
19. **Yoon JH, Kang SJ, Park S, Oh TK.** *Mucilaginibacter litoreus* sp. nov., isolated from marine sand. *Int J Syst Evol Microbiol* 2012;62:2822–2827.
20. **Nývlt D, Fišáková MN, Barták M, Stachoň Z, Pavel V *et al.*** Death age, seasonality, taphonomy and colonization of seal carcasses from Ulu Peninsula, James Ross Island, Antarctic Peninsula. *Antarct Sci* 2016;28:3–16.
21. **Kýrová K, Sedláček I, Pantůček R, Králová S, Holochová P *et al.*** *Rufibacter ruber* sp. nov., isolated from fragmentary rock. *Int J Syst Evol Microbiol* 2016;66:4401–4405.
22. **Jeon YS, Lee K, Park SC, Kim BS, Cho YJ *et al.*** EzEditor: a versatile sequence alignment editor for both rRNA- and protein-coding genes. *Int J Syst Evol Microbiol* 2014;64:689–691.
23. **Tamura K, Stecher G, Peterson D, Filipski A, Kumar S.** MEGA6: molecular evolutionary genetics analysis version 6.0. *Mol Biol Evol* 2013;30:2725–2729.
24. **Tamura K, Nei M.** Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Mol Biol Evol* 1993;10:512–526.
25. **Bernardet JF, Nakagawa Y, Holmes B.** Proposed minimal standards for describing new taxa of the family *Flavobacteriaceae* and emended description of the family. *Int J Syst Evol Microbiol* 2002;52:1049–1070.
26. **Hugh R, Leifson E.** The taxonomic significance of fermentative versus oxidative metabolism of carbohydrates by various gram negative bacteria. *J Bacteriol* 1953;66:24–26.
27. **Brooks K, Sodeman T.** A rapid method for determining decarboxylase and dihydrolase activity. *J Clin Pathol* 1974;27:148–152.
28. **Christensen WB.** Urea decomposition as a means of differentiating *Proteus* and paracolon cultures from each other and from *Salmonella* and *Shigella* types. *J Bacteriol* 1946;52:461–466.
29. **Barrow GL, Cowan F.** *Steel's Manual for the Identification of Medical Bacteria*, 3rd ed. Great Britain: Cambridge University Press; 1993.
30. **Pácová Z, Kocur M.** New medium for detection of esterase and gelatinase activity. *Zentralbl Bakteriol Mikrobiol Hyg A* 1984;258:69–73.
31. **Kurup VP, Babcock JB.** Use of casein, tyrosine, and hypoxanthine in the identification of nonfermentative gram-negative bacilli. *Med Microbiol Immunol* 1979;167:71–75.
32. **Owens JJ.** The egg yolk reaction produced by several species of bacteria. *J Appl Bacteriol* 1974;37:137–148.
33. **Lowe GH.** The rapid detection of lactose fermentation in paracolon organisms by the demonstration of β-D-galactosidase. *J Med Lab Technol* 1962;19:21–25.

34. **Oberhofer TR, Rowen JW.** Acetamide Agar for differentiation of nonfermentative bacteria. *Appl Microbiol* 1974;28:720–721.
35. **Ewing WH.** *Enterobacteriaceae. Biochemical Methods for Group Differentation*. Atlanta: Public Health Service Publication No 734 CDC; 1960.
36. **Da X, Fang C, Deng S, Zhang Y, Chang X *et al.*** *Pedobacter ardleyensis* sp. nov., isolated from soil in Antarctica. *Int J Syst Evol Microbiol* 2015;65:3841–3846.
37. **EUCAST.** *European Committee on Antimicrobial Susceptibility Testing. EUCAST Clinical Breakpoints - Bacteria*; 2015. (version 5.0; http://www.eucast.org).
38. **CLSI.** *Performance Standards for Antimicrobial susceptibility testing;* Twenty-Fifth Informational Supplement (M100-S25). Vol. 35 No. 3. Wayne, PA: Clinical and Laboratory Standards Institute; 2015.
39. **Sasser M.** *Identification of Bacteria by Gas Chromatography of Cellular Fatty Acids*, MIDI Technical Note 101. Newark, DE: Microbial ID, Inc.; 1990.
40. **Altenburger P, Kämpfer P, Makristathis A, Lubitz W, Busse H-J.** Classification of bacteria isolated from a medieval wall painting. *J Biotechnol* 1996;47:39–52.
41. **Stolz A, Busse HJ, Kämpfer P.** *Pseudomonas knackmussii* sp. nov. *Int J Syst Evol Microbiol* 2007;57:572–576.
42. **Tindall BJ.** Lipid composition of *Halobacterium lacusprofundi*. *FEMS Microbiol Lett* 1990;66:199–202.
43. **Tindall BJ.** A comparative study of the lipid composition *of Halobacterium saccharovorum* from various sources. *Syst Appl Microbiol* 1990;13:128–130.
44. **Kato M, Muto Y, Tanaka-Bandoh K, Watanabe K, Ueno K.** Sphingolipid composition in *Bacteroides* species. *Anaerobe* 1995;1:135–139.
45. **Kang CH, Jung YT, Yoon JH.** *Mucilaginibacter sabulilitoris* sp. nov., isolated from marine sand in a firth. *Int J Syst Evol Microbiol* 2013; 63:2865–2871.
46. **Aydogan EL, Busse HJ, Moser G, Müller C, Kämpfer P *et al.*** Proposal of *Mucilaginibacter galii* sp. nov. isolated from leaves of *Galium album*. *Int J Syst Evol Microbiol* 2017;67:1318–1326.
47. **Busse J, Auling G.** Polyamine pattern as a chemotaxonomic marker within the *Proteobacteria*. *Syst Appl Microbiol* 1988;11:1–8.
48. **Busse H-J, Bunka S, Hensel A, Lubitz W.** Discrimination of members of the family *Pasteurellaceae* based on polyamine patterns. *Int J Syst Bacteriol* 1997;47:698–708.
49. **An DS, Yin CR, Lee ST, Cho CH.** *Mucilaginibacter daejeonensis* sp. nov., isolated from dried rice straw. *Int J Syst Evol Microbiol* 2009; 59:1122–1125.
50. **Kim JH, Kang SJ, Jung YT, Oh TK, Yoon JH.** *Mucilaginibacter lutimaris* sp. nov., isolated from a tidal flat sediment. *Int J Syst Evol Microbiol* 2012;62:515–519.
51. **Khan H, Chung EJ, Kang DY, Jeon CO, Chung YR.** *Mucilaginibacter jinjuensis* sp. nov., with xylan-degrading activity. *Int J Syst Evol Microbiol* 2013;63:1267–1272.