Demequina aestuarii gen. nov., sp. nov., a novel actinomycete of the suborder *Micrococcineae*, and reclassification of *Cellulomonas fermentans* Bagnara *et al.* 1985 as *Actinotalea fermentans* gen. nov., comb. nov.

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An actinobacterial strain containing demethylmenaquinone DMK-9(H_4) as the diagnostic isoprenoid quinone was isolated from a tidal flat sediment sample, from South Korea. Phylogenetic analyses based on 16S rRNA gene sequences showed that strain JC2054^T represents a distinct phyletic line within the suborder *Micrococcineae* of the order *Actinomycetales*. The closest phylogenetic neighbour was *Cellulomonas fermentans*, with 94.7 % 16S rRNA gene sequence similarity. The novel isolate was strictly aerobic and slightly halophilic, with optimum growth occurring in 2–4 % (w/v) NaCl. Cells were non-motile, non-sporulating and rod-shaped. The peptidoglycan type was of the A-type of cross-linkage. L-Ornithine was the diamino acid and D-glutamate represented the N-terminus of the interpeptide bridge. The predominant fatty acids were anteiso-branched and straight-chain fatty acids. The major polar lipids were phosphatidylinositol, diphosphatidylglycerol and an unknown phospholipid. The menaquinone composition of *C. fermentans* was determined to be MK-10(H_4), MK-9(H_4) and MK-8(H_4) in the ratio 56 : 2 : 1. On the basis of the polyphasic evidence presented in this study, it is proposed that strain JC2054^T should be classified as representing a novel genus and species of the suborder *Micrococcineae*, with the name *Demequina aestuarii* gen. nov., sp. nov. The type strain is JC2054^T (=IMSNU 14027^T=KCTC 9919^T=JCM 12123^T). In addition, it was clear from the phylogenetic analysis and chemotaxonomic data that *C. fermentans* does not belong to the genus *Cellulomonas* or any other recognized genera. Therefore, *C. fermentans* should be reclassified as representing a novel genus, for which the name *Actinotalea fermentans* gen. nov., comb. nov. is proposed, with strain DSM 3133^T (=ATCC 43279^T=CFBP 4259^T=CIP 103003^T=NBRC 15517^T=JCM 9966^T=LMG 16154^T) as the type strain.

The west and south-west coasts of the Korean peninsula consist mainly of tidal flats that are unique among other marine sediments. The high microbial diversity of Korean tidal flat sediments has been reported in culture-independent (Kim *et al.*, 2004, 2005) and culture-dependent (Park *et al.*, 2005; Yi & Chun, 2006) studies. A novel actinomycete was isolated from Korean tidal flat sediment and subjected to a comprehensive taxonomic study. Here, we report the taxonomic description of this strain.

An actinomycete strain, designated JC2054^T, was isolated from a tidal flat sediment sample from Ganghwa Island, South Korea (37° 36′ 22.3″ N 126° 22′ 59.4″ E), using the standard dilution plating method based on marine agar 2216 (MA; Difco). The isolate was routinely cultured on MA and maintained as a glycerol suspension (20 %, w/v) at −80 °C.

The 16S rRNA gene was amplified enzymically from a single colony. The primers, PCR conditions and sequencing methods used have been described elsewhere (Chun & Goodfellow, 1995). Preliminary sequence comparison against 16S rRNA gene sequences held in the GenBank database indicated that our isolate belonged to the suborder *Micrococcineae* in the order *Actinomycetales*. The nearly complete 16S rRNA gene sequence of strain JC2054^T (1423 bp) was aligned manually against those of

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain JC2054^T is DQ010160.

representatives of the suborder *Micrococcineae* using the bacterial 16S rRNA secondary structure model. The regions available for all sequences (positions 45–71 and 98–1410; *Escherichia coli* numbering system) showed unambiguous alignment and were used to construct phylogenetic trees. The phylogenetic trees were inferred by using the Fitch–Margoliash (Fitch & Margoliash, 1967), maximum-likelihood (Felsenstein, 1981), maximum-parsimony (Fitch, 1971) and neighbour-joining (Saitou & Nei, 1987) methods. Evolutionary distance matrices for the neighbour-joining and Fitch–Margoliash methods were generated according to the model of Jukes & Cantor (1969). The resultant neighbour-joining tree topology was evaluated by bootstrap analyses (Felsenstein, 1985) based on 1000 resamplings. Alignment and phylogenetic analyses were carried out using the jPHYDIT program (Jeon *et al.*, 2005; available at http://chunlab.snu.ac.kr/jphydit) and PAUP 4.0 (Swofford, 1998), as described previously (Yi & Chun, 2006).

On the basis of 16S rRNA gene sequence analyses, strain JC2054^T showed the highest sequence similarity to *Cellulomonas fermentans* DSM 3133^T (94.7 %) and *Sanguibacter* species. (94.1–94.7 %). No other taxa showed more than 94 % sequence similarity to our isolate. The close relationship between strain JC2054^T and other members of the suborder *Micrococcineae* was also evident from the phylogenetic tree (Fig. 1). Strain JC2054^T formed a clade with *C. fermentans* in the neighbour-joining (with 64 % bootstrap support), maximum-likelihood and maximum-parsimony trees, and was recovered as a sister group of the genus *Sanguibacter* in the Fitch–Margoliash tree. Apart from the close relationship with strain JC2054^T, *C. fermentans* showed the highest sequence similarities to the genera *Cellulomonas* (94.4–95.8 %), *Oerskovia* (94.2–94.7 %), *Tetrasphaera* (94.6 %) and *Sanguibacter* (94.1–94.4 %). It is evident from the phylogenetic tree (Fig. 1) that our isolate together with *C. fermentans* are not members of the genus *Cellulomonas*.

Cellular morphology and motility were examined by scanning-electron and phase-contrast microscopy, respectively, using cells that had been grown on MA at 30 °C for 3 days. The presence of mycelium and spore formation were examined following growth at 30 °C for 3 weeks on several culture media: ISP medium No. 3 (Difco) supplemented with 4 % (w/v) sea salts (Sigma), ISP medium No. 4 (Difco)

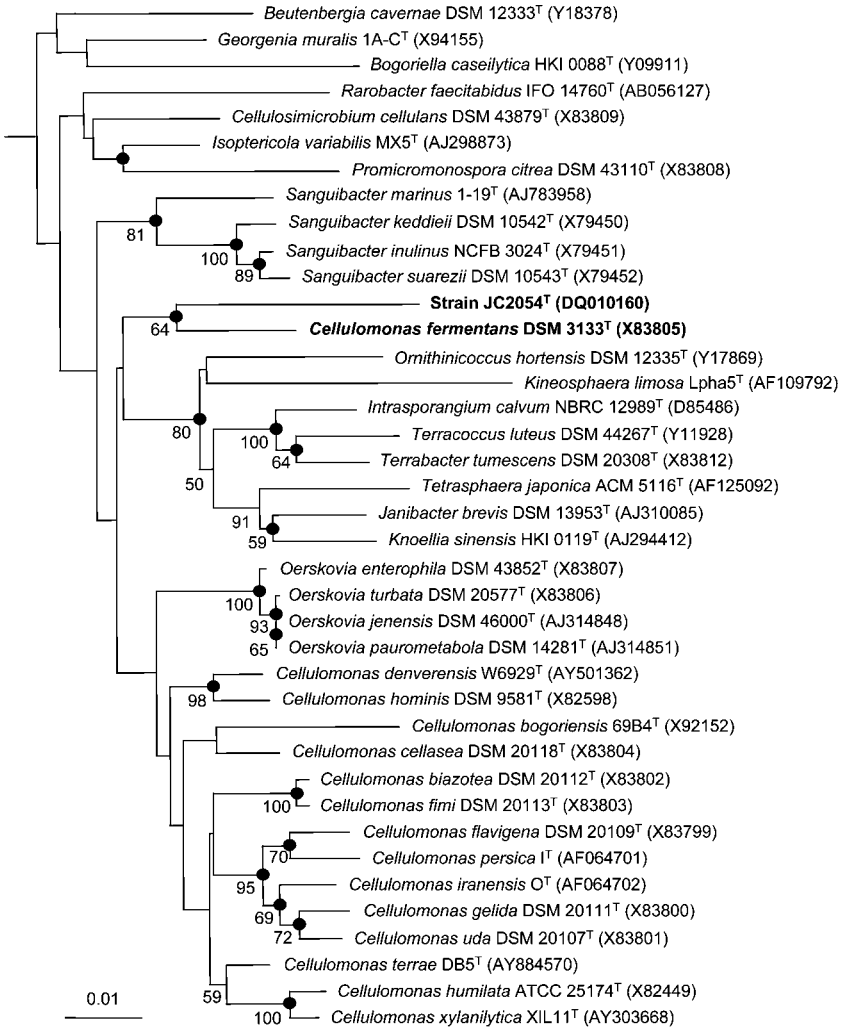

Fig. 1. Neighbour-joining tree based on nearly complete 16S rRNA gene sequences. Numbers at nodes are percentage bootstrap support (>50 %) from 1000 resampled datasets. Solid circles indicate that the corresponding nodes (groupings) were recovered in more than three tree-inferring methods. *Actinomyces coleocanis* CCUG 41708^T (GenBank accession no. AJ249326), *Bifidobacterium bifidum* DSM 20456^T (S83624) and *Nocardia asteroides* ATCC 19247^T (Z36934) were used as outgroups (not shown). Bar, 0.01 nucleotide substitutions per position.

supplemented with sea salts, PYGV (Staley, 1968) supplemented with sea salts, basal medium (BM; Baumann *et al.*, 1972) and MA.

Growth under anaerobic conditions was checked using MA, in an anaerobic chamber [10 % CO_2, 10 % H_2, 80 % N_2 (v/v); Sheldon Manufacturing]. The temperature range for growth (5–50 °C), the pH range (pH 4–12, adjusted with 10 M KOH and HCl) and tolerance of NaCl (0–15 %, w/v) were determined using synthetic ZoBell medium [Zobell, 1941; 5 g Bacto peptone (Difco), 1 g yeast extract (Difco), 0.1 g ferric citrate, per litre seawater] for up to 3 weeks. The optimal temperature for growth was also determined by using a temperature-gradient incubator (TVS 126MA; Advantec).

Standard physiological and biochemical tests were performed as described by Smibert & Krieg (1994). The acid-fast test was performed using Ziehl-Neelsen staining. Hydrolysis of alginic acid, casein, carboxymethylcellulose, chitin, starch and Tween 80 was tested using MA as the basal medium. DNase test agar (Difco) supplemented with 2.5 % (w/v) NaCl was used for the DNase assay. Decomposition of adenine, hypoxanthine, L-tyrosine and xanthine was tested using MA, according to Gordon *et al.* (1974). Production of H_2S was detected using triple sugar iron agar supplemented with 2.5 % (w/v) NaCl. Tests for arginine dihydrolase and β-galactosidase activities, nitrate reduction, urease activity, acid production from glucose and indole production were performed using an API 20NE kit (bioMérieux). Other enzymic activities were determined using an API ZYM kit (bioMérieux). Strips for API kits were inoculated with a heavy bacterial suspension in half-strength artificial seawater or AUX medium (bioMérieux), supplemented with 2.5 % (w/v) NaCl. Carbon source utilization was tested in 96-well tissue culture microplates (Falcon) for up to 7 days using BM supplemented with 1 % (v/v) vitamin solution (Staley, 1968) as basal medium.

The results of the morphological, cultural, biochemical and physiological tests are given in the genus and species description.

Biomass for chemotaxonomy was scraped from MA plates. Extraction and gas chromatographic separation of fatty acid methyl esters were performed using the Microbial Identification System (MIDI), according to the instructions of the manufacturer. Peptidoglycan was purified and analysed according to Schleifer & Seidl (1985). Amino acids and peptides of peptidoglycan hydrolysates were separated by two-dimensional ascending TLC on cellulose plates, using the solvent systems of Schleifer & Kandler (1972). The molar ratios of amino acids were determined by GC and GC/MS of *N*-heptafluorobutyryl amino acid isobutyl esters (Groth *et al.*, 1996; MacKenzie, 1987). The amino terminal amino acid of the interpeptide bridge was determined by dinitrophenylation as described by Schleifer (1985). The glycolate content of bacterial cell walls was determined by using the colorimetric method of Uchida & Aida (1984). Menaquinones were extracted and purified according to Collins *et al.* (1977) and analysed by using a modification of the HPLC method described by Groth *et al.* (1996) [isocratic elution from an RP 18 column (no. 201HS5415; Vydac) using acetonitrile/2-propanol (65 : 35, v/v) at 35 °C] and electron-impact mass spectrometry as described by Pukall *et al.* (2006). The DNA G+C content was determined by HPLC analysis of deoxyribonucleosides as described by Mesbah *et al.* (1989). Phospholipids were extracted, purified and identified as described by Yassin *et al.* (1993).

The peptidoglycan of strain JC2054^T was composed of L-ornithine, alanine, glycine, serine, D-glutamate and aspartate in an approximate molar ratio of 1.0 : 1.2 : 1.4 : 1.7 : 5.5 : 0.4. Glutamic acid represented the N-terminus of the interpeptide bridge. The cross-linkage was of the A-type, as the characteristic peptide was L-Ala→D-Glu (Schleifer & Kandler, 1972). L-Ornithine was the diamino acid. The major polar lipids were phosphatidylinositol, diphosphatidylglycerol and an unknown phospholipid. The fatty acid composition of strain JC2054^T was as follows: $C_{14:0}$ (1.59 %), $C_{15:0}$ (4.16 %), $C_{16:0}$ (10.42 %), $C_{17:0}$ (0.70 %), i-$C_{14:0}$ (1.08 %), i-$C_{15:0}$ (3.31 %), i-$C_{16:0}$ (6.96 %), i-$C_{17:0}$ (0.91 %), ai-$C_{15:0}$ (46.83 %), ai-$C_{17:0}$ (13.14 %), i-$C_{15:1}$ I (0.68 %) and ai-$C_{15:1}$ (10.21 %). The DNA G+C content was 67 mol%. HPLC analysis of the menaquinone composition of strain JC2054^T revealed a single component that eluted at a retention time that was earlier by 10 % than that of MK-9(H_4) extracted from *Oerskovia turbata* DSM 20577^T. The electron-impact mass spectrum of the isoprenoid quinone of strain JC2054^T clearly showed a base peak at *m/z* 211, and the peak of the molecular ion at *m/z* 775. The corresponding values for MK-9(H_4) are *m/z* 225 and 788, respectively. Taking an instrumental error of approximately 1 mass unit into account, this result suggests the presence of demethylmenaquinone DMK-9(H_4) in strain JC2054^T (Collins, 1994). In contrast, the isoprenoid quinone composition of *C. fermentans* DSM 3133^T was MK-10(H_4), MK-9(H_4) and MK-8(H_4) in the ratio 56 : 2 : 1, as revealed in this study. These results differentiate *C. fermentans* from strain JC2054^T, as well as from bona fide members of the genus *Cellulomonas* that contain MK-9(H_4) as the major isoprenoid quinone (Stackebrandt *et al.*, 2002).

Taxonomic conclusions

Phylogenetic analyses based on 16S rRNA gene sequences showed that strain JC2054^T represents a distinct phyletic line within the suborder *Micrococcineae* of the order *Actinomycetales*. The highest sequence similarity values to our isolate were observed for *C. fermentans* DSM 3133^T (94.7 %) and members of the genus *Sanguibacter* (94.1–94.7 %). However, strain JC2054^T could be clearly differentiated from all the phylogenetically related genera by the presence of DMK-9(H_4), which hitherto has not been detected as a major menaquinone of members of the suborder *Micrococcineae*, and by several other chemotaxonomic characteristics (Table 1). It is therefore proposed

Table 1. Major chemotaxonomic characteristics that differentiate strain JC2054^T from phylogenetically related bacteria

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Major menaquinone(s)	DMK-9(H_4)	MK-10(H_4)	MK-9(H_4)	MK-9(H_4)	MK-9(H_4)	MK-9(H_4)	MK-8(H_4)	MK-8	MK-9(H_4), MK-9(H_2), MK-9	MK-8(H_4)	MK-8(H_4)	MK-8(H_4)	MK-8(H_4)	MK-8(H_4)	MK-8(H_4)
Diamino acid	L-Orn	L-Orn	L-Orn	L-Lys	L-Lys	L-Lys	L-Lys	LL-A2pm	L-Lys	*meso*-A2pm	*meso*-A2pm	L-Orn	LL-A2pm	LL-A2pm	*meso*-A2pm or 3-hydroxy *meso*-A2pm
Interpeptide bridge	D-Glu	D-Asp	D-Asp or D-Glu	L-Thr←D-Glu or L-Thr←D-Asp	Ser←D-Glu	D-Ser←D-Asp	L-Glu	Gly_3	D-Asp or D-Glu	None	ND	$Gly_{(1,2)}$←D-Glu	Gly_3	Gly_3	None
DNA G+C content (mol%)	67	76	71–76	71	69–73	74–75	70	68	70–74	70	68–69	72	70–73	73	68–73
Major fatty acids	ai-$C_{15:0}$ ai-$C_{15:1}$ $C_{16:0}$ ai-$C_{17:0}$	$C_{14:0}$ ai-$C_{15:0}$ $C_{16:0}$	ai-$C_{15:0}$ i-$C_{15:0}$ $C_{16:0}$ ai-$C_{17:0}$ $C_{18:0}$	ai-$C_{15:0}$ i-$C_{15:0}$ $C_{16:0}$ ai-$C_{17:0}$	ai-$C_{15:0}$ $C_{16:0}$ $C_{18:0}$	ai-$C_{15:0}$ i-$C_{15:0}$ $C_{16:0}$ i-$C_{16:0}$	ai-$C_{15:0}$	ai-$C_{15:0}$ i-$C_{15:0}$ i-$C_{16:0}$	ai-$C_{15:0}$ i-$C_{15:0}$ $C_{16:0}$ i-$C_{16:0}$ ai-$C_{17:0}$	i-$C_{16:0}$ $C_{17:0}$ $C_{17:1}$	i-$C_{15:0}$ i-$C_{16:0}$ ai-$C_{17:0}$ i-$C_{17:1}$	ai-C_{15}, i-$C_{15:0}$	ai-$C_{15:0}$ i-$C_{15:0}$ i-$C_{16:0}$ i-$C_{14:0}$	ai-$C_{15:0}$ i-$C_{15:0}$ $C_{16:0}$	i-$C_{15:0}$ i-$C_{16:0}$ i-$C_{16:1}$ ai-$C_{17:0}$ 10-methyl $C_{17:0}$
Relation to oxygen*	S	F	A or F	F	F	F	F	A	F	A	A	A	A	S	A

*A, aerobic; F, facultatively anaerobic; S, strictly aerobic.

Taxa: 1, strain JC2054^T; 2, *Cellulomonas fermentans*; 3, *Cellulomonas*; 4, *Oerskovia*; 5, *Sanguibacter*; 6, *Cellulosimicrobium*; 7, *Georgenia*; 8, *Intrasporangium*; 9, *Isoptericola*; 10, *Janibacter*; 11, *Knoellia*; 12, *Ornithinicoccus*; 13, *Terrabacter*; 14, *Terracoccus*; 15, *Tetrasphaera*. Data are from this and previous studies (Altenburger *et al.*, 2002; An *et al.*, 2005; Brown *et al.*, 2005, 2006; Elberson *et al.*, 2000; Groth *et al.*, 1999, 2005; Hanada *et al.*, 2002; Huang *et al.*, 2005; Ishikawa & Yokota, 2006; Jones *et al.*, 2005; Maszenan *et al.*, 2000; Prauser *et al.*, 1997; Rivas *et al.*, 2004; Schumann *et al.*, 2001; Stackebrandt & Schumann, 2000; Stackebrandt *et al.*, 2002, 2004; Zhang *et al.*, 2005). A2pm, diaminopimelic acid; Asp, aspartate; Glu, D-glutamate; Lys, lysine; Orn, L-ornithine; Ser, serine; Thr, threonine; ND, not determined.

that the novel isolate should be assigned as representing a novel genus and species in the suborder *Micrococcineae*, with the name *Demequina aestuarii* gen. nov., sp. nov. In addition, on the basis of 16S rRNA gene phylogeny (Fig. 1) and chemotaxonomy (Table 1), it is evident that *C. fermentans* is not a member of the genus *Cellulomonas* or the newly proposed genus *Demequina*. Therefore it is proposed that *Cellulomonas fermentans* should be reclassified as representing a novel genus, with the name *Actinotalea fermentans* gen. nov., comb. nov.

Description of *Demequina* gen. nov.

Demequina (De.me.qui′na. N.L. fem. n. *Demequina* arbitrary name derived from demethylmenaquinone, an unusual quinone found in this organism).

Gram-positive, strictly aerobic and slightly halophilic. Oxidase-negative, catalase-positive and acid-fast-negative. Do not form spores. Cells are non-motile rods with round ends. The only isoprenoid quinone is demethylmenaquinone DMK-9(H_4). The peptidoglycan type is of the A-type of cross-linkage and contains L-ornithine, alanine, glycine, serine, D-glutamate and aspartate. L-Ornithine is the diamino acid and D-glutamate represents the N-terminus of the interpeptide bridge. Predominant cellular fatty acids are of the anteiso-branched and straight-chain types. Major polar lipids are phosphatidylinositol, diphosphatidylglycerol and an unknown phospholipid. Phylogenetically, the genus is affiliated to the suborder *Micrococcineae*. The type species is *Demequina aestuarii*.

Description of *Demequina aestuarii* sp. nov.

Demequina aestuarii (ae.stu.a′ri.i. L. gen. n. *aestuarii* of a tidal flat, isolated from tidal flat sediment).

Exhibits the following properties in addition to those given in the genus description. Cells are 0.25–0.35 × 0.60–1.20 μm. Growth occurs at pH 6–11 (optimum, pH 7) and in 0–12 % (w/v) NaCl (optimum, 2–4 %). Growth occurs at 5–35 °C, with optimum growth at 33.5 °C. Minimum doubling time is 6.4 h. Extended incubation (up to 15 days) is required at 5 °C. Growth occurs on MA, ISP medium No. 3 supplemented with sea salts, ISP medium No. 4 supplemented with sea salts and PYGV supplemented with sea salts, but not on Oatmeal agar supplemented with sea salts. Colonies on MA at 30 °C are yellow, translucent and circular, with entire margins, approximately 0.2 mm in diameter after 2 days, with a maximum diameter of 3–4 mm after 7 days. Negative for arginine dihydrolase and urease activities. Positive for β-galactosidase activity. Nitrate is not reduced to nitrite. Does not produce H_2S. Acid is not produced from glucose nor indole from tryptophan. Decomposes carboxymethylcellulose, DNA and aesculin, but not adenine, alginate, casein, chitin, gelatin, hypoxanthine, starch, Tween 80, tyrosine or xanthine. On the basis of the API ZYM system, the organism is positive for leucine arylamidase, β-galactosidase, α-glucosidase and

β-glucosidase; weakly positive for naphthol-AS-BI-phosphohydrolase; negative for alkaline phosphatase, esterase (C4), esterase lipase (C8), lipase (C14), valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, acid phosphatase, α-galactosidase, β-glucuronidase, *N*-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase. Utilizes D-cellobiose, D-fructose, D-galactose, D-glucose, D-mannitol, D-mannose, D-salicin, D-trehalose, D-xylose, glycerol, L-arabinose and sucrose as a sole carbon source. Utilizes lactose weakly. Does not utilize acetamide, acetate, benzoate, citrate, D-raffinose, D-ribose, D-sorbitol, ethanol, glycine, inositol, inulin, 2-propanol, L-arginine, L-ascorbate, L-asparagine, L-lysine, L-ornithine, L-rhamnose, *N*-acetylglucosamine, polyethylene glycol, salicylate, succinate, tartrate or thiamine. Major fatty acids are ai-$C_{15:0}$, ai-$C_{17:0}$, $C_{16:0}$ and ai-$C_{15:1}$.

The DNA G+C content of the type strain is 67 mol%. The type strain is JC2054^T (=IMSNU 14027^T=KCTC 9919^T=JCM 12123^T), isolated from a tidal flat sediment sample from Ganghwa Island, South Korea.

Description of *Actinotalea* gen. nov.

Actinotalea (Ac.ti.no.tal′e.a. Gr. n. *actis*, *actinis* ray; L. fem. n. *talea* a slender staff, rod, stick; N.L. fem. n. *Actinotalea* ray stick).

The description is based on that given for *Cellulomonas fermentans* by Bagnara *et al.* (1985). Gram-positive. Coryneform rods exhibiting polymorphism. Non-motile. Facultatively anaerobic. Diamino acid of the peptidoglycan is L-ornithine and interpeptide bridge is D-aspartate. Major fatty acids are $C_{14:0}$, ai-$C_{15:0}$ and $C_{16:0}$. The major isoprenoid quinone is MK-10(H_4). The type species is *Actinotalea fermentans*.

Description of *Actinotalea fermentans* sp. nov., comb. nov.

Actinotalea fermentans (fer.men′tans. L. part. adj. *fermentans* fermenting).

Basonym: *Cellulomonas fermentans* Bagnara *et al.* 1985.

The description is identical to that given for *Cellulomonas fermentans* by Bagnara *et al.* (1985). Cell wall sugars are glucose, rhamnose and ribose. The DNA G+C content of the type strain is 76 mol%. The type strain is DSM 3133^T (=ATCC 43279^T=CFBP 4259^T=CIP 103003^T=NBRC 15517^T=JCM 9966^T=LMG 16154^T).

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References

Altenburger, P., Kämpfer, P., Schumann, P., Vybiral, D., Lubitz, W. & Busse, H. J. (2002). *Georgenia muralis* gen. nov., sp. nov., a novel actinobacterium isolated from a medieval wall painting. *Int J Syst Evol Microbiol* **52**, 875–881.

An, D.-S., Im, W.-T., Yang, H.-C., Kang, M. S., Kim, K. K., Jin, L., Kim, M. K. & Lee, S.-T. (2005). *Cellulomonas terrae* sp. nov., a cellulolytic and xylanolytic bacterium isolated from soil. *Int J Syst Evol Microbiol* **55**, 1705–1709.

Bagnara, C., Toci, R., Gaudin, C. & Belaich, J. P. (1985). Isolation and characterization of a cellulolytic microorganism, *Cellulomonas fermentans* sp. nov. *Int J Syst Bacteriol* **35**, 502–507.

Baumann, L., Baumann, P., Mandel, M. & Allen, R. D. (1972). Taxonomy of aerobic marine eubacteria. *J Bacteriol* **110**, 402–429.

Brown, J. M., Frazier, R. P., Morey, R. E., Steigerwalt, A. G., Pellegrini, G. J., Daneshvar, M. I., Hollis, D. G. & McNeil, M. M. (2005). Phenotypic and genetic characterization of clinical isolates of CDC coryneform group A-3: proposal of a new species of *Cellulomonas*, *Cellulomonas denverensis* sp. nov. *J Clin Microbiol* **43**, 1732–1737.

Brown, J. M., Steigerwalt, A. G., Morey, R. E., Daneshvar, M. I., Romero, L.-J. & McNeil, M. M. (2006). Characterization of clinical isolates previously identified as *Oerskovia turbata*: proposal of *Cellulosimicrobium funkei* sp. nov. and emended description of the genus *Cellulosimicrobium*. *Int J Syst Evol Microbiol* **56**, 801–804.

Chun, J. & Goodfellow, M. (1995). A phylogenetic analysis of the genus *Nocardia* with 16S rRNA gene sequences. *Int J Syst Bacteriol* **45**, 240–245.

Collins, M. D. (1994). Isoprenoid quinones. In *Chemical Methods in Prokaryotic Systematics*, pp. 265–309. Edited by M. Goodfellow & A. G. O'Donnell. Chichester: Wiley.

Collins, M. D., Pirouz, T., Goodfellow, M. & Minnikin, D. E. (1977). Distribution of menaquinones in actinomycetes and corynebacteria. *J Gen Microbiol* **100**, 221–230.

Elberson, M. A., Malekzadeh, F., Yazdi, M. T., Kameranpour, N., Noori-Daloii, M. R., Matte, M. H., Shahamat, M., Colwell, R. R. & Sowers, K. R. (2000). *Cellulomonas persica* sp. nov. and Cellulomonas *iranensis* sp. nov., mesophilic cellulose-degrading bacteria isolated from forest soils. *Int J Syst Evol Microbiol* **50**, 993–996.

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.

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

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

Gordon, R. E., Barnett, D. A., Handerhan, J. E. & Pang, C. H.-N. (1974). *Nocardia coeliaca*, *Nocardia autotrophica*, and the nocardin strain. *Int J Syst Bacteriol* **24**, 54–63.

Groth, I., Schumann, P., Weiss, N., Martin, K. & Rainey, F. A. (1996). *Agrococcus jenensis* gen. nov., sp. nov., a new genus of actinomycetes with diaminobutyric acid in the cell wall. *Int J Syst Bacteriol* **46**, 234–239.

Groth, I., Schumann, P., Martin, K., Schuetze, B., Augsten, K., Kramer, I. & Stackebrandt, E. (1999). *Ornithinicoccus hortensis* gen. nov., sp. nov., a soil actinomycete which contains L-ornithine. *Int J Syst Bacteriol* **49**, 1717–1724.

Groth, I., Schumann, P., Schütze, B., Gonzalez, J. M., Laiz, L., Saiz-Jimenez, C. & Stackebrandt, E. (2005). *Isoptericola hypogeus* sp. nov., isolated from the Roman catacomb of Domitilla. *Int J Syst Evol Microbiol* **55**, 1715–1719.

Hanada, S., Liu, W.-T., Shintani, T., Kamagata, Y. & Nakamura, K. (2002). *Tetrasphaera elongata* sp. nov., a polyphosphate-accumulating bacterium isolated from activated sludge. *Int J Syst Evol Microbiol* **52**, 883–887.

Huang, Y., Dai, X., He, L., Wang, Y.-N., Wang, B.-J., Liu, Z. & Liu, S.-J. (2005). *Sanguibacter marinus* sp. nov., isolated from coastal sediment. *Int J Syst Evol Microbiol* **55**, 1755–1758.

Ishikawa, T. & Yokota, A. (2006). Reclassification of *Arthrobacter duodecadis* Lochhead 1958 as *Tetrasphaera duodecadis* comb. nov. and emended description of the genus *Tetrasphaera*. *Int J Syst Evol Microbiol* **56**, 1369–1373.

Jeon, Y.-S., Chung, H., Park, S., Hur, I., Lee, J.-H. & Chun, J. (2005). jPHYDIT: a JAVA-based integrated environment for molecular phylogeny of ribosomal RNA sequences. *Bioinformatics* **21**, 3171–3173.

Jones, B. E., Grant, W. D., Duckworth, A. W., Schumann, P., Weiss, N. & Stackebrandt, E. (2005). *Cellulomonas bogoriensis* sp. nov., an alkaliphilic cellulomonad. *Int J Syst Evol Microbiol* **55**, 1711–1714.

Jukes, T. H. & Cantor, C. R. (1969). Evolution of protein molecules. In *Mammalian Protein Metabolism*, vol. 3, pp. 21–132. Edited by H. N. Munro. New York: Academic Press.

Kim, B. S., Oh, H. M., Kang, H., Park, S. S. & Chun, J. (2004). Remarkable bacterial diversity in the tidal flat sediment as revealed by 16S rDNA analysis. *J Microbiol Biotechnol* **14**, 205–211.

Kim, B. S., Oh, H. M., Kang, H. & Chun, J. (2005). Archaeal diversity in tidal flat sediment as revealed by 16S rDNA analysis. *J Microbiol* **43**, 144–151.

Mackenzie, S. L. (1987). Gas chromatographic analysis of amino acids as the *N*-heptafluorobutyryl isobutyl esters. *J Assoc Off Anal Chem* **70**, 151–160.

Maszenan, A. M., Seviour, R. J., Patel, B. K. C., Schumann, P., Burghardt, J., Tokiwa, Y. & Stratton, H. M. (2000). Three isolates of novel polyphosphate-accumulating Gram-positive cocci, obtained from activated sludge, belong to a new genus, *Tetrasphaera* gen. nov., and description of two new species, *Tetrasphaera japonica* sp. nov. and *Tetrasphaera australiensis* sp. nov. *Int J Syst Evol Microbiol* **50**, 593–603.

Mesbah, M., Premachandran, U. & Whitman, W. B. (1989). Precise measurement of the G+C content of deoxyribonucleic acid by high-performance liquid chromatography. *Int J Syst Bacteriol* **39**, 159–167.

Park, Y.-D., Baik, K. S., Yi, H., Bae, K. S. & Chun, J. (2005). *Pseudoalteromonas byunsanensis* sp. nov., isolated from tidal flat sediment in Korea. *Int J Syst Evol Microbiol* **55**, 2519–2523.

Prauser, H., Schumann, P., Rainey, F. A., Kroppenstedt, R. M. & Stackebrandt, E. (1997). *Terracoccus luteus* gen. nov., sp. nov., an LL-diaminopimelic acid-containing coccoid actinomycete from soil. *Int J Syst Bacteriol* **47**, 1218–1224.

Pukall, R., Schumann, P., Schütte, C., Gols, R. & Dicke, M. (2006). *Acaricomes phytoseiuli* gen. nov., sp. nov., isolated from the predatory mite *Phytoseiulus persimilis*. *Int J Syst Evol Microbiol* **56**, 465–469.

Rivas, R., Trujillo, M. E., Mateos, P. F., Martínez-Molina, E. & Velázquez, E. (2004). *Cellulomonas xylanilytica* sp. nov., a cellulolytic and xylanolytic bacterium isolated from a decayed elm tree. *Int J Syst Evol Microbiol* **54**, 533–536.

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. (1985). Analysis of the chemical composition and primary structure of murein. *Methods Microbiol* **18**, 123–156.

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

Schleifer, K. H. & Seidl, P. H. (1985). Chemical composition and structure of murein. In *Chemical Methods in Bacterial Systematics*, pp. 201–219. Edited by M. Goodfellow & D. E. Minnikin. London: Academic Press.

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.

Smibert, R. M. & Krieg, N. R. (1994). Phenotypic characterization. In *Methods for General and Molecular Bacteriology*, pp. 607–654. Washington, DC: American Society for Microbiology.

Stackebrandt, E. & Schumann, P. (2000). Introduction to the taxonomy of actinobacteria. In *The Prokaryotes: an Evolving Electronic Resource for the Microbiological Community*, release 3.3. Edited by M. Dworkin. New York: Springer. http://link.springer-ny.com/link/service/books/10125/

Stackebrandt, E., Schumann, P. & Prauser, H. (2002). The Family *Cellulomonadaceae*. In *The Prokaryotes: an Evolving Electronic Resource for the Microbiological Community*, release 3.0. Edited by M. Dworkin, S. Falkow, E. Rosenberg, K.-H. Schleifer & E. Stackebrandt. New York: Springer. http://link.springer-ny.com/link/service/books/10125/

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

Staley, J. T. (1968). *Prosthecomicrobium* and *Ancalomicrobium*: new prosthecate freshwater bacteria. *J Bacteriol* **95**, 1921–1942.

Swofford, D. L. (1998). PAUP*: Phylogenetic Analysis Using Parsimony (*and other methods), version 4. Sunderland, MA: Sinauer Associates.

Uchida, K. & Aida, K. (1984). An improved method for the glycolate test for simple identification of the acyl type of bacterial cell walls. *J Gen Appl Microbiol* **30**, 131–134.

Yassin, A. F., Haggenei, B., Budzikiewicz, H. & Schaal, K. P. (1993). Fatty acid and polar lipid composition of the genus *Amycolatopsis*: application of fast atom bombardment-mass spectrometry to structure analysis of underivatized phospholipids. *Int J Syst Bacteriol* **43**, 414–420.

Yi, H. & Chun, J. (2006). *Thalassobius aestuarii* sp. nov., isolated from tidal flat sediment. *J Microbiol* **44**, 171–176.

Zhang, Y.-Q., Schumann, P., Li, W.-J., Chen, G.-Z., Tian, X.-P., Stackebrandt, E., Xu, L.-H. & Jiang, C.-L. (2005). *Isoptericola halotolerans* sp. nov., a novel actinobacterium isolated from saline soil from Qinghai Province, north-west China. *Int J Syst Evol Microbiol* **55**, 1867–1870.

Zobell, C. E. (1941). Studies on marine bacteria. I. The cultural requirements of heterotrophic aerobes. *J Mar Res* **4**, 42–75.