Cellulomonas bogoriensis sp. nov., an alkaliphilic cellulomonad

Brian E. Jones,¹ William D. Grant,² A. W. Duckworth,² Peter Schumann,³ Norbert Weiss³ and Erko Stackebrandt³

Correspondence

Brian E. Jones
bjones@genencor.com

¹Genencor International BV, Archimedesweg 30, 2333 CN Leiden, The Netherlands

²Department of Infection, Immunity and Inflammation, University of Leicester, PO Box 138, Leicester LE1 9HN, UK

³DSMZ – Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Mascheroder Weg 1b, 38124 Braunschweig, Germany

An alkaliphilic, slightly halotolerant, chemo-organotrophic, Gram-positive, rod-shaped bacterium, strain 69B4^T, was isolated from the sediment of the littoral zone of Lake Bogoria, Kenya. Phylogenetically, it is a member of the genus *Cellulomonas*, showing less than 97.5% sequence similarity to the type strains of other *Cellulomonas* species. The highest level of similarity, albeit moderate, was found with respect to *Cellulomonas cellasea* DSM 20118^T. Chemotaxonomic properties confirm the 16S rRNA gene-based generic affiliation, i.e. a DNA G + C content of 71.5 mol%, anteiso-C_{15:0} and C_{16:0} as the major fatty acids, MK-9(H₄) as the major isoprenoid quinone, a peptidoglycan containing L-ornithine as the diamino acid and D-aspartic acid in the interpeptide bridge and phosphatidylglycerol as the only identified main polar lipid. The strain is aerobic to facultatively anaerobic, being capable of growth under strictly anaerobic conditions. Optimal growth occurs between pH values 9.0 and 10.0. On the basis of its distinct phylogenetic position and metabolic properties, strain 69B4^T represents a novel species of the genus *Cellulomonas*, for which the name *Cellulomonas bogoriensis* sp. nov. is proposed. The type strain is 69B4^T (= DSM 16987^T = CIP 108683^T).

The genus *Cellulomonas* is a member of the suborder *Micrococccineae*, order *Actinomycetales*, class *Actinobacteria* (Stackebrandt *et al.*, 1997), being related to *Oerskovia*, and, more distantly, to *Terrabacter*, *Rarobacter* and relatives (Schumann *et al.*, 2001; Stackebrandt *et al.*, 2002). *Cellulomonas* contains 12 species, only one of which, *Cellulomonas xylanilytica*, has been described recently (Rivas *et al.*, 2004). Most species are phylogenetically well separated, *C. xylanilytica* LMG 21723^T and *Cellulomonas humilata* ATCC 25174^T being the most closely related pair of type strains (99% 16S rRNA gene sequence similarity, 36% DNA–DNA reassociation; Rivas *et al.*, 2004).

Strain 69B4^T was isolated from a sample of sediment and water from the littoral zone of Lake Bogoria, at Acacia Camp (0° 12' N 36° 07' E), Kenya on 10 October 1988. The water temperature was 33 °C, the pH was 10.5 and the conductivity was 44 mS cm⁻¹. The strain was partially characterized during a broad phylogenetic survey of soda-lake alkaliphiles (Duckworth *et al.*, 1996) and was tentatively

placed in the *Arthrobacter*/*Terrabacter* region of the dendrogram based on 16S rRNA gene sequences (no *Cellulomonas* spp. sequences were included for comparison). Later, the strain was sent to the identification service of the DSMZ for cell-wall analyses to confirm its affiliation to the genus *Cellulomonas*. Since partial identification (Duckworth *et al.*, 1996) highlighted its isolated phylogenetic position, perhaps indicative of a novel species, the characterization was extended to allow proper taxonomic description.

Strain 69B4^T was isolated at 37 °C on an alkaline casein medium containing the following (g l⁻¹): glucose (10), Difco peptone (5), Difco yeast extract (5), K₂HPO₄ (1), MgSO₄·7H₂O (0.2), NaCl (40), Na₂CO₃ (10), casein (20) and agar (20). Cultivation was done in glucose alkaline medium (GAM) consisting of two parts. GAM solution A contained the following, dissolved in 800 ml distilled water and sterilized: glucose (10 g), Difco peptone (5 g), Difco yeast extract (5 g), K₂HPO₄ (1 g) and MgSO₄·7H₂O (0.2 g). GAM solution B contained 40 g NaCl and 10 g Na₂CO₃ dissolved in 200 ml distilled water and sterilized. The two solutions were then mixed. Solid medium was prepared by adding agar (2%, w/v) to GAM solution A before sterilization (Duckworth *et al.*, 1996). The cultural properties are indicated in the species description. Standard

Published online ahead of print on 1 April 2005 as DOI 10.1099/ij.s.0.63646-0.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain 69B4^T is X92152.

Table 1. Cultural and metabolic properties that distinguish strain 69B4^T from *Cellulomonas* species

Taxa: 1, strain 69B4^T; 2, *C. flavigena*; 3, *C. fermentans*; 4, *C. persica*; 5, *C. iranensis*; 6, *C. biazotea*; 7, *C. uda*; 8, *C. fimi*; 9, *C. gelida*; 10, *C. hominis*; 11, *C. humilata*; 12, *C. cellasea*; 13, *C. xylanilytica*. Data are from Stackebrandt & Kandler (1979), Bagnara *et al.* (1985), Funke *et al.* (1995), Collins & Pascual (2000), Elberson *et al.* (2000), Schumann *et al.* (2001) and Rivas *et al.* (2004). Symbols: ND, not determined; w, weak.

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13
Catalase	+	+	—	ND	ND	+	+	+	+	+	—	+	—
Motility	+	+	—	+	+	+	—	+	+	+	—	—	—
Nitrate reduction	—	+	+	+	+	+	+	+	+	+	—	+	+
Urease	—	—	—	+	+	—	—	—	—	—	—	—	—
DNase	+	—	+	+	+	—	+	ND	+	+	ND	—	ND
Gelatin hydrolysis	+	+	+	W	+	+	+	+	+	+	W	—	+
Utilization of:													
Mannitol	—	—	+	ND	ND	—	—	—	—	—	+	+	—
Ribose	—	+	—	—	—	—	—	—	—	—	W	—	—
D-Raffinose	—	—	+	—	—	+	—	—	—	+	W	—	+
Rhamnose	W	—	—	ND	ND	+	—	—	—	+	+	—	+
Peptidoglycan type	L-Orn— D-Asp	L-Orn— D-Asp	L-Orn— D-Asp	L-Orn— D-Asp	L-Orn— D-Asp	L-Orn— D-Glu	L-Orn— D-Glu	L-Orn— D-Glu	L-Orn— D-Glu	L-Orn— D-Glu	L-Orn— D-Glu	L-Orn— D-Glu	L-Orn— D-Glu

physiological tests were carried out according to the methods described by Smibert & Krieg (1994). Acid production from carbon sources and enzyme activities were studied using the API 50 CH, API ZYM and API Coryne substrate panels (bioMérieux). Antibiotic sensitivity was tested by using the disc-diffusion method (Oxoid). The results are summarized in Table 1 and in the species description. Sequence analysis of the 16S rRNA gene was performed as described by Duckworth *et al.* (1996). The sequence (X92152) was reanalysed (Rainey *et al.*, 1996) because of the presence of some ambiguous nucleotides. Chemotaxonomic properties were investigated as described by Groth *et al.* (1999) and are indicated in the species description. Despite being phylogenetically coherent, members of *Cellulomonas* exhibit differences in terms of the amino acid composition of peptidoglycan (Schleifer & Kandler, 1972; Fiedler & Kandler, 1973). Whilst four type strains and strain 69B4^T contain D-aspartic acid as the interpeptide bridge, the other species contain D-glutamic acid. The distribution does not parallel the phylogenetic relatedness of the strains (Fig. 1).

The pairwise 16S rRNA gene similarity values determined for strain 69B4^T and other type strains of *Cellulomonas* species are moderate (95.0–97.2 % similarity), the highest value being that obtained with *C. cellasea* DSM 20118^T (97.2 %). Neighbour-joining and distance matrix analyses (Saitou & Nei, 1987; De Soete, 1983) place strain 69B4^T adjacent to *Cellulomonas fermentans* DSM 3133^T (96.4 % sequence similarity), but strain 69B4^T and *C. cellasea* DSM 20118^T have a shorter added branch distance than the former pair of strains. Strain 69B4^T shares with strain DSM 3133^T the same peptidoglycan type but differs from this strain in terms of nitrate reduction and the use of mannitol and raffinose. The phenotypic differences between strain

69B4^T and strain DSM 20118^T are even greater, in addition to the different peptidoglycan type. On the basis of a combination of phylogenetic distinctness, differences in diagnostic amino acids in peptidoglycan, and metabolic traits, we conclude that strain 69B4^T represents a novel species, for which the name *Cellulomonas bogoriensis* sp. nov. is proposed.

Fig. 1. Phylogenetic relatedness of strain 69B4^T and type strains of *Cellulomonas* species, based on 16S rRNA gene sequence comparison. *Beutenbergia cavernae* DSM 12333^T (GenBank accession no. Y18378) and *Jonesia denitrificans* DSM 20603^T (X83811) served as a root (not shown). The dendrogram was generated by using neighbour-joining analysis (Saitou & Nei, 1987). Numbers within the dendrogram indicate percentages of occurrence of the branching order in 500 bootstrapped trees (only values of 50% and above are shown). Bar, 2 substitutions per 100 nt.

Cellulomonas bogoriensis sp. nov.

Cellulomonas bogoriensis (bo.go.ri.en'sis. N.L. fem. adj. *bogoriensis* pertaining to Lake Bogoria, Kenya).

Fresh cultures consist of Gram-positive, slender, generally straight and rod-shaped cells, each approximately 0.5–0.7 µm in size. Primary mycelium is not formed. Older cultures contain mainly short rods and coccoid cells; 'V'-forms and pairs may occur. Primary branching not observed. On alkaline GAM agar, colonies are opaque, glistening, pale yellow, circular and convex or domed, the margins are entire, and they are about 2 mm in diameter after 2–3 days at 37 °C. Colonies are viscous or slimy and tend to clump when scraped with a loop. On neutral tryptone soy agar (Oxoid), growth is less vigorous, colonies are translucent and yellow, and generally <1 mm in diameter. The temperature range for growth is 20–37 °C, with an optimum around 30–37 °C. No growth occurs at 15 or 45 °C. Alkaliphilic and slightly halotolerant. Growth occurs at pH values between 6.0 and 10.5, with an optimum around pH 9–10. No growth occurs at pH 11 or pH 5.5. Growth below pH 7 is less vigorous and less abundant. Growth occurs in medium containing 0–8.0% (w/v) NaCl. Chemo-organotrophic. Growth occurs on complex substrates such as yeast extract and peptone. Facultatively anaerobic; acid is produced aerobically and anaerobically (API 50 CH) from the following: L-arabinose, D-xylose, D-glucose, D-fructose, D-mannose, cellobiose, maltose, sucrose, trehalose, gentiobiose, D-turanose, D-lyxose, rhamnose (weak) and 5-ketogluconate (weak). Utilizes amygdalin, arbutin, salicin and aesculin. Unable to utilize ribose, lactose, galactose, melibiose, D-raffinose, glycogen, glycerol, erythritol, inositol, mannitol, sorbitol, xylitol, arabitol, gluconate or lactate. Hydrolyses starch, gelatin, casein, carboxymethylcellulose and amorphous cellulose. The following enzymes are produced (API ZYM, API Coryne): C4-esterase, C8-esterase, leucine arylamidase, α-chymotrypsin, α-glucosidase, β-glucosidase and pyrazinamidase. Susceptible to ampicillin (25 µg), chloramphenicol (25 µg), erythromycin (5 µg), fusidic acid (10 µg), methicillin (10 µg), novobiocin (5 µg), streptomycin (10 µg), tetracycline (25 µg), sulphafurazole (100 µg), oleandomycin (5 µg), polymyxin (300 IU), rifampicin (2 µg), vancomycin (30 µg) and bacitracin (10 IU). Resistant to gentamicin (10 µg), nitrofurantoin (50 µg), nalidixic acid (30 µg), sulphamethoxazole (50 µg), trimethoprim (2.5 µg), penicillin G (1 IU), neomycin (30 µg) and kanamycin (30 µg). The murein contains the amino acids L-ornithine and D-aspartic acid as diagnostic amino acids (L-Orn-D-Asp type, variation A4β). The main menaquinone is MK-9(H₄). Major fatty acids (>1.0%) are anteiso-C_{15:0} (54.9 mol%), C_{16:0} (12.9 mol%), iso-C_{15:0} (5.5 mol%), iso-C_{16:0} (5.1 mol%), C_{14:0} (8.8 mol%), anteiso-C_{15:1} (4.1 mol%), iso-C_{14:0} (2.6 mol%) and anteiso-C_{17:0} (2.8 mol%). Phosphatidylglycerol is the only identified phospholipid; three unknown phospholipids occur as well. The DNA G+C content of the type strain is 71.5 mol%.

The type strain, 69B4^T (= DSM 16987^T = CIP 108683^T), was isolated from the littoral zone of Lake Bogoria, Kenya, at Acacia Camp.

Acknowledgements

We appreciate the excellent technical assistance of Daan Meijer.

References

- 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.
- Collins, M. D. & Pascual, C. (2000). Reclassification of *Actinomyces humiferus* (Gledhill and Casida) as *Cellulomonas humilata* nom. corrig., comb. nov. *Int J Syst Evol Microbiol* **50**, 661–663.
- De Soete, G. (1983). A least square algorithm for fitting additive trees to proximity data. *Psychometrika* **48**, 621–626.
- Duckworth, A. W., Grant, W. D., Jones, B. E. & van Steenberg, R. (1996). Phylogenetic diversity of soda lake alkaliphiles. *FEMS Microbiol Ecol* **19**, 181–191.
- Elberson, M. A., Malekzadeh, F., Yazdi, M. T., Kameranpour, N., Noori-Dalooi, 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.
- Fiedler, F. & Kandler, O. (1973). Die Mureintypen in der Gattung *Cellulomonas* Bergey et al. *Arch Mikrobiol* **89**, 41–50 (in German).
- Funke, G., Ramos, C. P. & Collins, M. D. (1995). Identification of some clinical strains of CDC coryneform group A-3 and A-4 bacteria as *Cellulomonas* species and proposal of *Cellulomonas hominis* sp. nov. for some group A-3 strains. *J Clin Microbiol* **33**, 2091–2097.
- Groth, I., Schumann, P., Martin, K., Schütze, B., Augsten, K., Kramer, I. & Stackebrandt, E. (1999). *Ornithinococcus hortensis* gen. nov., sp. nov., a soil actinomycete which contains L-ornithine. *Int J Syst Bacteriol* **49**, 1717–1724.
- Rainey, F. A., Ward-Rainey, N., Kroppenstedt, R. M. & Stackebrandt, E. (1996). The genus *Nocardiopsis* represents a phylogenetically coherent taxon and a distinct actinomycete lineage: proposal of *Nocardiopsaceae* fam. nov. *Int J Syst Bacteriol* **46**, 1088–1092.
- 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. & Kandler, O. (1972). Peptidoglycan types of bacterial cell walls and their taxonomic implications. *Bacteriol Rev* **36**, 407–477.
- Schumann, P., Weiss, N. & Stackebrandt, E. (2001). Reclassification of *Cellulomonas cellulans* (Stackebrandt and Keddle 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. 607–655. Edited by P. Gerhardt, R. G. E. Murray, W. A. Wood & N. R. Krieg. Washington, DC: American Society for Microbiology.
- Stackebrandt, E. & Kandler, O. (1979). Taxonomy of the genus *Cellulomonas*, based on phenotypic characters and deoxyribonucleic

acid-deoxyribonucleic acid homology, and proposal of seven neotype strains. *Int J Syst Bacteriol* **29**, 273–282.

Stackebrandt, E., Rainey, F. A. & Ward-Rainey, N. L. (1997). Proposal for a new hierarchic classification system, *Actinobacteria* classis nov. *Int J Syst Bacteriol* **47**, 479–491.

Stackebrandt, E., Schumann, P. & Prauser, H. (2002). The family *Cellulomonadaceae*. In *The Prokaryotes*, 3rd edn, release 3.10. Edited by M. Dworkin, S. Falkow, E. Rosenberg, K. H. Schleifer & E. Stackebrandt. New York: Springer. http://141.150.157.117:8080/prokPUB/chaprender/jsp/showchap.jsp?chapnum=440&initsec=01_