Cellulomonas bogoriensis sp. nov., an alkaliphilic cellulosomalad

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An alkaliphilic, slightly halotolerant, chemo-organotrophic, Gram-positive, rod-shaped bacterium, strain 69B47, 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 cellulae DSM 20118T. Chemotaxonomic properties confirm the 16S rRNA gene-based generic affiliation, i.e. a DNA G+C content of 71.5 mol%, anteiso-C15:0 and C16:0 as the major fatty acids, MK-9(H4) as the major isoprenoid quione, 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 69B47 represents a novel species of the genus Cellulomonas, for which the name Cellulomonas bogoriensis sp. nov. is proposed. The type strain is 69B47 (= DSM 16987T = CIP 108683T).

The genus Cellulomonas is a member of the suborder Micrococinae, 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 21723T and Cellulomonas humilata ATCC 25174T being the most closely related pair of type strains (99% 16S rRNA gene sequence similarity, 36% DNA–DNA reassociation; Rivas et al., 2004).

Strain 69B47 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 69B47 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 properties confirm the 16S rRNA gene-based generic affiliation, i.e. a DNA G+C content of 71.5 mol%, anteiso-C15:0 and C16:0 as the major fatty acids, MK-9(H4) as the major isoprenoid quione, 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 69B47 represents a novel species of the genus Cellulomonas, for which the name Cellulomonas bogoriensis sp. nov. is proposed. The type strain is 69B47 (= DSM 16987T = CIP 108683T).
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 of *Cellulomonas* exhibit differences in terms of 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 DSM 20118T and other type strains of *Cellulomonas* species are moderate (95-0–97.2 % similarity), the highest value being that obtained with *C. cellasea* DSM 20118T (97.2%). Neighbour-joining and distance matrix analyses (Saitou & Nei, 1987; De Soete, 1983) place strain DSM 20118T adjacent to *Cellulomonas fermentans* DSM 3133T (96-4% sequence similarity), but strain DSM 20118T and *C. cellasea* DSM 20118T have a shorter added branch distance than the former pair of strains. Strain DSM 20118T shares with strain DSM 3133T 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 69B4T and strain DSM 20118T 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 69B4T represents a novel species, for which the name *Cellulomonas bogoriensis* sp. nov. is proposed.

### Table 1. Cultural and metabolic properties that distinguish strain 69B4T from *Cellulomonas* species

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#### Fig. 1. Phylogenetic relatedness of strain 69B4T and type strains of *Cellulomonas* species, based on 16S rRNA gene sequence comparison. *Beutenbergia cavernae* DSM 12333T (GenBank accession no. Y18378) and *Jonesia denitrificans* DSM 20603T (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.


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. Growth below pH 7 is less vigorous and less abundant. Growth occurs in medium containing 0–8% (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-xylene, D-glucose, D-fructose, D-mannose, cellobiose, maltose, sucrose, trehalose, gentiobiose, D-turanose, L-lyxose, rhamnose (weak) and 5-ketogluconate (weak). Utilizes amygdalin, arbutin, salicin and aesculin. Unable to utilize ribose, lactose, galactose, melibiose, D-raffinose, glycecon, glycerol, erythritol, inositol, mannotol, sorbitol, xyitol, arabinose, fructose 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, z-chymotrypsin, z-glucosidas and amyginidase. Susceptible to ampicillin (25 μg), chloramphenicol (25 μg), erythromycin (5 μg), fusidic acid (10 μg), methicillin (10 μg), novobiocin (5 μg), streptomycin (10 μg), tetacycline (25 μg), sulfaguanazole (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), sulphanmethoxazole (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-C15:0 (54-9 mol%), C16:0 (12-9 mol%), iso-C15:0 (5-5 mol%), iso-C16:0 (5-1 mol%), C14:0 (8-8 mol%), anteiso-C15:1 (4-1 mol%), iso-C14:0 (2-6 mol%) and anteiso-C17:0 (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, 69B4T (= DSM 16987T = CIP 108683T), was isolated from the littoral zone of Lake Bogoria, Kenya, at Acacia Camp.

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References


