Bogoriella caseilytica gen. nov., sp. nov., a New Alkaliphilic Actinomycete from a Soda Lake in Africa

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A new gram-positive, alkaliphilic, nonsporulating, rod-shaped bacterium is described. The organism was isolated from soda soil (Lake Bogoria, Kenya) and has the following characteristics. It is nonmotile, not acid fast, halotolerant, and microaerophilic, and optimal growth occurs at pH values between 9 and 10. The peptidoglycan type is of type A4 α , with lysine as the characteristic diamino acid and glutamic acid as a component of the interpeptide bridge. The major menaquinone is MK-8(H₄). The polar lipids are phosphatidylglycerol, diphosphatidylglycerol, phosphatidylinositol, and an unknown phospholipid. 12-Methyltetradecanoic acid is the predominant fatty acid. The G+C content of the DNA is 70 mol%. The results of 16S ribosomal DNA sequence comparisons revealed that strain HKI 0088^T represents a new lineage in the order Actinomycetales. Therefore, we concluded that strain HKI 0088^T should be assigned to a new genus and species, for which we propose the name *Bogoriella caseilytica* gen. nov., sp. nov. The type strain and only strain of this genus and species is HKI 0088 (= DSM 11294).

The actinomycetes are a group of morphologically and phylogenetically diverse gram-positive bacteria with high DNA guanine-plus-cytosine (G+C) contents (>55 mol%). They are widespread in nature and are able to occupy extreme habitats, such as antarctic soil (39), desert regions (6, 49), hot springs (2), and highly saline areas (31). Because of the biotechnologically important secondary metabolites of these organisms and their role in decomposition of hazardous chemical compounds in nature, many isolation programs have focused on actinomycetes. Since 1990 32 new genera of actinomycetes have been described; 24 of these new genera are monotypic. Because of the use of improved chemotaxonomic and molecular techniques, 10 of these genera were established by describing new combinations for members of previously described genera (32, 41, 42). In addition, novel taxa have been isolated from different environmental and clinical samples, and the taxonomic positions of these taxa have been clarified by polyphasic approaches (16, 21, 30, 47, 48).

In this paper we describe the morphological, physiological, chemotaxonomic, and phylogenetic characteristics of an isolate obtained from a soda soil sample collected near Lake Bogoria, Kenya. On the basis of our results and the unique taxonomic properties of the organism, we concluded that strain HKI 0088^T is a member of a new genus and a new species, for which we propose the name *Bogoriella caseilytica*. Strain HKI 0088 is the type strain and only strain of *B. caseilytica*. This strain has been deposited in the German Collection of Microorganisms and Cell Cultures as strain DSM 11294^T.

MATERIALS AND METHODS

Bacterial strain and cultural conditions. Strain HKI 0088^T was isolated from a sample of soda soil (pH 10) collected by W. D. Grant in 1988 near Lake Bogoria in the Kenyan-Tanzanian Rift Valley. The sample was stored at 4°C and was kindly provided for investigation in 1994. Isolation of the organism involved

dilution plating on medium A (8) containing (per liter) 10.0 g of glucose, 5.0 g of peptone (Difco Laboratories, Detroit, Mich.), 5.0 g of yeast extract (Difco), 1.0 g of KH₂PO₄, 0.2 g of MgSO₄ · 7H₂O, 40.0 g of NaCl, 10.0 g of Na₂CO₃, and 20.0 g of agar (pH 9.6). The NaCl and Na₂CO₃ were autoclaved separately and were added to the organic compounds at 60°C before the agar medium was poured. General laboratory cultivation was performed on solid medium A or in liquid medium A. For comparison, strain HKI 0088^T was also cultivated on rich (R) medium (46) and on Bacto Nutrient Agar (Difco).

Morphological and physiological characteristics. Cell morphology was examined by phase-contrast microscopy with a model BH-2 microscope (Olympus, Tokyo, Japan). Motility was studied by the hanging drop method. Cell dimensions were measured with an ocular (×10) and an objective (×100/1.25). Gram staining was performed by using Hucker's modification and reagents produced by bioMerieux (Marcy l'Etoile, France). The decoloriser for Gram staining was replaced by 96% (wt/vol) ethanol. Acid fastness was determined with Ziehl-Neelsen stain (23). Colony morphology was studied by using a stereo microscope (Olympus model SZ 11). For scanning electron microscopy a 48-h-old strain HKI 0088^T culture from medium A was suspended in a phosphate-buffered salt solution. The cells were fixed with 0.5% glutaraldehyde (pH 7.4), washed several times, and dehydrated in a series of ethanol concentrations. After the cells were sputter coated with gold-palladium, they were observed with a scanning electron microscope (model JSM 6300 F; JEOL, Tokyo, Japan) at 3 kV by using a working distance of 7 mm.

The pH range for growth was determined in medium A adjusted to pH values between 7.2 and 10.7 with 5 M HCl or 5 M NaOH by measuring the optical density at 600 nm and comparing it with the optical density of uninoculated medium. Tolerance to NaCl was investigated by using NaCl concentrations between 2 and 18% (wt/vol) in medium A and R medium. The effects of different temperatures on growth were determined with medium A and Bacto Nutrient Agar incubated at 28, 37, 45, and 50°C. Oxygen requirements were studied with Generbag microaer and Generbag anaer incubation systems (bioMerieux). Acid production from carbohydrates was examined by using a modification of the oxidation-fermentation (OF) medium of Hugh and Leifson (17). The basal medium contained (per liter) 2.0 g of Bacto Peptone (Difco), 1.0 g of Bacto Meat Extract (Difco), 3.0 g of Bacto Agar (Difco), and 15.0 ml of a bromothymol blue solution (0.2%, wt/vol) (pH 7.2). In addition, the ability of the organism to utilize a broad range of carbon sources was tested by using the panel of 95 carbon-containing compounds on BIOLOG GP microplates (BIOLOG, Inc., Hayward, Calif.) and the procedure described by Miller et al. (26). The inocula for the microplates were grown on the recommended Biolog Universal Growth Medium (BIOLOG) and on Bacto Nutrient Agar. The enzyme activities in a 48-h-old culture grown on solid medium A were examined by using API ZYM galleries (bioMerieux). Utilization of organic acids was studied by using the medium described by Cowan and Steel (5) and adding the sodium salt of each organic acid at a final concentration of 0.2% (wt/vol) (in the case of tartrate the potassium salt was added). Nitrate reduction, urease activity, production of indole and hydrogen sulfide, hydrolysis of Tween 80, esculin, and gelatin, and the methyl red and Voges-Proskauer reactions were examined as described by Lanyi (23). To

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examine nitrate reduction, the reagents NIT 1 and NIT 2 (bioMerieux) were used. Urease activity was tested on solidified Bacto Urea Agar Base (Difco). Indole production and the methyl red and Voges-Proskauer reactions were examined by using Kovács reagent and reagents VP 1 and VP 2 (bioMerieux), respectively. Hydrogen sulfide production was determined by using commercial test strips (Difco). Hydrolysis of gelatin was tested in medium A and Bacto Nutrient Broth (Difco) by using UV-sterilized unprocessed film strips. Decomposition of adenine, hypoxanthine, xanthine, and tyrosine was studied by using the method recommended by Gordon et al. (13). Hydrolysis of casein and potato starch was studied as described by Gledhill and Casida (12) by using Bacto Nutrient Agar (Difco) instead of brain heart infusion agar. Hydrolysis of hippurate was tested on hippurate agar (5). Cytochrome *c* oxidase activity was studied by monitoring the oxidation of *N,N,N',N'*-tetramethylphenylenediamine dihydrochloride on DrySlide oxidase (Difco). Catalase production was determined as described by Gledhill and Casida (12). Susceptibility to antibiotics was studied by placing antibiotic discs (Oxoid) on Bacto Nutrient Agar plates seeded with suspensions of strain HKI 0088^T.

Cell wall analysis. Purified cell wall preparations were obtained by the method of Schleifer and Kandler (37). The amino acids and peptides in cell wall hydrolysates were analyzed by two-dimensional ascending thin-layer chromatography on cellulose plates by using previously described systems (37). The amino-terminal amino acid of the interpeptide bridge was determined by dinitrophenylation as described by Schleifer (36). Whole-cell sugars were determined as alditol acetates by gas chromatography and gas chromatography-mass spectrometry as described previously (16). The molar ratios of amino acids were determined by gas chromatography and gas chromatography-mass spectrometry of *N*-heptafluorobutyl amino acid isobutyl esters (24). The enantiomers of cell wall amino acids were analyzed by gas chromatography of *N*-pentafluoropropionyl amino acid isopropyl esters (11) on an L-Chirasil-Val column (25 m by 0.25 mm [inside diameter]; Macherey-Nagel, Düren, Germany) by using He as the carrier gas at a linear velocity of 200 mm/s, and the temperature was programmed to increase from 80 to 190°C at a rate of 2°C/min. The instruments used for gas chromatography and gas chromatography-mass spectrometry have been described previously (16). The glycolate content of bacterial cell walls was determined by the colorimetric method of Uchida and Aida (45).

Lipid analysis. Cellular fatty acid methyl esters obtained by the method described by Stead et al. (44) were separated by gas chromatography (16) and were identified as described previously (39). Menaquinones were extracted as described by Collins et al. (4) and were analyzed by high-performance liquid chromatography (16). Polar lipids extracted by the method of Minnikin et al. (28) were identified by two-dimensional thin-layer chromatography and by spraying the preparations with specific reagents (3). The absence of mycolic acids was demonstrated by thin-layer chromatography (27).

DNA base composition. DNA was isolated and its G+C content was determined by high-performance liquid chromatography of deoxyribonucleosides as described previously (16).

16S rDNA sequence determination and phylogenetic analysis. Genomic DNA extraction, PCR-mediated amplification of the 16S ribosomal DNA (rDNA), and sequencing of the PCR products were performed as described previously (33). The sequence reaction mixtures were electrophoresed by using a model 373A automatic DNA sequencer (Applied Biosystems, Foster City, Calif.). The 16S rDNA sequences were manually aligned with the 16S rDNA sequences of members of the order *Actinomycetales* by using the ae2 editor (25). Evolutionary distances were calculated by the method of Jukes and Cantor (19). Phylogenetic dendrograms were reconstructed by using treeing algorithms contained in the PHYLIP package (10).

Nucleotide sequence accession number. The 16S rDNA sequence determined in this study for strain HKI 0088^T has been deposited in the EMBL database under accession no. Y09911.

RESULTS

Morphological and cultural characteristics. Strain HKI 0088^T grew as round, slimy, slightly convex, glistening colonies that were 1 to 3 mm in diameter and were mainly pale yellow to intense yellow (Fig. 1). The cells were gram-positive, non-acid-fast, nonmotile, thin irregular rods or coccoid (0.5 to 0.8 by 1.0 to 2.5 µm) that occurred singly, in pairs, or in small clusters (Fig. 2). Sometimes filaments up to 10 µm long appeared in the populations. Endospores were never observed. Scanning electron micrographs (Fig. 3) revealed the presence of spiky structures distributed over the whole surface of each cell. Such spikes have not been found previously in coryneform bacteria. They may be comparable to the nonprosthecae appendages of the gram-negative spinate bacteria (9). The so-called spiniae are distinct from flagella and pili. In the case of isolate HKI 0088^T further investigation will be necessary to clarify the chemical composition of these structures, their pos-

FIG. 1. Single colonies of strain HKI 0088^T grown on solid medium A at 28°C for 6 days. Bar = 1 mm.

sible function as a reflection of adaptation to the special alkaline ecological niche, and their taxonomic significance. Strain HKI 0088^T was isolated on alkaline medium A (pH 9.6) but was able to grow at pH 7.2 on Bacto Nutrient Agar and R medium at 28 and 37°C. Growth at neutral pH on agar media was delayed and reduced, especially at 37°C and after serial transfers. At 45°C only traces of growth were observed on media at neutral and alkaline pH values. At 50°C no growth occurred. The optimum initial pH for growth in liquid medium A ranged from 8.8 to 10.1, indicating that strain HKI 0088^T is an alkaliphilic organism (14, 15) which is able to grow well under aerobic conditions and in the presence of reduced O₂ concentrations. In an anaerobic atmosphere, however, traces of growth were observed. NaCl in alkaline medium A did not influence growth at NaCl concentrations up to 8% (wt/vol). At an NaCl concentration of 10% (wt/vol) growth was markedly reduced, and poor growth occurred in the presence of 18% (wt/vol) NaCl. In R medium growth occurred in the presence of NaCl concentrations up to 6% (wt/vol). Only traces of poor growth were observed in the presence of NaCl concentrations between 8 and 14% (wt/vol).

FIG. 2. Micrograph of strain HKI 0088^T cells grown in liquid medium A at 28°C for 24 h. Bar = 10 µm.

FIG. 3. Scanning electron micrograph of cells from a 48-h-old culture of strain HKI 0088^T grown at 28°C on solid medium A. Bar = 1 μm.

Physiological characteristics. The physiological properties of strain HKI 0088^T are shown in Tables 1 and 2. The ability of strain HKI 0088^T to utilize a broad range of carbon sources was demonstrated by the classical method of Hugh and Leifson (17) and by the microplate technique (Biolog system). Except for inulin and L-rhamnose, there was a good correlation between the results obtained with the two different methods for the carbon sources tested. The activities of some enzymes of carbohydrate metabolism determined with the API ZYM method (Table 1) confirmed these results. Cultivation of the inoculum for the GP microplates on Bacto Nutrient Agar gave similar good results compared with the results obtained with Biolog Universal Growth Medium. The alkali salts of the organic acids acetate, aconitate, benzoate, citrate, formate, malate, succinate, and tartrate were not used for growth. Nitrogenous compounds like ammonia, nitrate, hippurate, and urea were not utilized. Strain HKI 0088^T preferred complex nitrogenous compounds, such as peptone and casein, for growth. Gelatin was hydrolyzed in alkaline medium A when peptone was limited in the stationary growth phase. Due to the reduced growth at neutral pH, no peptone limitation occurred, and gelatin was not attacked. Esculin was hydrolyzed, but adenine, hypoxanthine, tyrosine, Tween 80, and xanthine were not decomposed. Strain HKI 0088^T was susceptible to all of the antibiotics tested except nitrofurantoin and sulfonamid.

Chemotaxonomic characteristics. The peptidoglycan of strain HKI 0088^T was characterized by the presence of lysine (Lys), alanine (Ala), and glutamic acid (Glu) at a molar ratio of 1.0:4.3:2.1. Glu was found to be the amino-terminal amino acid of the interpeptide bridge. The analysis of the enanti-

omers of the cell wall amino acids resulted in detection of L-Lys, detection of D-Ala and L-Ala (molar ratio, 1:3.5), and detection of D-Glu and L-Glu (molar ratio, 1:1.8). From these results and from the two-dimensional thin-layer chromatographic peptide patterns of partial hydrolysates of cell walls, we concluded that strain HKI 0088^T represents a new variation of peptidoglycan type A4α (7, 37) with an L-Lys←L-Ala←L-Ala←L-Glu interpeptide bridge. The acyl type is acetyl. Galactose was the only whole-cell sugar. The polar lipids were phosphatidylglycerol, diphosphatidylglycerol, phosphatidylinositol, and an unknown phospholipid. The fatty acid profile was characterized by the predominance of 12-methyltetradecanoic acid (ai-C_{15:0}) and by the presence of iso- and anteiso-branched acids as minor components (Table 3). The menaquinones were MK-8(H₄) and MK-9(H₄) (peak area ratio, 87:11). No mycolic acids were present. The G+C content of the DNA of strain HKI 0088^T was 70 mol%.

16S rDNA sequence analysis. An almost complete 16S rDNA sequence comprising 1,476 nucleotides (>95% of the *Escherichia coli* sequence [1]) was determined for strain HKI 0088^T. The phylogenetic dendrogram in Fig. 4 was reconstructed from evolutionary distances by the neighbor-joining method (35). A total of 1,322 nucleotides present in all of the strains between positions 41 and 1458 (*E. coli* numbering [1]) were used for this analysis. The phylogenetic dendrogram (Fig. 4) shows that strain HKI 0088^T falls within the radiation of the order *Actinomycetales*. This strain represents a distinct lineage that exhibits 90.1 to 93.4% 16S rDNA sequence similarity to the other members of the cluster included in the analysis (data not shown).

TABLE 1. Physiological properties of strain HKI 0088^T

Characteristic	Reaction of strain HKI 0088 ^T
Decomposition of:	
Adenine	- ^a
Casein	+
Esculin	+
Gelatin	+
Hippurate	-
Hypoxanthine	-
Potato starch	+
Tween 80	-
Tyrosine	-
Urea	-
Xanthine	-
Acid produced from:	
L-Arabinose	+
D-Cellobiose	+
Dextrin	+
D-Fructose	w
D-Galactose	+
D-Glucitol	w
D-Glucose	+
Glycerol	+
Inulin	+
Lactose	w
Maltose	w
D-Mannitol	w
D-Mannose	+
D-Raffinose	w
L-Rhamnose	+
D-Ribose	+
Salicin	+
Sucrose	w
Potato starch	+
Trehalose	+
D-Xylose	+
Utilization of:	
Acetate	-
Aconitate	-
Benzoate	-
Citrate	-
Formate	-
Malate	-
Succinate	-
DL-Tartrate	-
Enzyme activities (API ZYM)	
Phosphatase (alkaline)	+
Esterase (C4)	+
Esterase-lipase (C8)	+
Lipase (C14)	-
Leucine arylamidase	+
Valine arylamidase	-
Cystine arylamidase	-
Trypsin	-
Chymotrypsin	-
Phosphatase (acid)	+
Naphthol-AS-BI-phosphohydrolase	-
α-Galactosidase	-
β-Galactosidase	+
β-Glucuronidase	-
α-Glucosidase	+
β-Glucosidase	+
N-Acetyl-β-glucosamidase	+
α-Mannosidase	+
α-Fucosidase	-
Nitrate reduction	-
Production of H ₂ S	w
Catalase reaction	+
Voges-Proskauer test	-

Continued

TABLE 1—Continued

Characteristic	Reaction of strain HKI 0088 ^T
Methyl red test	-
Oxidase test	-
Indole test	-
Growth in the presence of:	
2% NaCl	+
4% NaCl	+
6% NaCl	+
8% NaCl	+
10% NaCl	w
12% NaCl	w
14% NaCl	w
16% NaCl	w
18% NaCl	(w)
Growth at:	
28°C	+
37°C	+
45°C	w
50°C	-
Antibiotic susceptibility	
Ampicillin (10 µg)	+
Chloramphenicol (30 µg)	+
Ciprofloxacin (5 µg)	+
Erythromycin (15 µg)	+
Gentamicin (10 µg)	+
Kanamycin (30 µg)	+
Lincomycin (2 µg)	+
Neomycin (30 µg)	+
Nitrofurantoin (300 µg)	-
Oxacillin (5 µg)	+
Oxytetracycline (30 µg)	+
Penicillin G (2 IU)	+
Polymyxin B (300 IU)	+
Rifampin (2 µg)	+
Streptomycin (10 µg)	+
Sulfonamid	-

^a -, negative; +, positive; w, weakly positive.

DISCUSSION

Soda lakes in Kenya are interesting naturally occurring alkaline environments with different levels of salinity (14, 15). As they are assumed to harbor numerous novel taxa, their microbial diversity will be explored more (8, 14, 15, 18, 29, 50, 51). The recent findings of Duckworth et al. (8) support our conclusion based on an investigation of six different soil or water-sediment samples from this area that the gram-positive isolates were mainly members of the genus *Bacillus*. Despite of the long storage of the samples (6 years) at 4°C, we isolated actinomycetes which we identified on the basis of morphological and chemotaxonomic characteristics as members of the genera *Aureobacterium*, *Arthrobacter*, *Nesterenkonia*, *Micrococcus*, *Celulomonas*, *Clavibacter*, *Rhodococcus*, *Nocardia*, *Nocardiopsis*, *Micromonospora*, and *Streptomyces*. To our knowledge, isolate HKI 0088^T represents the first novel genus of high-G+C-content gram-positive bacteria from the soda lakes. The results of our 16S rDNA sequence comparisons clearly demonstrated the distinct position of strain HKI 0088^T within the radiation of the actinomycetes. This was demonstrated both by the phylogenetic dendrogram and by the range of levels of 16S rDNA sequence similarity to other taxa. Genus status was also supported by the results of the chemotaxonomic analyses.

The structure of the peptidoglycan of strain HKI 0088^T represents a variation of type A4α (37) with an L-Lys←L-Ala←L-Ala←L-Glu interpeptide bridge which has not been

TABLE 2. Utilization of different substrates as sole carbon sources for respiration (formation of NADH) by strain HKI 0088^T after cultivation on two different media

Carbon source	Utilization on:	
	BUGM ^a	NA ^a
α-Cyclodextrin	+	+
β-Cyclodextrin	+	+
Dextrin	+	+
Glycogen	+	+
Inulin	—	—
Mannan	—	—
Tween 40	—	—
Tween 80	—	—
N-Acetylglucosamine	—	—
N-Acetylmannosamine	—	—
Amygdalin	+	+
L-Arabinose	+	+
D-Arabitol	+	+
Arbutin	+	+
Cellobiose	+	+
D-Fructose	+	+
L-Fucose	+	+
D-Galactose	+	+
D-Galacturonic acid	w	—
Gentiobiose	+	+
D-Gluconic acid	—	—
α-D-Glucose	+	+
m-Inositol	—	—
α-D-Lactose	+	+
Lactulose	+	+
Maltose	+	+
Maltotriose	+	+
D-Mannitol	+	+
D-Mannose	+	+
D-Melezitose	+	+
D-Melibiose	+	+
α-Methyl-D-galactoside	—	—
β-Methyl-D-galactoside	+	+
3-Methylglucose	+	+
α-Methyl-D-glucoside	—	—
β-Methyl-D-glucoside	+	+
α-Methyl-D-mannoside	+	+
Palatinose	+	+
D-Psicose	—	—
D-Raffinose	+	+
L-Rhamnose	—	—
D-Ribose	+	+
Salicin	+	+
Sedoheptulosan	—	—
D-Sorbitol	+	+
Stachyose	w	w
Sucrose	+	+
D-Tagatose	+	+
D-Trehalose	+	+
Turanose	+	+
Xylitol	—	—
D-Xylose	+	+
Acetic acid	—	—
α-Hydroxybutyric acid	+	+
β-Hydroxybutyric acid	—	—
γ-Hydroxybutyric acid	—	—
p-Hydroxyphenylacetic acid	—	—
α-Ketoglutaric acid	—	—
α-Ketovaleric acid	—	—
Lactamide	—	—
D-Lactic acid methyl ester	—	—
L-Lactic acid	—	—
D-Malic acid	—	—
L-Malic acid	—	—

Continued

TABLE 2—Continued

Carbon source	Utilization on:	
	BUGM ^a	NA ^a
Methylpyruvate	+	+
Methylsuccinate	+	—
Propionic acid	—	—
Pyruvic acid	+	+
Succinamic acid	—	—
Succinic acid	—	—
N-Acetyl-L-glutamic acid	—	—
Alaninamide	—	—
D-Alanine	—	—
L-Alanine	—	—
L-Alanyl-glycine	—	—
L-Asparagine	—	—
L-Glutamic acid	—	—
Glycyl-L-glutamic acid	—	—
L-Pyrogutamic acid	—	—
L-Serine	—	—
Putrescine	—	—
2,3-Butanediol	—	—
Glycerol	+	+
Adenosine	—	—
2'-Deoxyadenosine	—	—
Inosine	—	—
Thymidine	—	—
Uridine	—	—
AMP	—	—
TMP	—	—
UMP	—	—
Fructose 6-phosphate	—	—
Glucose 1-phosphate	—	—
Glucose 6-phosphate	—	—
DL-α-Glycerophosphate	w	—

^a BUGM, Biolog Universal Growth Medium; NA, Bacto Nutrient Agar (Difco).^b +, positive; —, negative; w, weakly positive.

reported previously (7, 37, 38). Type A11.35 (7), a similar variation of peptidoglycan type A4α with an L-Lys←L-Ala←L-Glu interpeptide bridge, has been found in *Arthrobacter nicotianae* DSM 20123, *Arthrobacter uratoxydans* DSM 20647^T, and *Brevibacterium liquefaciens* DSM 20579^T (considered synonymous with *A. nicotianae*) (40). These species are clearly different from our strain; their menaquinones are MK-8 and MK-9 menaquinones, they lack the phospholipid phosphatidylinositol, and their DNA G+C contents are lower (60 to 66 mol%) (20, 40). The typical rod-coccus growth cycle of *Arthrobacter* species was not found in strain HKI 0088^T. Isolate HKI 0088^T

TABLE 3. Cellular fatty acid composition of strain HKI 0088^T

Fatty acid	%
i-C _{14:0}	1.0
C _{14:0}	0.4
i-C _{15:0}	0.4
i-C _{15:1}	5.1
ai-C _{15:0}	81.6
C _{15:0}	0.5
i-C _{16:0}	3.1
C _{16:0}	1.1
C _{16:1}	0.7
ai-C _{17:0}	6.0

^a The abbreviations for fatty acids are illustrated by the following examples: C_{16:0}, hexadecanoic acid; C_{18:1}, octadecenoic acid; i-C_{15:0}, 13-methyltetradecanoic acid; ai-C_{15:0}, 12-methyltetradecanoic acid.

FIG. 4. 16S rDNA sequence-based phylogenetic dendrogram reconstructed from evolutionary distances by using the neighbor-joining method (35). Scale bar = 5 inferred nucleotide substitutions per 100 nucleotides.

is also phylogenetically clearly distinct from the other A4 α peptidoglycan-containing species, such as *A. nicotianae* and *Cellulomonas turbata*, which have been shown previously to cluster with the respective type species of their genera (22, 34). The same is true for the differentiation of strain HKI 0088^T from the MK-8(H₄)-containing organisms *Nocardioides albus* and *Nocardioides simplex*, which have been shown to be members of the suborder *Propionibacterineae*, a distinct phylogenetic group within the *Actinomycetales* (43) that is separate from the suborder *Micrococceinae* (43), into which strain HKI 0088^T falls.

On the basis of the results described above and in view of the uniqueness of some of the taxonomic properties, we propose that strain HKI 0088^T represents a new genus in the order *Actinomycetales*.

Description of *Bogoriella* gen. nov. *Bogoriella* (Bo.go.ri.el'la. M. L. dim. fem. n. *Bogoriella*, named after Lake Bogoria in Kenya, the place from which isolate HKI 0088^T originated). Cells are irregular and rod shaped or coccoid and occur singly, in pairs, or in small irregular clusters. The cells are gram positive, not acid fast, and nonmotile. Endospores are not formed. Aerobic or microaerophilic. Very poor growth can occur under anaerobic conditions. Catalase positive and oxidase negative. The peptidoglycan type is type A4 α with lysine as the characteristic diamino acid. The acyl type is acetyl. The phospholipids are phosphatidylglycerol, diphosphatidylglycerol, phosphatidylinositol, and an unknown phospholipid. Mycolic acids are absent, and the cellular fatty acids are of the iso- and anteiso-branched type; 12-methyltetradecanoic acid is the major fatty acid. The major menaquinone is MK-8(H₄). The G+C content of the DNA is 70 mol%. The type species is *Bogoriella caseilytica*.

Description of *Bogoriella caseilytica* sp. nov. *Bogoriella caseilytica* (ca. sei.ly' ti.ca. L. n. *caseus*, cheese; Gr. v. *lyein*, to loosen, dissolve; M. L. fem. adj. *caseilytica*, loosening or dissolving casein). Cells are irregular and rod shaped or coccoid and occur singly, in pairs, or in small clusters. The cells are 0.5 to 0.8 by 1.0 to 2.5 μ m, gram positive, not acid fast, and nonmotile, and no endospores are formed. A few cells may be filamentous and up to 10 μ m long. Colonies are round, smooth, slightly convex, glistening, and 1 to 3 mm in diameter. The colony color varies from pale yellow to intense yellow. Alkaliphilic. Optimal growth occurs at pH 9 to 10 and at 28 to 37°C. NaCl tolerant. Aerobic or microaerophilic. Acids are produced from L-arabinose, D-cellobiose, dextrin, D-fructose, D-galactose, D-glucose, D-glucitol, glycerol, inulin, lactose, maltose, D-mannitol, D-mannose, D-raffinose, L-rhamnose, D-ribose, salicin, sucrose, potato starch, trehalose, and D-xylose. Acetate, aconitate, benzoate, citrate, formate, malate, succinate, and tartrate are not utilized as carbon sources. Starch, esculin, casein, and gelatin are hydrolyzed. Urea, hippurate, adenine, hypoxanthine, xanthine, tyrosine, and Tween 80 are not decomposed or hydrolyzed. Nitrate is not reduced to nitrite. Hydrogen sulfide is produced; indole is not produced. Methyl red and Voges-Proskauer reactions are negative. Catalase positive and oxidase negative. Cells are susceptible to ampicillin, chloramphenicol, ciprofloxacin, erythromycin, gentamicin, kanamycin, lincomycin, neomycin, oxacillin, oxytetracycline, penicillin G, polymyxin B, rifampin, and streptomycin. Cells are resistant to nitrofur and sulfonamid. The habitat is soil. The G+C content is 70 mol%. The type strain is strain HKI 0088 (= DSM 11294).

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