Daeguia caeni gen. nov., sp. nov., isolated from sludge of a textile dye works

Jung-Hoon Yoon, So-Jung Kang, Sooyeon Park and Tae-Kwang Oh

Correspondence

Jung-Hoon Yoon

jhyoon@kribb.re.kr

Korea Research Institute of Bioscience and Biotechnology (KRIIBB), PO Box 115, Yusong, Taejeon, Republic of Korea

A Gram-negative, non-spore-forming, rod-shaped bacterial strain, K107^T, was isolated from sludge collected from a textile dye works in Korea and its taxonomic position was investigated by means of a polyphasic analysis. Strain K107^T contained Q-10 as the predominant ubiquinone. The major fatty acids (>10% of total fatty acids) were C_{18:1}ω7c and C_{18:1} 2-OH. The DNA G + C content was 57.0 mol%. A phylogenetic analysis based on 16S rRNA gene sequences showed that strain K107^T was closely related to the genera *Mycoplana*, *Brucella* and *Ochrobactrum*. Strain K107^T exhibited 16S rRNA gene sequence similarity values of 96.3–97.1% with respect to the type strains of two *Mycoplana* species and 94.8–96.8% with respect to members of the genera *Brucella* and *Ochrobactrum*. A phylogenetic analysis based on *recA* gene sequences showed that strain K107^T forms a distinct phylogenetic lineage within the *Alphaproteobacteria*. The *recA* gene sequence of strain K107^T showed similarity values of 84.5% with respect to type strains of *Brucella* species and values of 77.6–83.1% with respect to members of the genera *Pseudochrobactrum*, *Ochrobactrum* and *Mycoplana*. Strain K107^T could be differentiated from phylogenetically related genera by differences in phenotypic properties and fatty acid profiles. On the basis of phenotypic, chemotaxonomic and phylogenetic data, strain K107^T represents a novel genus and species, for which the name *Daeguia caeni* gen. nov., sp. nov. is proposed. The type strain of *Daeguia caeni* is strain K107^T (=KCTC 12981^T =CCUG 54520^T).

In the course of the screening of micro-organisms from the wastewater treatment plant of a textile dye works in Korea, many bacterial strains were isolated and characterized taxonomically (Yoon *et al.*, 2007a, b, c, d). This study focuses on one of these isolates, designated strain K107^T. Comparative 16S rRNA gene sequence analysis indicated that strain K107^T is phylogenetically closely related to the family *Brucellaceae* within the *Alphaproteobacteria*. The aim of the present work was to determine the exact taxonomic position of strain K107^T by using a polyphasic characterization that included determination of its phenotypic properties and detailed phylogenetic analyses based on its 16S rRNA and *recA* gene sequences.

Strain K107^T was isolated on trypticase soy agar (TSA; Difco) at 30 °C using the standard dilution plating

technique. *Mycoplana dimorpha* DSM 7138^T and *Mycoplana ramosa* DSM 7292^T, which were used as reference strains for fatty acid analyses, were obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (Braunschweig, Germany). The morphological, physiological and biochemical characteristics of strain K107^T were investigated using routine cultivation on TSA at 37 °C. Cell morphology was examined by using light microscopy (E600; Nikon) and transmission electron microscopy with cells from exponentially growing cultures. Flagellation was determined by using a Philips CM-20 transmission electron microscope with cells from exponentially growing cultures: for this purpose, the cells were negatively stained with 1% (w/v) phosphotungstic acid and the grids were examined after being air-dried. The Gram reaction was determined using the bioMérieux Gram stain kit according to the manufacturer's instructions. Growth at various temperatures (4–60 °C) was investigated on TSA. The pH range for growth was determined in nutrient broth (Difco) adjusted to various pH values (pH 4.5–10.5, in increments of 0.5 pH units) by the addition of HCl or Na₂CO₃. Growth in the absence of NaCl and at various NaCl concentrations (0.5%, w/v, and 1.0–7.0%, w/v, using increments of 1.0%) was investigated using trypticase soy broth prepared according to the

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strain K107^T, *Mycoplana dimorpha* DSM 7138^T and *Mycoplana ramosa* DSM 7292^T are EF532794, EU022307 and EU022308, respectively, and that for the *recA* gene sequence of strain K107^T is EU022306.

Cellular fatty acid compositions for strain K107^T and two *Mycoplana* type strains are presented in a supplementary table available with the online version of this paper.

formula of the Difco medium except that NaCl was excluded. Growth under anaerobic conditions was determined after incubation in an anaerobic chamber on TSA and on TSA supplemented with potassium nitrate (0.1 %, w/v), both of which had been prepared anaerobically under nitrogen. Catalase and oxidase activities and hydrolysis of casein, gelatin, hypoxanthine, starch, Tweens 20, 40, 60 and 80, tyrosine, urea and xanthine were determined as described by Cowan & Steel (1965). The hydrolysis of aesculin and the reduction of nitrate were studied as described previously (Lanyi, 1987). Susceptibility to antibiotics was tested on TSA plates using antibiotic discs containing the following: polymyxin B, 100 U; streptomycin, 50 µg; penicillin G, 20 U; chloramphenicol, 100 µg; ampicillin, 10 µg; cephalothin, 30 µg; gentamicin, 30 µg; novobiocin, 5 µg; tetracycline, 30 µg; kanamycin, 30 µg; lincomycin, 15 µg; oleandomycin, 15 µg; neomycin, 30 µg; and carbenicillin, 100 µg. The utilization of various substrates, enzyme activities and other physiological and biochemical properties were tested by using the API 20E, API 20NE, API 50 CH and API ZYM systems (bioMérieux). The cells were suspended in AUX medium, according to the manufacturer's instructions, for inoculation of the API 50 CH system.

Cell biomass for DNA extraction and for isoprenoid quinone analysis was obtained from cultivation in trypticase soy broth (Difco) at 37 °C. Chromosomal DNA was isolated and purified according to the method described by Yoon *et al.* (1996), with the exception that RNase T1 was used in combination with RNase A to minimize contamination with RNA. The 16S rRNA gene was amplified by using a PCR with two universal primers, 5'-GAGTTTG-ATCCTGGCTCAG-3' and 5'-AGAAAGGAGGTGATC-CAGCC-3', as described previously (Yoon *et al.*, 1998). PCR amplification of a partial *recA* gene was performed by using two primers, 5'-CGKCTSGTAGAGGAYAAATCGG-TGGA-3' and 5'-CGRATCTGGTTGATGAAGATCACC-AT-3', as described by Gaunt *et al.* (2001). Sequencing of the amplified 16S rRNA and *recA* genes and phylogenetic analysis were performed as described by Yoon *et al.* (2003). Isoprenoid quinones were extracted according to the method of Komagata & Suzuki (1987) and analysed using reversed-phase HPLC and a YMC ODS-A (250 × 4.6 mm) column. For the cellular fatty acid analysis, cell mass of strain K107^T, *M. dimorpha* DSM 7138^T and *M. ramosa* DSM 7292^T was harvested from TSA plates after incubation for 3 days at 30 °C. The fatty acids were extracted and fatty acid methyl esters were prepared according to the standard protocol of the MIDI/Hewlett Packard Microbial Identification System (Sasser, 1990). The DNA G+C content was determined using the method of Tamaoka & Komagata (1984), with the modification that DNA was hydrolysed and the resultant nucleotides were analysed by reversed-phase HPLC.

Morphological, cultural, physiological and biochemical characteristics of strain K107^T are given in the species description or are shown in Table 1. The almost-complete

16S rRNA gene sequences of strain K107^T, *M. dimorpha* DSM 7138^T and *M. ramosa* DSM 7292^T determined in this study comprised 1441, 1440 and 1440 nt, respectively, representing approximately 96 % of the *Escherichia coli* 16S rRNA gene sequence. In the phylogenetic tree constructed using the neighbour-joining algorithm, strain K107^T joined the cluster comprising the type strains of *M. dimorpha* and *M. ramosa* (Fig. 1). This relationship was maintained in the tree based on the maximum-parsimony algorithm, but not in the tree based on the maximum-likelihood algorithm (Fig. 1). Strain K107^T exhibited 16S rRNA gene sequence similarity values of 96.3 and 97.1 % with respect to *M. dimorpha* DSM 7138^T and *M. ramosa* DSM 7292^T, respectively, 96.8 % to *Brucella melitensis* ATCC 23456^T, 94.8–96.7 % to the type strains of *Ochrobactrum* species and 94.7–95.1 % to the type strains of *Pseudochrobactrum* species. The *recA* gene sequence (550 nt) for strain K107^T was determined in this study: it showed the highest level of similarity (84.5 %) with respect to type strains of *Brucella* species, the sequences of which are identical. The *recA* gene sequence of strain K107^T had similarity values of 82.0–83.1 % with respect to the type strains of *Pseudochrobactrum* species, 79.1–80.7 % to the type strains of *Ochrobactrum* species and 77.6–77.9 % to the type strains of *Mycoplana* species.

The predominant isoprenoid quinone detected in strain K107^T was Q-10 (at a peak area ratio of approximately 95 %); a minor amount of Q-9 (4 %) was present. The major fatty acids (constituting >10 % of total fatty acids) detected in strain K107^T were C_{18:1}ω7c (66.2 %) and C_{18:1} 2-OH (10.3 %) (see Supplementary Table S1 in IJSEM Online). This fatty acid profile was similar to those of the two *Mycoplana* species analysed in this study (Supplementary Table S1). However, the proportions of some fatty acids differed between strain K107^T and the two *Mycoplana* species: in particular, fatty acid C_{18:1} 2-OH was present in significant amounts in strain K107^T, but was not detected in either of the two *Mycoplana* species analysed (Supplementary Table S1). The DNA G+C content of strain K107^T was 57.0 mol%.

In the 16S rRNA gene sequence analysis, strain K107^T was phylogenetically most closely related to *M. ramosa*. However, strain K107^T was distinguishable from *M. dimorpha* and *M. ramosa* on the basis of differences in phenotypic properties, DNA G+C contents and fatty acid profiles. Strain K107^T was also distinguishable from members of the genera *Brucella*, *Ochrobactrum* and *Pseudochrobactrum* on the basis of differences in fatty acid profiles, particularly in the compositions of fatty acids cyclo-C_{19:0}ω8c and C_{18:1} 2-OH (although these differences may be the result of differences in cultivation conditions and extraction procedures) (Dees *et al.*, 1980, 1981; Tripathi *et al.*, 2006; Kämpfer *et al.*, 2007) (Table 1). In the phylogenetic analysis based on *recA* gene sequences, strain K107^T formed a phylogenetic lineage that is distinct from those of members of the *Alphaproteobacteria*, including *Brucella*, *Ochrobactrum*, *Pseudochrobactrum* and

Table 1. Differential phenotypic characteristics of strain K107^T and phylogenetically related genera

Taxa: 1, strain K107^T; 2, *Mycoplana* [data from Urakami *et al.* (1990) and this study]; 3, *Ochrobactrum* (Kämpfer *et al.*, 2007; Teyssier *et al.*, 2007; Tripathi *et al.*, 2006; Zurdo-Piñero *et al.*, 2007); 4, *Pseudochrobactrum* (Kämpfer *et al.*, 2006, 2007); 5, *Brucella* (Corbel & Brinley-Morgan, 1984; Dees *et al.*, 1980, 1981). +, Positive; –, negative; v, variable; ND, no data available.

Characteristic	1	2	3	4	5
Growth at 45 °C	+	–	v	ND	–
Urease	–	+	v	ND	ND
Nitrate reduction	–	v	v	ND	+
Assimilation of:					
L-Arabinose	–	+	v	v	v
D-Galactose	–	+	v	v	v
D-Glucose	+	+	+	v	v
D-Fructose	–	+	v	v	ND
Maltose	–	v	v	–	ND
Mannose	+	+	v	v	ND
Rhamnose	–	ND	v	v	ND
Ribose	–	ND	+ or ND	v	v
Sucrose	–	v	v	–	ND
Trehalose	–	–	v	v	ND
D-Xylose	–	+	+ or ND	v	v
Glycerol	–	+	ND	ND	ND
Inositol	+	–	v	–	ND
D-Mannitol	–	+	v	–	ND
D-Sorbitol	–	+	v	–	ND
Major fatty acid(s) (>10 % of total)	C _{18:1} ω7c and C _{18:1} 2-OH	C _{18:1} ω7c	C _{18:1} ω7c and cyclo-C _{19:0} ω8c, C _{18:1} 2-OH or C _{16:0}	C _{18:1} ω7c and cyclo-C _{19:0} ω8c or C _{18:0}	C _{18:1} ω7c, C _{16:0} and cyclo-C _{19:0} ω8c or C _{18:0}
DNA G + C content (mol%)	57.0	64–65	55–59	50.9*	55–58

*Data available for *Pseudochrobactrum asaccharolyticum* only.

Mycoplana (Fig. 2). Accordingly, strain K107^T represents a novel genus and species that is distinct from the genera *Brucella*, *Ochrobactrum*, *Pseudochrobactrum* and *Mycoplana*, for which the name *Daeguia caeni* gen. nov., sp. nov. is proposed.

Description of *Daeguia* gen. nov.

Daeguia (Dae.gu'i.a. N.L. fem. n. *Daeguia* pertaining to Daegu, the location of the textile dye works from which the type strain of the type species was isolated).

Fig. 1. Neighbour-joining phylogenetic tree, based on 16S rRNA gene sequences (alignment length 1369 bp), showing the positions of strain K107^T and some related taxa. Bootstrap percentages (based on 1000 replications) >50 % are shown at branching points. Filled circles indicate that the corresponding nodes were also recovered in the trees generated with the maximum-likelihood and maximum-parsimony algorithms. Open circles indicate that the corresponding nodes were also recovered only in the tree generated with the maximum-parsimony algorithm. *Azorhizobium caulinodans* LMG 6465^T was used as an outgroup. Bar, 0.01 substitutions per nucleotide position.

Fig. 2. Neighbour-joining phylogenetic tree, based on partial *recA* gene sequences (alignment length 504 bp), showing the positions of strain K107^T and some related taxa. Bootstrap percentages (based on 1000 replications) >50% are shown at branching points. Filled circles indicate that the corresponding nodes were also recovered in the trees generated with the maximum-likelihood and maximum-parsimony algorithms. *[Mycoplana] bullata* ATCC 4278^T was used as an outgroup. Bar, 0.1 substitutions per nucleotide position.

Cells are Gram-negative, non-spore-forming rods. The predominant ubiquinone is Q-10. The major fatty acid is C_{18:1}ω7c; a significant amount of C_{18:1} 2-OH is present. Exhibits 16S rRNA gene sequence similarity values of 94.7–97.1% with respect to members of the genera *Brucella*, *Mycoplana*, *Ochrobactrum* and *Pseudochrobactrum*. The *recA* gene (550 nt) exhibits sequence similarity values of 77.6–84.5% with respect to the genera *Brucella*, *Mycoplana*, *Ochrobactrum* and *Pseudochrobactrum*. The DNA G+C content of the type strain of the type species is 57.0 mol%. The type species is *Daeguia caeni*.

Description of *Daeguia caeni* sp. nov.

Daeguia caeni (cae'ni. L. gen. n. *caeni* of sludge).

Exhibits the following properties in addition to those given in the genus description. Cells are 0.4–0.6 × 0.7–2.0 μm in size. Colonies on TSA are circular, convex, smooth, glistening, greyish yellow in colour and 1.0–1.5 mm in diameter after 2 days incubation at 37 °C. The optimal temperature for growth is 30–37 °C. Growth occurs at 15 and 55 °C, but not at 10 or 56 °C. The optimal pH for growth is 7.0–8.0; growth occurs at pH 5.0 and 9.0, but not at pH 4.5 or 9.5. Growth occurs in the presence of 0–4% (w/v) NaCl; optimal growth occurs in the presence of 0.5–1.0% (w/v) NaCl. No anaerobic growth occurs on TSA or on TSA supplemented with nitrate. Hypoxanthine is hydrolysed, but aesculin, casein, gelatin, starch, tyrosine, xanthine, urea and Tweens 20, 40, 60 and 80 are not. Arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase and tryptophan deaminase are absent. H₂S and indole are not produced. Adonitol, *N*-acetylglucosamine and phenylacetate are assimilated and adipate is weakly assimilated, but erythritol, D-arabinose, L-xylose, methyl β-D-xyloside, sorbose, dulcitol, methyl α-D-mannoside, methyl α-D-glucoside, amygdalin, arbutin, aesculin, salicin, cellobiose, lactose, melibiose, inulin, melezitose, raffinose, starch, glycogen, xylitol, gentiobiose, turanose,

D-lyxose, D-tagatose, D-fucose, L-fucose, D-arabitol, L-arabitol, gluconate, 2-ketogluconate, 5-ketogluconate, caprate, citrate and malate are not assimilated. Alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, acid phosphatase and naphthol-AS-BI-phosphohydrolase are present, but lipase (C14), valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase are absent. Susceptible to carbenicillin, cephalothin, chloramphenicol, gentamicin, kanamycin, neomycin, novobiocin, streptomycin and tetracycline, but not to ampicillin, lincomycin, oleandomycin, penicillin G or polymyxin B. Other phenotypic characteristics are given in Table 1.

The type strain, K107^T (=KCTC 12981^T =CCUG 54520^T), was isolated from sludge from a textile dye works in Daegu, Korea.

Acknowledgements

This work was supported by the 21C Frontier Program of Microbial Genomics and Applications (grant MG05-0401-2-0) from the Ministry of Science and Technology (MOST) of the Republic of Korea.

References

- Corbel, M. J. & Brinley-Morgan, W. J. (1984). Genus *Brucella* Meyer and Shaw 1920. In *Bergey's Manual of Systematic Bacteriology*, vol. 1, pp. 377–388. Edited by N. R. Krieg & J. G. Holt. Baltimore: Williams & Wilkins.
- Cowan, S. T. & Steel, K. J. (1965). *Manual for the Identification of Medical Bacteria*. London: Cambridge University Press.
- Dees, S., Thanabalasundrum, S., Moss, C. W., Hollis, D. G. & Weaver, R. E. (1980). Cellular fatty acid composition of group IVe, a nonsaccharolytic organism from clinical sources. *J Clin Microbiol* 11, 664–668.

- Dees, S. B., Hollis, D. G., Weaver, R. E. & Moss, C. W. (1981). Cellular fatty acids of *Brucella canis* and *Brucella suis*. *J Clin Microbiol* **14**, 111–112.
- Gaunt, M. W., Turner, S. L., Rigottier-Gois, L., Lloyd-Macgilp, S. A. & Young, J. P. W. (2001). Phylogenies of *atpD* and *recA* support the small subunit rRNA-based classification of rhizobia. *Int J Syst Evol Microbiol* **51**, 2037–2048.
- Kämpfer, P., Rosselló-Mora, R., Scholz, H. C., Welinder-Olsson, C., Falsen, E. & Busse, H.-J. (2006). Description of *Pseudochrobactrum* gen. nov., with the two species *Pseudochrobactrum asaccharolyticum* sp. nov. and *Pseudochrobactrum saccharolyticum* sp. nov. *Int J Syst Evol Microbiol* **56**, 1823–1829.
- Kämpfer, P., Scholz, H., Huber, B., Thummes, K., Busse, H.-J., Maas, E. W. & Falsen, E. (2007). Description of *Pseudochrobactrum kiredjianiae* sp. nov. *Int J Syst Evol Microbiol* **57**, 755–760.
- Komagata, K. & Suzuki, K. (1987). Lipid and cell-wall analysis in bacterial systematics. *Methods Microbiol* **19**, 161–207.
- Lanyi, B. (1987). Classical and rapid identification methods for medically important bacteria. *Methods Microbiol* **19**, 1–67.
- Sasser, M. (1990). *Identification of bacteria by gas chromatography of cellular fatty acids*, MIDI Technical Note 101. Newark, DE: MIDI Inc.
- Tamaoka, J. & Komagata, K. (1984). Determination of DNA base composition by reversed-phase high-performance liquid chromatography. *FEMS Microbiol Lett* **25**, 125–128.
- Teyssier, C., Marchandin, H., Jean-Pierre, H., Masnou, A., Dusart, G. & Jumas-Bilak, E. (2007). *Ochrobactrum pseudintermedium* sp. nov., a novel member of the family *Brucellaceae*, isolated from human clinical samples. *Int J Syst Evol Microbiol* **57**, 1007–1013.
- Tripathi, A. K., Verma, S. C., Chowdhury, S. P., Lebuhn, M., Gattinger, A. & Schloter, M. (2006). *Ochrobactrum oryzae* sp. nov., an endophytic bacterial species isolated from deep-water rice in India. *Int J Syst Evol Microbiol* **56**, 1677–1680.
- Urakami, T., Oyanagi, H., Araki, H., Suzuki, K. & Komagata, K. (1990). Recharacterization and emended description of the genus *Mycoplana* and description of two new species, *Mycoplana ramosa* and *Mycoplana segnis*. *Int J Syst Bacteriol* **40**, 434–442.
- Yoon, J.-H., Kim, H., Kim, S.-B., Kim, H.-J., Kim, W. Y., Lee, S. T., Goodfellow, M. & Park, Y.-H. (1996). Identification of *Saccharomonospora* strains by the use of genomic DNA fragments and rRNA gene probes. *Int J Syst Bacteriol* **46**, 502–505.
- Yoon, J.-H., Lee, S. T. & Park, Y.-H. (1998). Inter- and intraspecific phylogenetic analysis of the genus *Nocardioides* and related taxa based on 16S rRNA gene sequences. *Int J Syst Bacteriol* **48**, 187–194.
- Yoon, J.-H., Kang, K. H. & Park, Y.-H. (2003). *Psychrobacter jeotgali* sp. nov., isolated from jeotgal, a traditional Korean fermented seafood. *Int J Syst Evol Microbiol* **53**, 449–454.
- Yoon, J.-H., Kang, S.-J., Kim, W. & Oh, T.-K. (2007a). *Pigmentiphaga daeguensis* sp. nov., isolated from wastewater of a dye works, and emended description of the genus *Pigmentiphaga*. *Int J Syst Evol Microbiol* **57**, 1188–1191.
- Yoon, J.-H., Kang, S.-J., Park, S. & Oh, T.-K. (2007b). *Caenispirillum bisanense* gen. nov., sp. nov., isolated from sludge of a dye works. *Int J Syst Evol Microbiol* **57**, 1217–1221.
- Yoon, J.-H., Kang, S.-J. & Oh, T.-K. (2007c). *Chryseobacterium daeguense* sp. nov., isolated from wastewater of a textile dye works. *Int J Syst Evol Microbiol* **57**, 1355–1359.
- Yoon, J.-H., Park, S. E., Kang, S.-J. & Oh, T.-K. (2007d). *Rheinheimera aquimaris* sp. nov., isolated from seawater of the East Sea in Korea. *Int J Syst Evol Microbiol* **57**, 1386–1390.
- Zurdo-Piñeiro, J. L., Rivas, R., Trujillo, M. E., Vizcaino, N., Carrasco, J. A., Chamber, M., Palomares, A., Mateos, P. F., Martínez-Molina, E. & Velázquez, E. (2007). *Ochrobactrum cytisi* sp. nov., isolated from nodules of *Cytisus scoparius* in Spain. *Int J Syst Evol Microbiol* **57**, 784–788.