Comamonas zonglianii sp. nov., isolated from phenol-contaminated soil

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A bacterial strain, designated BF-3^T, was isolated from phenol-contaminated soil and investigated using a polyphasic taxonomic approach. Cells were Gram-reaction-negative, non-sporulating, non-motile, short rods. Phylogenetic analysis based on 16S rRNA gene sequences showed that strain BF-3^T formed a monophyletic branch at the periphery of the evolutionary radiation occupied by the genus *Comamonas*; it showed highest sequence similarities to *Comamonas aquatica* LMG 2370^T (96.8%), *C. nitrivorans* DSM 13191^T (96.4%), *C. odontotermitis* LMG 23579^T (96.4%), *C. kerstersii* LMG 3475^T (96.3%), *C. koreensis* KCTC 12005^T (96.1%) and *C. terrigena* LMG 1253^T (96.0%). The major cellular fatty acids were C_{16:0}, C_{18:1}/C_{18:1ω7c}, C_{17:0} cyclo and summed feature 3 (C_{16:1ω7c} and/or iso-C_{15:0} 2-OH). Based on the phylogenetic analysis, DNA–DNA hybridization, whole-cell fatty acid composition and biochemical characteristics, strain BF-3^T was clearly distinct from type strains of other recognized species of the genus *Comamonas* and, as such, represents a novel species of the genus *Comamonas*, for which the name *Comamonas zonglianii* sp. nov. is proposed. The type strain is BF-3^T (=CCTCC AB 209170^T =DSM 22523^T).

The genus *Comamonas* belongs to the family *Comamonadaceae* of the class *Betaproteobacteria*. Since the first description of the genus *Comamonas* by De Vos *et al.* (1985), a number of novel species have been added to this genus. At the time of writing, the genus *Comamonas* includes ten recognized species: *Comamonas aquatica* (Wauters *et al.*, 2003), *C. badia* (Tago & Yokota, 2004), *C. composti* (Young *et al.*, 2008), *C. denitrificans* (Gumaelius *et al.*, 2001), *C. kerstersii* (Wauters *et al.*, 2003), *C. koreensis* (Chang *et al.*, 2002), *C. nitrivorans* (Etchebehere *et al.*, 2001), *C. odontotermitis* (Chou *et al.*, 2007), *C. terrigena* (De Vos *et al.*, 1985) and *C. testosteroni* (Tamaoka *et al.*, 1987).

During the study of the microbial communities of soil contaminated by phenol that had leaked from a chemical factory in Nanjing, Jiangsu province, PR China, phenol-tolerant bacteria were isolated using LB medium supplemented with 500 mg phenol l⁻¹. Several bacterial strains were isolated. On the basis of 16S rRNA gene sequence

data, one strain, designated BF-3^T, shared 94.7–96.8% sequence similarity to species of the genus *Comamonas*. Strain BF-3^T tolerated up to 1000 mg phenol l⁻¹ but was not able to degrade phenol. For comparison studies, type strains of *C. aquatica* and *C. odontotermitis* were obtained from the LMG and the type strain of *C. nitrivorans* was obtained from the DSMZ. Since the same cultivation conditions are very important for the comparison of phenotypic characteristics (Tindall *et al.*, 2010), all the strains tested were grown under the same cultivation conditions.

For the investigation of morphological features, strains were cultivated aerobically on LB agar plates for 2 days at 30 °C. Morphological features of the cells were determined using light microscopy and transmission electron microscopy. Cell motility was tested using the hanging-drop method. The Gram reaction was performed using the classical Gram procedure (Buck, 1982). The optimum temperature and pH for growth and tolerance of NaCl were determined by growing strains in LB medium as described by Chou *et al.* (2007).

Cells of BF-3^T were aerobic, Gram-reaction-negative, non-sporulating, non-motile, short rods (0.6–0.8 × 0.9–1.5 µm). Colonies on LB agar were circular, semi-transparent, pale yellow, convex and ~2 mm in diameter.

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The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain BF-3^T is GQ245981.

Two supplementary figures and two supplementary tables are available with the online version of this paper.

Strain BF-3^T grew at 15–43 °C, pH 6–10 and in 0–2.5 % NaCl. Optimum growth was observed at 25–37 °C, pH 7–8 and 0–1 % NaCl.

Genomic DNA was extracted according to the standard procedure of Sambrook & Russell (2001). PCR amplification of the 16S rRNA gene was performed by using a bacterial universal primer set (27f and 1492r; Lane, 1991). The purified PCR product was sequenced using an automatic sequencer. The resulting sequence was compared with available 16S rRNA gene sequences from the Ribosomal Database Project (<http://rdp.cme.msu.edu/seqmatch>) and GenBank (<http://www.ncbi.nlm.nih.gov/BLAST/>). Multiple sequence alignments of strain BF-3^T and closely related strains were performed using MEGA version 3.0 (Kumar *et al.*, 2004). The phylogenetic trees were generated using the neighbour-joining, maximum-parsimony (Saitou & Nei, 1987; Kimura, 1980) and maximum-likelihood (Felsenstein, 1981) algorithms.

The nearly complete 16S rRNA gene sequence (1453 nt) was obtained for strain BF-3^T. Search results in the Ribosomal Database Project and GenBank indicated that strain BF-3^T belonged to the genus *Comamonas* in the family *Comamonadaceae* of the class *Betaproteobacteria*. Trees depicting the phylogenetic relationship between strain BF-3^T and related taxa showed that strain BF-3^T formed a distinct lineage in the genus *Comamonas* (Fig. 1 and Supplementary Figs S1 and S2, available in IJSEM Online). According to sequence similarity calculations, the organism was most closely related to *C. aquatica* LMG 2370^T (96.8 %), *C. nitrivorans* DSM 13191^T (96.4 %), *C. odontotermitis* LMG 23579^T (96.4 %), *C. kerstersii* LMG 3475^T (96.3 %), *C. koreensis* KCTC 12005^T (96.1 %) and *C. terrigena* LMG 1253^T (96.0 %) and shared less than 95 % similarity to other species of the genus *Comamonas*. These low sequence similarities (<97 %) indicated that strain BF-3^T could be clearly distinguished from other species of the genus *Comamonas*.

To clarify further the taxonomic relationship between strain BF-3^T and closely related species, DNA–DNA hybridizations were performed between strain BF-3^T and *C. aquatica* LMG 2370^T, *C. nitrivorans* DSM 13191^T and *C. odontotermitis* LMG 23579^T according to the method of Rosselló-Mora (2006). The results indicated that strain BF-3^T showed relatively low DNA–DNA relatedness to *C. aquatica* LMG 2370^T (26.9 %), *C. nitrivorans* DSM

13191^T (28.7 %) and *C. odontotermitis* LMG 23579^T (25.4 %), the values being well below the threshold of 70 % recommended for the delineation of bacterial species (Wayne *et al.*, 1987).

The genomic DNA G+C content of strain BF-3^T, determined by thermal denaturation (Mandel & Marmur, 1968), was 65.2 ± 1 mol%, which fell within the range of 60.8–66.3 mol% observed for other members of the genus *Comamonas* (Chou *et al.*, 2007).

Strain BF-3^T was examined for a broad range of phenotypic properties. Oxidase and catalase tests were carried out as described previously (Ohta & Hattori, 1983). Indole production and Voges–Proskauer tests were assessed as described by Smibert & Krieg (1994). Urease production was determined by the method of Cowan & Steel (1965). Nitrate reduction was determined by the method of Lányi (1987). Carbon source utilizations were determined by means of the API 20NE and API ZYM systems (bioMérieux) and the Biolog GN2 system in accordance with the manufacturer's instructions. Susceptibility to antibiotics was determined on agar medium plates by using antibiotic discs with the following amounts of antibiotic (µg): ampicillin (10), gentamicin (10), rifampicin (5), penicillin G (1) and streptomycin (10).

Detailed phenotypic properties of strain BF-3^T are given in the species description. Differential phenotypic characteristics of strain BF-3^T and other members of the genus *Comamonas* are summarized in Table 1 and Supplementary Table S1. It is clear from the two tables that there are several phenotypic characters that readily distinguish strain BF-3^T from other members of the genus *Comamonas*.

To determine whole-cell fatty acid compositions of strain BF-3^T and the type strain of closely related species, cells were harvested at maximum growth by centrifugation, washed with distilled water and freeze-dried. The cellular fatty acid compositions were then determined by Dr Qi-Liang Lai (Third Institute of Oceanography, State of Oceanic Administration, PR China) according to the method of Sasser (1990). The major cellular fatty acids of strain BF-3^T were C_{16:0} (31.8 %), C_{18:1}/C_{18:1}ω7c (18.2 %), C_{17:0} cyclo (17.2 %) and summed feature 3 (C_{16:1}ω7c and/or iso-C_{15:0} 2-OH; 11.5 %). The fatty acid profiles of strain BF-3^T and the type strains of other species of the genus *Comamonas* are listed in Table 2 and Supplementary Table

Fig. 1. Phylogenetic tree constructed by the neighbour-joining method based on 16S rRNA gene sequences of BF-3^T and members of other recognized species of the genus *Comamonas*. Numbers at branching points are percentage bootstrap values based on 1200 resampled datasets. Bar, 0.005 substitutions per nucleotide position.

Table 1. Differential phenotypic characteristics of strain BF-3^T and closely related type strains of species of the genus *Comamonas*

Taxa: 1, BF-3^T; 2, *C. aquatica* LMG 2370^T; 3, *C. nitrivorans* DSM 13191^T; 4, *C. odontotermitis* LMG 23579^T. All of the data were obtained from this study. +, Positive; –, negative; R, resistant; S, sensitive.

Characteristic	1	2	3	4
Motility	–	+	+	+
Growth in 3% NaCl	–	+	+	+
Phenol tolerance (mg l ⁻¹)	1000	600	200	500
Alkaline phosphatase	+	–	–	+
Valine arylamidase	+	–	+	+
Cystine arylamidase	–	+	–	–
Assimilation of:				
D-Gluconate	+	+	–	+
Adipate	+	+	+	–
Malate	–	+	+	+
Citrate	+	–	–	+
Phenylacetate	–	–	+	+
Oxidation of:				
Acetate	+	–	+	–
γ-Hydroxybutyrate	+	+	–	+
Itaconate	–	+	–	–
L-Phenylalanine	+	–	+	–
Hydroxy-L-proline	–	+	+	+
Glycyl L-aspartate	–	–	–	+
Glycyl L-glutamate	–	–	–	+
Tween 40	+	–	+	+
Susceptibility to:				
Ampicillin	R	S	S	R
Gentamicin	R	S	S	S
Penicillin G	R	S	S	R
Rifampicin	R	S	S	R
Streptomycin	R	S	S	S

S2. The fatty acid profile of strain BF-3^T was similar to those of the other members of the genus *Comamonas* except that strain BF-3^T possessed relatively high levels of C_{17:0} cyclo.

On the basis of its phenotypic and phylogenetic properties, strain BF-3^T represents a novel species of the genus *Comamonas*, for which the name *Comamonas zonglianii* sp. nov. is proposed.

Description of *Comamonas zonglianii* sp. nov.

Comamonas zonglianii (zong.li.a'ni.i. N.L. masc. gen. n. *zonglianii* named in honour of Zong-Lian Gu, a respected microbiologist who has contributed significantly to the promotion and development of environmental microbiology in China).

Cells are aerobic, Gram-reaction-negative, non-sporulating, non-motile, short rods (0.6–0.8 × 0.9–1.5 µm). Colonies on LB agar are circular, semi-transparent, pale

Table 2. Fatty acid compositions of strain BF-3^T and closely related type strains of species of the genus *Comamonas*

Taxa: 1, BF-3^T; 2, *C. aquatica* LMG 2370^T; 3, *C. nitrivorans* DSM 13191^T; 4, *C. odontotermitis* LMG 23579^T. All of the data were obtained from this study. Values are percentages of total fatty acids; –, fatty acids representing less than 0.5%. The position of the double bond in the unsaturated fatty acids is obtained by counting from the methyl (ω) end of the molecule.

Fatty acid	1	2	3	4
C _{10:0} 3-OH	3.5	4.6	4.4	3.4
C _{12:0}	3.9	3.2	3.0	2.6
C _{14:0}	0.8	4.6	3.7	0.8
C _{16:0}	31.8	24.4	19.8	30.3
C _{16:0} 2-OH	4.1	–	–	1.8
C _{16:1} 2-OH	1.8	–	–	–
C _{17:0}	0.7	–	–	–
C _{17:0} cyclo	17.2	–	–	–
C _{17:1} ω7c	0.9	–	–	–
C _{18:0}	1.6	–	–	1.0
C _{18:1} /C _{18:1} ω7c	18.2	17.7	21.6	18.9
C _{19:0} cyclo ω8c	2.3	–	–	–
iso-C _{20:0}	–	–	0.8	–
Summed feature 3*	11.5	44.5	45.3	40.2

*C_{16:1} ω7c and/or iso-C_{15:0} 2-OH

yellow and convex after 2 days of incubation at 30 °C. Optimum growth is observed at 25–37 °C, pH 7–8 and in 0–1% NaCl. In API 20NE tests, positive for oxidase, catalase, arginine dihydrolase and urease activities, hydrolysis of gelatin and assimilation of gluconate, caprate, adipate and citrate; negative for nitrate reduction, indole production, glucose fermentation, β-galactosidase activity, hydrolysis of aesculin and assimilation of glucose, arabinose, mannose, mannitol, N-acetylglucosamine, maltose, malate and phenylacetate. In API ZYM tests, positive for alkaline phosphatase, C4 esterase, C8 lipase, leucine arylamidase, valine arylamidase, acid phosphatase and naphthol-AS-BI-phosphohydrolase activities; negative for C14 lipase, cystine arylamidase, trypsin, α-chymotrypsin, α-galactosidase, α- and β-glucosidases, β-glucuronidase, α-mannosidase, N-acetyl-β-glucosaminidase and α-fucosidase activities. In the Biolog GN2 system, the following compounds can be oxidized: Tweens 40 and 80, methyl pyruvate, monomethyl succinate, acetic acid, citric acid, D-galactonic acid, D-gluconic acid, D-glucosaminic acid, α-, β- and γ-hydroxybutyric acids, p-hydroxyphenylacetic acid, α-ketobutyric acid, DL-lactic acid, propionic acid, quinic acid, sebacic acid, succinic acid, L-asparagine, L-aspartic acid, L-alanine, L-glutamic acid, bromosuccinic acid, succinamic acid, L-alaninamide, L-leucine, L-phenylalanine, L-proline, L-pyroglutamic acid, urocanic acid, glycerol and DL-α-glycerol phosphate. Cells are resistant to ampicillin, gentamicin, rifampicin, penicillin G and streptomycin. The major fatty acids are C_{16:0}, C_{18:1}/C_{18:1} ω7c, C_{17:0} cyclo and

summed feature 3 (C_{16:1ω7c} and/or iso-C_{15:0} 2-OH). The DNA G+C content of the type strain is 65.2±1 mol%.

The type strain, BF-3^T (=CCTCC AB 209170^T =DSM 22523^T), was isolated from phenol-contaminated soil in Jiangsu Province, China.

Acknowledgements

We are grateful to Dr Susanne Verbarg and Dr Qi-Liang Lai (Third Institute of Oceanography, State of Oceanic Administration, PR China) for the analysis of cellular fatty acids. This work was supported by the National Natural Science Foundation of China (30970099) and the National R&D Project of Transgenic Crops of the Ministry of Science and Technology of China (2009ZX08012-014B).

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