Dyella japonica gen. nov., sp. nov., a γ -proteobacterium isolated from soil

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Three strains isolated from the soil of a garden in Tokyo, Japan, were characterized physiologically, biochemically and in terms of fatty acid profile, DNA–DNA relatedness and 16S rRNA gene sequence. The isolates were Gram-negative, aerobic, rod-shaped cells with polar flagellation. According to DNA–DNA similarity, the strains belonged to the same species. The bacteria grew at temperatures from 10 to 37 °C, with an optimum around 25–30 °C. Growth was observed at pH values from 5.6 to 8.0. The DNA G+C content ranged from 63.4 to 64.0 mol%. Phylogenetic analyses of 16S rRNA gene sequences revealed a clear affiliation with members of the family 'Xanthomonadaceae'. The closest relationship was seen with *Fulvimonas soli* and *Frateruia aurantia*, but, in terms of physiology and fatty acid profile, the bacteria described were rather distant from *Fulvimonas* and *Frateruia*. On the basis of phenotypic and phylogenetic distinctness, it is proposed that the isolates represent a novel species in a novel genus, namely *Dyella japonica* gen. nov., sp. nov. The type strain is XD53^T (=IAM 15069^T=DSM 16301^T=ATCC BAA-939^T).

Three bacterial strains, XD10, XD22 and XD53^T, were isolated from soil during the course of the isolation of diazotrophs from a garden at the University of Tokyo, Japan. These isolates neither fix nitrogen nor have the *nifH* gene (Poly *et al.*, 2001). On the basis of 16S rRNA gene sequence analysis, these yellow-pigmented soil isolates seemed to represent a novel and important group within the γ -Proteobacteria and were closely related to the plasticized acetylated starch-degrading bacterium *Fulvimonas soli* (Mergaert *et al.*, 2002), the lindane-degrading bacterium *Rhodanobacter lindaniclasticus* (Nalin *et al.*, 1999) and the acidophilic bacterium *Frateruia aurantia* (Swings *et al.*, 1984). Upon comparison of the metabolism of the novel bacterium with those of its phylogenetic neighbours, we found that it was not acidophilic like members of the genus *Frateruia*; however, it was not known if the organism possessed the ability to degrade plasticized acetylated starch or lindane. The aim of this study was to elucidate the phylogenetic position of the isolates, using polyphasic taxonomy (physiology, fatty acid composition, quinone system, DNA G+C content, DNA–DNA relatedness and 16S rRNA gene sequence analysis). On the basis of this substantial evidence, it is proposed that the isolates represent a novel species in a novel genus, *Dyella*

japonica gen. nov., sp. nov. The type strain is XD53^T (=IAM 15069^T=DSM 16301^T=ATCC BAA-939^T).

The cells used for tests of growth at various temperatures and pH values were incubated in nutrient broth. Cell shape, size and motility were observed by light microscopy (BX 60 apparatus; Olympus). The presence of flagella was determined by transmission electron microscopy (JEM-1011 apparatus; JEOL) after negative staining with uranyl acetate. Gram staining was performed using the method of Oyaizu-Masuchi & Komagata (1988). API 20NE, API 50 CH and API ZYM tests (all bioMérieux) were used to determine physiological and biochemical characteristics. The API ZYM test results were read after 4 h incubation at 28 °C; all other API tests were read after 48 h. H₂S formation was detected with lead acetate paper strips in GPY medium containing (w/v) 5 % D-glucose, 0.5 % peptone, 0.2 % yeast extract, 0.1 % L-cysteine and 0.005 % Na₂SO₄. The bacteria were incubated at 28 °C on a rotary shaker in AE broth [1.5 % (w/v) glucose, 0.2 % (w/v) yeast extract, 0.3 % (w/v) peptone, 6.5 % (v/v) acetic acid and 2 % (v/v) ethanol] (Entani *et al.*, 1985) for 30 days; AE broth was used to identify the genus *Frateruia* (Swings *et al.*, 1984). Examination of the respiratory quinone system, DNA G+C content and cellular fatty acid composition, PCR-mediated amplification of 16S rRNA gene sequences and sequencing of the PCR products were carried out as described previously (Xie & Yokota, 2003). DNA was prepared according to the method of Marmur (1961) from cells grown on nutrient broth and DNA G+C contents were determined by using

Published online ahead of print on 22 October 2004 as DOI 10.1099/ij.s.0.63377-0.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains XD10, XD22 and XD53^T are AB110496–AB110498.

Fig. 1. Phylogenetic tree of a subset of the γ -Proteobacteria, based on 16S rRNA gene sequence comparison, determined by neighbour-joining. *Escherichia coli* was used as the outgroup. Bootstrap percentages of 1000 replicates are indicated at nodes; only values greater than 70 % are shown.

the HPLC method of Mesbah *et al.* (1989). DNA–DNA hybridizations were carried out with photobiotin-labelled probes in microplate wells as described by Ezaki *et al.* (1989). The hybridization temperature was set at 51 °C.

The DNA sequences of the three strains were compared with sequences obtained from GenBank (National Centre for Biotechnology Information). The sequences were aligned using the CLUSTAL W software package (Thompson *et al.*, 1994) and evolutionary distances and K_{nuc} values (Kimura, 1980) were generated. Alignment gaps and ambiguous bases were not taken into consideration when 1433 bases of the 16S rRNA gene sequences were compared. Phylogenetic trees were constructed using either the neighbour-joining method (Saitou & Nei, 1987) or the maximum-likelihood method (PHYLIP package; Felsenstein, 1989). The topology of the phylogenetic tree was evaluated by using the bootstrap resampling method of Felsenstein (1985), with 1000 replicates. Similarity values were calculated using PAUP 4.0b1 (Swofford, 1998).

The 16S rRNA gene sequences of the three strains were determined and subjected to comparative analysis. The sequence of strain XD53^T showed high similarity (more than 99.0 %) to those of the other two strains. The phylogenetic tree (Fig. 1) shows that these strains are clustered within the family ‘Xanthomonadaceae’ of the γ -Proteobacteria. The levels of 16S rRNA gene sequence similarity between XD53^T and *Fulvimonas soli* LMG 19981^T (Mergaert *et al.*, 2002), *Frateruia aurantia* LMG 1558^T and *Rhodanobacter lindaniclasticus* RP 5557^T (Nalin *et al.*, 1999) were 96.5, 95.9 and 95.0 %, respectively. A similarity value of no more than 97 % between 16S rRNA gene sequences is widely accepted for genus-level differentiation (Gillis *et al.*, 2001). The bootstrap value for the branching of *Frateruia aurantia* LMG 1558^T and the novel strains was less than 70 % (data not shown). The phylogenetic tree calculated by the maximum-likelihood method (data not shown) also supported the contention that these novel isolates represented an independent taxon separated from the genera *Fulvimonas*, *Frateruia* and *Rhodanobacter*.

The DNA–DNA hybridization values for XD10 and XD53^T

and of XD22 and XD10 were 87.2 and 99.7 %, respectively. The three strains should therefore be considered as belonging to the same species. The DNA G + C contents of strains XD10, XD22 and XD53^T were 64.0, 63.4 and 63.5 mol%, respectively; these values are quite different from those of members of the genus *Fulvimonas* (71.7 %). The cellular fatty acid patterns of these strains displayed similar compositions (Table 1). They were found to consist primarily of branched fatty acids, with 17:1 iso ω 9c (25.6–30.8 %), 15:0 iso (20.0–23.6 %) and 17:0 iso (19.6–20.0 %) as major constituents. The major hydroxy fatty acids were 11:0 iso 3-OH, 13:0 iso 3-OH and 17:0 iso 3-OH. This cellular fatty acid composition can be differentiated from that of *Frateruia aurantia*, which contains

Table 1. Fatty acid composition of strains XD10, XD22 and XD53^T

The following fatty acids constituted no more than 0.5 % of the total: unknown 9.531, 12:0 anteiso, 13:0 iso, 12:0 iso 3-OH, unknown 13.65, 14:0 iso, 14:0, 16:0 N alcohol, 17:0 anteiso, 16:0 iso 3-OH, 18:0 iso, 18:0 and 20:0. Summed feature 3 comprises 15:0 iso 2-OH and/or 16:1 ω 7c.

Fatty acid	XD10	XD22	XD53 ^T
11:0 iso	3.5	4.1	3.8
Unknown ECL 11.799*	1.8	1.9	1.9
11:0 iso 3-OH	3.5	3.9	3.7
13:0 iso 3-OH	3.6	4.1	3.9
15:0 iso	22.9	20.0	23.6
15:0 anteiso	1.0	1.2	1.6
16:0 iso	2.5	4.1	4.9
16:0	1.70	1.6	1.5
17:1 iso ω 9c	30.8	28.3	25.6
17:0 iso	19.6	21.6	20.0
17:0 anteiso	0.4	0.5	0.7
17:0 iso 3-OH	1.0	1.0	1.1
19:0 iso	0.7	0.6	0.7
Summed feature 3	4.0	3.6	4.0

*Unknown peak that appeared at 11.799 min.

Fig. 2. Cells of *D. japonica* XD53^T after negative staining with uranyl acetate, as visualized by transmission electron microscopy, showing a single, polar, attached flagellum. Bar, 0.2 µm.

more than 40.6 % 15:0 iso and not more than 3 % 17:1 iso ω₉c, and has 12:0 3-OH and 12:0 2-OH as the major hydroxy fatty acids (Lisdiyanti *et al.*, 2003). On the other hand, we found that the hydroxy fatty acid profile of *Fulvimonas soli* contained 12:0 iso 3-OH and did not contain 17:0 iso 3-OH (Mergaert *et al.*, 2002). Ubiquinone Q-8 was detected as the major quinone system in these isolates; this is similar to the situation in *Frateuria aurantia*.

Colonies are yellow in nutrient agar. Cells of strain XD53^T are straight rods, approximately 0.4 µm in diameter and 1.2 µm in length, and each possesses a single polar flagellum (Fig. 2). Motility could be observed in the cells of all strains. All strains could grow slowly at 10 or 37 °C, but could not grow at 4 or 40 °C. The optimum temperature for growth was 25–30 °C. The optimum pH was 6.5–7.2; the strains could not grow at pH 4.6. Biochemical and physiological characteristics of the strains were determined by using the API microtest galleries (bioMérieux): the strains displayed similar characteristics. All were catalase-positive, oxidase-negative, nitrate reduction-positive, nitrite reduction-negative, H₂S-negative, produced acid from glucose, fructose and mannose and grew on maltose. In terms of biochemical characteristics, positive test results were obtained for *N*-acetylglucosamine, alkaline phosphatase, C4 esterase lipase, C8 esterase lipase, C14 lipase, leucine arylamidase, valine arylamidase, acid phosphatase and naphthol phosphohydrolase, weak reactions were obtained for *N*-acetyl-β-D-glucosamine and negative reactions were obtained for β-glucuronidase, urease, gelatin liquefaction, indole production and growth on mannitol, caprate, valerate, D-ribose, D-sucrose, acetate, L-alanine, citrate, histidine, D-ribose, hydroxybenzoate, *p*-nitrophenyl β-D-galactopyranoside, trypsin, α-galactosidase, aesculin, malate, arginine, β-galactosidase, α-mannosidase, α-fucosidase and D-xylose. These strains can be differentiated

Table 2. Differential characteristics among *Fulvimonas*, *Frateuria*, *Rhodanobacter* and *Dyella* gen. nov.

Species: 1, *Dyella japonica* gen. nov., sp. nov.; 2, *Frateuria aurantia*; 3, *Fulvimonas soli*; 4, *Rhodanobacter lindaniclasticus*. Data for reference species are from Mergaert *et al.* (2002). Members of all four genera are catalase-positive. +, Positive; –, negative; ND, not determined.

Characteristic	1	2	3	4
Oxidase	–	–	+	+
Motility	+	+	+	–
Acid from:				
D-Glucose	+	+	–	–
D-Ribose	–	+	–	ND
D-Xylose	–	+	–	ND
D-Galactose	–	+	–	ND
D-Mannose	+	+	–	ND
L-Arabinose	–	+	–	ND
Growth on:				
Maltose	+	+	+	–
Caprate	–	–	–	+
Citrate	–	–	–	+
Growth at pH 4.5	–	+	ND	ND
β-D-Glucosidase	Weak	+	–	ND
<i>N</i> -Acetyl-β-D-glucosaminidase	Weak	–	+	ND
Nitrate reduction	+	–	ND	ND
Nitrite reduction	–	–	ND	ND
H ₂ S production	–	+	ND	ND
Hydroxy fatty acid composition (3-OH)	11:0 iso, 13:0 iso, 17:0 iso	12:0	11:0 iso, 13:0 iso, 12:0 iso	ND
DNA G+C content (mol%)	63.4–64.0	63.5	71.7	63

from *Frateuria aurantia* and *Fulvimonas soli* on the basis of some phenotypic features (Table 2). The novel bacterium could use *N*-acetyl β -D-glucosamine but *Frateuria* strains could not. Moreover, the novel bacterium did not have the general characteristics of *Frateuria* (i.e. growth at pH 3.6, production of H₂S, ketogenesis from D-mannitol, acid production from almost all carbon sources; Swings *et al.*, 1984). *Frateuria* strains have been isolated from *Lilium auratum* and from the fruit of *Rubus parvifolius*. The novel bacterium could be distinguished easily from members of the genera *Fulvimonas* and *Rhodanobacter* by DNA G+C content and by motility, respectively.

On the basis of their phenotypic, chemotaxonomic and phylogenetic characteristics, the novel isolates cannot be assigned to any recognized bacterial genus. Therefore, we propose *Dyella japonica* gen. nov., sp. nov. for these three strains.

Description of *Dyella* gen. nov.

Dyella (Dy.el'la. L. dim. ending -ella; N.L. fem. n. *Dyella* of Dye, in honour of Dr Douglas W. Dye, of New Zealand, who contributed to the taxonomic study of the genus *Xanthomonas*).

Cells are Gram-negative, catalase-positive and oxidase-negative. Colonies on nutrient agar are yellow. They do not grow in AE broth and do not produce H₂S. The G+C content of the DNA is approximately 62–64 mol%. The cellular fatty acid composition consists mainly of branched fatty acids, with 17:1 iso ω 9c, 15:0 iso and 17:0 iso as the major fatty acids and 11:0 iso 3-OH, 13:0 iso 3-OH and 17:0 iso 3-OH as the major hydroxy fatty acids. Q-8 is the major component of the quinone system. The type species of the genus is *Dyella japonica*.

Description of *Dyella japonica* sp. nov.

Dyella japonica (ja.po'ni.ca. N.L. fem. adj. *japonica* pertaining to Japan, from where the type strain and other strains originated).

Cells are straight rods (0.4 $\times$ 1.2 μ m), motile by means of a single polar flagellum. Colonies grow well on nutrient agar. The conditions for growth are 10–37 °C and pH 5.6–8; growth does not occur at pH 4.6. The optimum pH for growth is 6.5–7.2. The optimum temperature for growth is 25–30 °C; growth at 4 and 40 °C is very poor. Cells are nitrate reduction-positive; acids are produced from glucose, fructose and mannose, but not from mannitol. The other characteristics of the species can be found in Table 2. The G+C content of the DNA of the type strain is 63.5 mol%.

The type strain, XD53^T (=IAM 15069^T=DSM 16301^T=ATCC BAA-939^T), and strains XD10 (=IAM 15067) and XD22 (=IAM 15068) were isolated from soil in Tokyo, Japan.

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