Duganella violaceinigra sp. nov., a novel mesophilic bacterium isolated from forest soil

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A mesophilic bacterium, designated strain YIM 31327^T, was isolated from a forest soil sample collected from Yunnan Province, China, and was then investigated using a polyphasic approach. The strain grew optimally at 28–30 °C and pH 7·2. The cells were Gram-negative, short, rod-shaped, motile and non-spore-forming with flagella. The major ubiquinone was Q-8 and the cellular fatty acids were $C_{16:0}$ and $C_{12:0}$. The DNA G+C content of strain YIM 31327^T was 62·8 mol%. Phylogenetic analysis revealed that strain YIM 31327^T was a member of the *β-Proteobacteria*, being most closely related to *Duganella zoogloeoides*, with which it exhibited less than 96 % 16S rRNA gene sequence similarity. On the basis of the phenotypic and genotypic differences between strain YIM 31327^T and *D. zoogloeoides*, a novel species, *Duganella violaceinigra* sp. nov., is proposed, with YIM 31327^T (=CIP 108077^T=KCTC 12193^T) as the type strain.

The genus *Duganella* was first proposed by Hiraishi *et al.* (1997) as a reclassification of a misnamed strain, IAM 12670^T (=ATCC 25935^T), which was defined as a Gram-negative, obligately aerobic, chemo-organotrophic, non-spore-forming, rod-shaped bacterium with flagella. At present, the genus comprises only one species, *Duganella zoogloeoides*.

During our screening research on the microbial flora of Yunnan, China, strain YIM 31327^T was recovered on HV agar (Hayakawa & Nonomura, 1987) and then investigated using a polyphasic taxonomic approach. HV agar medium contained the following (in 1 litre distilled water, final pH 7·2): humic acid, 1·0 g; KCl, 1·7 g; Na_2HPO_4, 0·5 g; $MgSO_4.7H_2O$, 0·5 g; $CaCO_3$, 0·02 g; $FeSO_4.7H_2O$, 0·01 g; B vitamins (0·5 mg each of thiamin, riboflavin, niacin, pyridoxin, calcium D-pantothenate, inositol, *p*-aminobenzoic acid and 0·25 mg biotin); cycloheximide, 50 mg; nalidixic acid, 20 mg; agar, 15·0 g.

The strain was maintained on a YM (yeast extract/malt extract) agar slant at 4 °C and as 20 % (w/v) glycerol suspensions at −20 °C. Biomass for chemical and molecular studies was obtained by cultivation, in shake flasks (at about 150 r.p.m.), with YM broth at 28 °C for 1 week.

Strain YIM 31327^T was grown on YM agar for observation of the cellular and colony morphology, and on some other media as controls, e.g. nutrient agar, trypticase/soy agar and Mueller–Hinton agar. Strain YIM 31327^T exhibited either weak growth or no growth on the media tested, except on YM agar. Yeast extract and the vitamin mixture of HV medium could stimulate its growth. No diffusible pigments were produced on any media. Morphological characteristics of strain YIM 31327^T were observed under light microscopy (model BH 2; Olympus) and using an electron microscope (JEM-1010; JEOL) after 1 week of growth on YM agar. The cells of strain YIM 31327^T were short, rod-shaped, motile, non-spore-forming and possessed flagella; they were about 0·4–0·6 μm wide and 0·8–1·0 μm long and occurred singly (Fig. 1). Colonies reached a maximum size (5–8 mm in diameter) after 1 week of incubation at 28 °C and were violet–black in colour and circular.

Gram-staining was carried out with 48 h cultures. Catalase activity was determined by means of the production of bubbles after the addition of a drop of 3 % H_2O_2. All physiological and biochemical tests were performed at 28 °C. Tests for carbon-source utilization, sugar fermentation and enzymes (qualitative) were carried out using API ID 32E, API 20NE and API 50CH test kits (bioMérieux). Strain YIM 31327^T was found to be Gram-negative and

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The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain YIM 31327^T is AY376163.

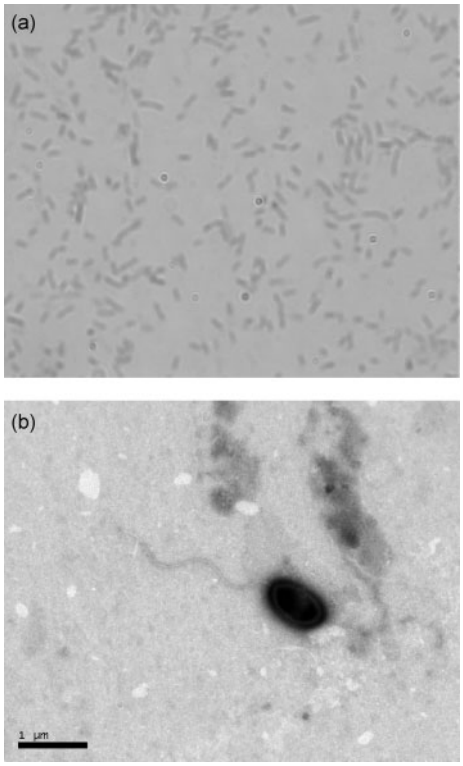

Fig. 1. Light micrograph (a) and scanning election micrograph (b) of strain YIM 31327^T grown on YM agar for 1 week at 28 °C. Bar, 1 μm (b).

obligately aerobic. The results of other phenotypic test are provided below in the description of the novel species and in Table 1.

The ubiquinones were isolated using the methods of Minnikin *et al.* (1984) and separated by HPLC (Kroppenstedt, 1982). The cellular fatty acid composition was determined as described by Sasser (1990) using the Microbial Identification System (MIDI). The ubiquinones were Q-8 (94 %) and Q-7 (6 %). The major fatty acids were $C_{16:0}$ (29·4 %), $C_{12:0}$ (13·6 %), 3-OH $C_{10:0}$ (5·1 %), 3-OH $C_{12:0}$ (7·6 %), 2-OH $C_{12:0}$ (5·7 %), $C_{14:0}$ (1·2 %), $C_{10:0}$ (1·0 %), $C_{18:1\omega7c}$ (3·3 %) and summed feature 3, which comprises $C_{16:1\omega7c}$ or 2-OH i-$C_{15:0}$ (32·6 %) or both.

Extraction of genomic DNA and amplification of the 16S rRNA gene were done as described by Xu *et al.* (2003). Phylogenetic analysis was performed using the software packages PHYLIP (Felsenstein, 1993) and MEGA (Molecular Evolutionary Genetics Analysis) version 2.1 (Kumar *et al.*, 2001) after multiple alignment of data by CLUSTAL_X (Thompson *et al.*, 1997). Distances (distance options according to the Kimura two-parameter model) (Kimura, 1980, 1983) were calculated and clustering was performed with the neighbour-joining method (Saitou & Nei, 1987). Bootstrap analysis was used to evaluate the tree topology of the neighbour-joining data by means of 1000 resamplings (Felsenstein, 1985).

Genomic DNA of strain YIM 31327^T for G+C content determination was prepared according to the method of Marmur (1961). The G+C content, determined using the thermal denaturation method of Marmur & Doty (1962), was 62·8 mol%.

The almost-complete 16S rRNA gene sequence (1439 bp) of strain YIM 31327^T was determined. A neighbour-joining tree, generated between positions 56 and 1478 (*Escherichia coli* positions; Brosius *et al.*, 1978), is shown in Fig. 2. The sequence of strain YIM 31327^T was closest to that of *D. zoogloeoides* IAM 12670^T (96·73 % similarity), and the two strains formed a distinct branch in the phylogenetic tree (Fig. 2).

Similarities in morphological characteristics, ubiquinone and fatty acid compositions and G+C content support the inclusion of strain YIM 31327^T in the genus *Duganella* (Hiraishi *et al.*, 1997), i.e. both strain YIM 31327^T and

Table 1. Differential phenotypic characteristics of strain YIM 31327^T and its closest phylogenetic relative, *D. zoogloeoides* IAM 12670^T

Data for *D. zoogloeoides* were taken from Hiraishi *et al.* (1997). +, Present; −, absent.

Characteristic	*D. violaceinigra* YIM 31327^T	*D. zoogloeoides* IAM 12670^T
Growth on nutrient agar	−	+
Colour of non-diffusible pigment	Violet–black	Yellow
Oxidative acid produced from glucose	−	+
Hydrolysis of starch	−	+
Urease	−	+
Oxidase	−	+
Major cellular fatty acids	$C_{16:0}$, $C_{12:0}$	$C_{16:0}$, $C_{16:1}$
Major hydroxy fatty acids	3-OH $C_{10:0}$, 3-OH $C_{12:0}$, 2-OH $C_{12:0}$	3-OH $C_{10:0}$
DNA G+C content (mol%)	62·8	63–64

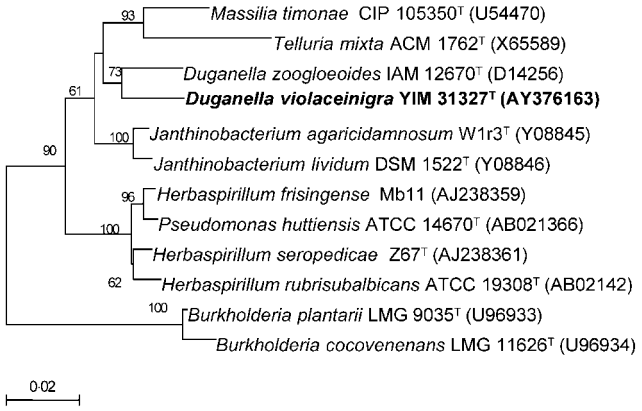

Fig. 2. Phylogenetic dendrogram, based on 16S rRNA gene sequence analysis, constructed using the neighbour-joining method, showing the phylogenetic position of strain YIM 31327^T within the genus *Duganella*. The sequence of *E. coli* was used as the outgroup (not shown). Scale bar, inferred nucleotide substitution per 100 nucleotides.

D. zoogloeoides IAM 12670^T show flagellation and flocculent growth, have no diffusible pigments, possess gelatinase and catalase, and have Q-8 as the major respiratory quinone. However, strain YIM 31327^T differs from the only recognized species of the genus *Duganella* by the absence of oxidase activity, the inability to hydrolyse starch, the fact that no oxidative acid is produced from glucose, and by the scarce or non-existent growth on nutrient agar. In addition, the non-diffusible pigment of strain YIM 31327^T is violet–black in colour, while that of *D. zoogloeoides* IAM 12670^T is yellow.

Thus, on the basis of the above phenotypic and genotypic data, we consider strain YIM 31327^T to represent a novel species of the genus *Duganella*, for which we propose the name *Duganella violaceinigra* sp. nov.

Description of *Duganella violaceinigra* sp. nov.

Duganella violaceinigra (vi.o.la.ce.i.ni′gra. L. adj. *violaceus -a -um* violet; L. adj. *niger -gra -grum* black; N.L. fem. adj. *violaceinigra* violet–black, after the colour of the colonies).

Cells are obligately aerobic, Gram-negative, non-spore-forming, short and rod-shaped with flagella, about 0·4–0·6 × 0·8–1·0 μm in size and occur singly. The cells produce flocculant growth in YM broth and the colonies on YM agar plates are wrinkled and a little leathery. Catalase activity is present, whereas oxidase activity is absent. Hydrolyses gelatin and aesculin. Unable to reduce nitrate or denitrify. Utilizes D-glucose, D-cellobiose, xylose, mannitol, galactose, maltose, lactose, salicin and *N*-acetyl-β-glucosamine as sole carbon sources, but unable to utilize glycerol, erythritol, D-ribose or fructose. Alkaline phosphatase, esterase, esterase lipase, lipase, α-glucoside, β-glucoside, leucine arylamidase, acid phosphatase and naphthol-AS-BI-phosphohydrolase are present, whereas valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, α-fucosidase, urease, arginine dihydrolase and indole production are not. The major ubiquinone is Q-8 and the major cellular fatty acids are $C_{16:0}$ (29·4 %) and $C_{12:0}$ (13·6 %); 3-OH $C_{10:0}$ (5·1 %), 3-OH $C_{12:0}$ (7·6 %) and 2-OH $C_{12:0}$ (5·7 %) are the major hydroxy fatty acids. The optimum growth temperature is 28–30 °C and the optimum pH for growth is 7·2. The DNA G + C content is 62·8 mol%. Isolated from a forest soil sample in Yunnan Province, China.

The type strain is YIM 31327^T (= CIP 108077^T = KCTC 12193^T).

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