Flaviumibacter petaseus gen. nov., sp. nov., isolated from soil of a subtropical rainforest

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A yellow-coloured bacterium, T41^T, was isolated from a soil sample of a subtropical rainforest in Nepal. Cells were Gram-reaction-positive, aerobic, non-motile, short rods. Phylogenetic analysis based on 16S rRNA gene sequences indicated that the strain formed a cluster with *Terrimonas ferruginea*, *Terrimonas lutea*, *Niabella soli*, *Flavisolibacter ginsengiterrae*, *Flavisolibacter ginsengisoli*, *Niastella yeongjuensis* and *Niastella koreensis* in the phylum *Bacteroidetes*. The strain showed the highest sequence similarity to the type strain of *Terrimonas lutea* (93.2%). The major isoprenoid quinone was MK-7 and the predominant cellular fatty acids (>10%) were iso-15:0 (33.8%), iso-15:1 G (13.3%) and iso-17:0 3-OH (12.9%). The DNA G+C content was 48.1 mol%. On the basis of phenotypic and phylogenetic data and genomic distinctiveness, strain T41^T represents a novel species in a new genus in the phylum *Bacteroidetes*, for which the name *Flaviumibacter petaseus* gen. nov., sp. nov. is proposed. The type strain of *Flaviumibacter petaseus* is strain T41^T (=CGMCC 1.7723^T =NBRC 106054^T).

During the course of a study on the culturable bacterial community in a subtropical rainforest soil sample from Nepal (27° 34' N 84° 31' E), a novel yellow-pigmented bacterium, designated strain T41^T, was isolated. On the basis of phylogenetic analysis, strain T41^T formed a distinct cluster closely related to members of the genera *Terrimonas*, *Flavisolibacter*, *Niabella* and *Niastella* of the phylum *Bacteroidetes*. The polyphasic phenotypic and genotypic characterization of strain T41^T is described in this report.

The soil sample was serially diluted with 0.85% NaCl (w/v) and the dilutions were plated onto modified R2A agar containing (l⁻¹) 0.5 g glucose, 0.5 g casein hydrolysate, 0.5 g soluble starch, 0.5 g peptone, 0.5 g yeast extract, 0.3 g K₂HPO₄, 0.05 g MgSO₄·7H₂O and 17 g agar (pH 7.2). The plates were incubated at 28 °C for 7 days. Single colonies were purified by transferring them onto new R2A plates and were incubated once again. Purified colonies were maintained as glycerol suspensions (20%, w/v) at –70 °C.

The Gram reaction was determined using the non-staining method, as described by Buck (1982). Cell morphology was observed under a light microscope (Nikon) at ×1000, with

cells grown for 3 days at 28 °C on R2A agar. Motility was examined using the hanging-drop technique with fresh cells in R2A broth. Catalase activity was determined by bubble production in 3% (v/v) H₂O₂, and oxidase activity was determined by daubing cultures with 1% (w/v) *N,N*-dimethyl *p*-phenylenediamine (Yoon & Im, 2007). Carbon-source utilization was assessed using a liquid medium containing (l⁻¹) 1 g (NH₄)₂HPO₄, 0.3 g K₂HPO₄, 0.05 g MgSO₄·7H₂O and 0.2 g yeast extract (pH 7.2). Carbon substrates were added at a concentration of 0.5% (w/v). The culture was incubated under 28 °C and, after 3 days, the result was compared using a spectrophotometer. Acid production was investigated by adding bromothymol blue as an indicator after 3 days of incubation. Growth at 4, 20, 28, 37 and 50 °C and pH 5.0–10.0 (in intervals of 0.5 pH units) was assessed after 5 days of incubation. Salt tolerance was tested on R2A broth supplemented with 0, 0.5, 1, 1.5, 2, 3 and 5% (w/v) NaCl. Nitrate reduction and degradation of casein, gelatin, urea, DNA and starch were examined according to the method of Dong & Cai (2001).

For the measurement of the G+C content of chromosomal DNA, genomic DNA was extracted and purified as described by Marmur (1961). The G+C content was determined by the thermal denaturation method using a BIO-20 UV spectrophotometer as described by De Ley (1970). For the analysis of isoprenoid quinones, cells grown aerobically were freeze-dried and subjected to extraction of the lipid fraction as described by Collins (1985). Fatty acid methyl esters were prepared and extracted from cells grown on R2A medium for 48 h at 28 °C, following the standard

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain T41^T is EU854577.

16S rRNA gene sequence-based maximum-parsimony and minimum-evolution phylogenetic trees are available as supplementary material with the online version of this paper.

protocol of the Microbial Identification System (MIDI, 1999).

PCR-mediated amplification of the 16S rRNA gene was carried out according to Güssow & Clackson (1989). The primers used were Eub27f and Eub1495r (Di Cello *et al.*, 1997). The 16S rRNA gene sequences of related taxa were obtained from GenBank. Multiple alignments were performed with CLUSTAL_X (Thompson *et al.*, 1997). Phylogenetic and molecular evolutionary analyses were conducted using the MEGA 3.1 (Kumar *et al.*, 2004) software package and clustering was determined using the neighbour-joining (Saitou & Nei, 1987), maximum-parsimony (Fitch, 1971) and minimum-evolution (Rzhetsky & Nei, 1992) algorithms with bootstrap values based on 1000 replications (Felsenstein, 1985), in order to determine the stability of clusters.

Cells of strain T41^T were aerobic, Gram-reaction-positive, non-motile, short rods. When grown on R2A agar plates for 3 days, the colonies were smooth, circular, yellow and 1–2 mm in diameter. On R2A agar medium, strain T41^T grew at 20–37 °C and at pH 5.5–9.5, with optimal growth at 28 °C and pH 6.5. NaCl was tolerated at 0–1.0 % (w/v). Other phenotypic and chemotaxonomic characteristics that differentiate strain

T41^T from its close phylogenetic relatives are shown in Table 1.

An almost-complete 16S rRNA gene sequence (1354 bp) for strain T41^T was determined and subjected to a phylogenetic analysis. Results showed that strain T41^T was clustered with the genera *Terrimonas*, *Flavisolibacter*, *Niastella* and *Niabella* in the phylum *Bacteroidetes* but was clearly separated from them (Fig. 1 and Supplementary Figs S1 and S2, available in IJSEM Online). Isolate T41^T showed the highest levels of 16S rRNA gene sequence similarity to type strains of the genus *Terrimonas* (92.3–93.2 %), followed by *Flavisolibacter* (92.1–92.2 %) and *Niabella* (91.2–91.7 %). The 16S rRNA gene sequence divergence of more than 3 % may place T41^T in a novel genus and species (Stackebrandt & Goebel, 1994).

The DNA G + C content of strain T41^T was 48.1 mol%. The major isoprenoid quinone was MK-7, as in the related species *Terrimonas ferruginea*, *Terrimonas lutea*, *Flavisolibacter ginsengiterrae*, *Flavisolibacter ginsengisoli*, *Niabella soli*, *Niastella yeongjuensis* and *Niastella koreensis* (Xie & Yokota, 2006; Yoon & Im, 2007; Weon *et al.*, 2006, 2008). The major fatty acids (>10 %) were iso-15:0 (33.8 %), iso-15:1 G (13.3 %) and iso-17:0 3-OH (12.9 %) (Table 2). The presence of 15:0 and iso-15:1 G and some

Table 1. Phenotypic characteristics of strain T41^T and related type strains

Strains: 1, T41^T; 2, *Terrimonas ferruginea* ATCC 13524^T; 3, *Terrimonas lutea* IAM 15284^T; 4, *Flavisolibacter ginsengiterrae* DSM 18136^T; 5, *Flavisolibacter ginsengisoli* DSM 18119^T; 6, *Niabella soli* DSM 19437^T; 7, *Niastella yeongjuensis* KACC 11466^T; 8, *Niastella koreensis* KACC 11465^T. Data for reference strains were taken from Xie & Yokota (2006), Yoon & Im (2007) and Weon *et al.* (2006, 2008). +, Positive; –, negative; w, weak reaction; ND, no data available.

Characteristic	1	2	3	4	5	6	7	8
Pigment	Yellow*	Yellowish orange	Yellow	Yellow	Yellow	Dark yellow	Milky	Light yellow
Cell length (µm)	0.5–0.6	1–3	1–3	0.3–1.0	0.3–6.0	0.8–1.4	10–40	10–50
Gliding motility	–	–	–	–	–	–	+	+
Growth at 37 °C	+	+	+	–	+	–	–	+
Oxidase	w	+	+	+	–	+	+	+
Catalase	+	+	w	–	+	+	–	–
Gram reaction	+	–	–	–	–	–	–	–
Maximum NaCl concentration (%)	1.0	1.0	1.0	3.0	1.0	1.0	ND	ND
Nitrate reduction	–	+	+	–	–	–	–	–
Hydrolysis of:								
Casein	+	–	ND	–	–	–	+	+
Starch	–	+	ND	–	–	–	–	–
Urea	–	–	–	–	–	–	–	–
Gelatin	+	+	+	+	+	–	+	+
DNA G + C content (mol%)	48.1	48.9	47.2	43	42.7	45	44.3	45.8
Major fatty acids (>15 %)†	i-15:0	i-15:0, i-15:1, i-17:0 3-OH	i-15:0, i-15:1, i-17:0 3-OH	i-15:0	i-15:0	i-15:0, i-15:1 G	i-15:0, i-15:1 G, i-17:0 3-OH	i-15:0, i-15:1 G, i-17:0 3-OH

*Pigment was observed on R2A culture plates.

†i, Iso-branched.

Fig. 1. Neighbour-joining phylogenetic tree based on the 16S rRNA gene sequences of strain T41^T and related species. Bootstrap values are shown as percentages of 1000 replicates; values below 50 % are not indicated. Bar, 1 substitution per 100 nucleotide positions.

Table 2. Cellular fatty acid composition of strain T41^T and related type strains

Strains: 1, T41^T; 2, *Terrimonas ferruginea* ATCC 13524^T; 3, *Terrimonas lutea* IAM 15284^T; 4, *Flavisolibacter ginsengiterrae* DSM 18136^T; 5, *Flavisolibacter ginsengisoli* DSM 18119^T; 6, *Niabella soli* DSM 19437^T; 7, *Niastella yeongjuensis* KACC 11466^T; 8, *Niastella koreensis* KACC 11465^T. Data for reference strains were taken from Xie & Yokota (2006), Yoon & Im (2007) and Weon *et al.* (2006, 2008). Values are percentages of total fatty acids; fatty acids that account for <1.0 % of the total are not shown. —, Not reported or <1 %.

Fatty acid	1	2	3	4	5	6	7	8
iso-15:1 G	13.3	—	—	—	—	18.4	14.7	15.6
iso-15:0	33.8	28.4	34.8	45.9	31.2	29.2	30.6	26.8
anteiso-15:0	—	—	—	5.9	5.0	1.2	3.3	4.9
15:0	8.0	2.7	—	—	—	—	—	—
iso-16:0	—	1.4	—	—	—	—	—	1.0
16:1 ω 5c	5.8	—	—	—	—	—	1.2	—
16:0	2.2	1.7	1.1	5.5	7.5	6.8	2.9	2.6
iso-15:0 3-OH	3.9	2.2	2.3	1.4	1.0	2.2	1.9	1.3
iso-17:0	—	—	—	3.1	9.1	—	—	1.6
16:0 2-OH	—	—	—	—	2.9	1.3	—	—
16:0 3-OH	2.7	2.5	3.3	—	—	2.2	2.3	1.3
18:0	—	—	—	—	2.0	3.8	—	—
iso-17:0 3-OH	12.9	15.3	14.2	13.7	11.8	11.8	27.1	29.4
17:0 3-OH	1.1	—	—	—	—	—	—	1.2
Summed feature 3*	8.3	11.2	14.9	—	—	11.1	5.3	4.3
Unknown 13.565	1.5	1.3	3.2	—	—	1.1	—	—
Unknown 16.582	—	1.3	1.8	—	—	—	1.6	1.4

*Summed feature 3 comprises iso-15:0 2-OH and/or 16:1 ω 7c.

quantitative differences in fatty acid composition distinguish isolate T41^T from phylogenetically related genera.

On the basis of the observations described above, it is appropriate to conclude that strain T41^T represents a novel species in a new genus, for which the name *Flaviumibacter petaseus* gen. nov., sp. nov. is proposed.

Description of *Flaviumibacter* gen. nov.

Flaviumibacter (Fla'vi.hu.mi.bac'ter. L. adj. *flavus* yellow; L. n. *humus* soil; L. masc. n. *bacter* a rod; N.L. masc. n. *Flaviumibacter* a yellow, rod-shaped bacterium from soil).

Cells are Gram-reaction-positive, aerobic, single rods. Positive for catalase and weakly positive for oxidase. The major fatty acids (>10 %) are iso-15:0, iso-15:1 G and iso-17:0 3-OH. The major respiratory quinone is MK-7. The genus is positioned phylogenetically within the phylum *Bacteroidetes*. The type species is *Flaviumibacter petaseus*.

Description of *Flaviumibacter petaseus* sp. nov.

Flaviumibacter petaseus (pe.ta'se.us. L. n. *petasus* hat; L. adj. suff. *-eus -ea -eum* suffix used with various meanings; N.L. masc. adj. *petaseus* belonging to a hat, referring to the formation of hat-shaped colonies).

Possesses the following properties in addition to those given in the genus description. Cells are 0.4–0.5 μ m wide and 0.5–0.6 μ m long, without gliding motility. Colonies grown on R2A agar plates are circular, smooth, yellow and 1–2 mm in diameter. Growth occurs at 20–37 °C and pH 5.5–9.5, with optimum growth at 28 °C and pH 6.5. Growth is inhibited by >1.0 % NaCl. Maltose, sucrose, L-rhamnose, starch, D-fructose, trehalose, D-galactose, D-xylose, melibiose, D-arabinose, salicin, raffinose, glucose, D-mannose, lactose and L-arabinose are assimilated, but mannitol, D-sorbitol, inulin, inositol, sodium D-gluconate, melezitose, sorbose, D-ribose, cellobiose and glycerol are not. Acid is produced from maltose, sucrose, L-rhamnose, starch, D-fructose, trehalose, D-galactose, D-xylose, melibiose, D-arabinose, salicin, glucose, D-mannose, lactose and L-arabinose. Negative reactions are observed for lipase, urease production, nitrate reduction, nitrite reduction, denitrification and hydrolysis of lecithin, arginine, starch and DNA. Positive for H₂S production and hydrolysis of casein and gelatin. The G+C content of the DNA of the type strain is 48.1 mol%.

The type strain is T41^T (=CGMCC 1.7723^T =NBRC 106054^T), which was isolated from a soil sample from a subtropical rainforest in Nepal.

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