

Proposal of *Yaniaceae* fam. nov. and *Yania flava* sp. nov. and emended description of the genus *Yania*

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A coccoid actinobacterium strain (designated YIM 70178^T) was isolated from a soil sample collected in Qinghai Province, China. The isolate grew well with an optimum salt concentration of 10–15% (KCl, w/v) but scarcely or not at all without salt. The cell-wall peptidoglycan type was A4 α , L-Lys–Gly–L-Glu. The major polar lipids were diphosphatidylglycerol, phosphatidylglycerol, an unknown phospholipid and an unknown glycolipid. The predominant menaquinones were MK-8 and MK-9. The major fatty acid was anteiso-C₁₅:0. The DNA G + C content was 57.9 mol%. Phylogenetic analysis based on 16S rRNA gene sequences showed that strain YIM 70178^T was most closely related to the type strain of *Yania halotolerans*. DNA–DNA hybridization and comparison of physiological and chemotaxonomic characteristics demonstrated that strain YIM 70178^T was different from *Yania halotolerans*. The name *Yania flava* sp. nov. is proposed, with strain YIM 70178^T (= DSM 16377^T = KCTC 19047^T) as the type strain. Based on the phenotypic characteristics and phylogenetic position, as determined by 16S rRNA gene analysis and 16S rRNA signature nucleotide data, the genus description of *Yania* is therefore emended and strains YIM 70085^T and YIM 70178^T represent a novel family of the suborder *Micrococcineae*, for which the name *Yaniaceae* fam. nov. is proposed.

The suborder *Micrococcineae* was proposed by Stackebrandt *et al.* (1997) based on their newly established hierarchic classification system for actinomycetes, the class *Actinobacteria* (high DNA G + C Gram-positive bacteria) and contained the families *Micrococcaceae*, *Brevibacteriaceae*, *Cellulomonadaceae*, *Promicromonosporaceae*, *Dermatophilaceae*, *Dermabacteraceae*, *Intrasporangiaceae*, *Jonesiaceae* and *Microbacteriaceae*. With the subsequent addition of

novel taxa into the suborder *Micrococcineae*, the phylogenetic coherence of some families was disrupted, leading to the proposal of another five families, *Bogoriellaceae*, *Dermacoccaceae*, *Rarobacteraceae*, *Sanguibacteraceae* (Stackebrandt & Schumann, 2000) and ‘*Beutenbergiaceae*’ (Garrity & Holt, 2001).

The genus *Yania* has been described recently and placed within the suborder *Micrococcineae* (W.-J. Li *et al.*, 2004a). Although the described isolate exhibited low (<94.3%) similarity to its phylogenetic neighbours and its 16S rRNA gene signature nucleotides differed significantly from those of members of the suborder *Micrococcineae*, a new family was not proposed because we were aware that a description based on a single isolate would probably not reflect the phenotypic diversity of the taxon.

Recently, another Gram-positive actinobacterium (designated YIM 70178^T) of the genus *Yania* was isolated from a hypersaline habitat in Qinghai Province, China. The 16S rRNA gene sequence of strain YIM 70178^T was closest to

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The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of *Yania flava* YIM 70178^T is AY684123.

A detailed phylogenetic tree based on 16S rRNA gene sequences displaying the position of *Yania flava* YIM 70178^T, *Yania halotolerans* YIM 70085^T and related taxa in the suborder *Micrococcineae* is available as supplementary material in IJSEM Online.

Yania halotolerans YIM 70085^T, the type species of the genus *Yania*. Subsequent DNA–DNA hybridization and comparison of physiological and chemotaxonomic data demonstrated that strain YIM 70178^T was different from *Yania halotolerans* YIM 70085^T. The name *Yania flava* sp. nov. is proposed for this novel strain. In addition, the family *Yaniaceae* fam. nov. is proposed based on its distinct phylogenetic lineage and 16S rRNA gene sequence signature nucleotides within the suborder *Micrococccineae*.

Strain YIM 70178^T was isolated by the dilution plating method from a saline soil sample from Qinghai Province, in north-west China. The medium used for selective isolation was SGA agar (Al-Tai & Ruan, 1994) (pH 7.2) supplemented with 20% (w/v) KCl. The culture was incubated at 28 °C for about 2 weeks. The strain was maintained on ISP5 agar slants containing 10% (w/v) KCl at 4 °C and in glycerol suspensions (20% v/v) at –20 °C. Biomass for chemical and molecular systematic studies was obtained by cultivation in shaken flasks (about 150 r.p.m.) of modified ISP5 medium (KCl 10% w/v, pH 7.0) broth at 28 °C for 1 week.

Cell morphology and metabolic properties were studied as described previously (Chen *et al.*, 2004; W.-J. Li *et al.*, 2004a,

b). The colour of colonies was determined with colour chips from the ISCC-NBS colour charts standard samples no. 2106 (Kelly, 1964). In addition, acid production from carbohydrates was examined by a slightly modified method after Gordon *et al.* (1974).

Morphological observation of 24–48 h cultures of strain YIM 70178^T grown on ISP5 medium supplemented with 10% KCl (w/v) revealed that the cells were similar to those of strain YIM 70085^T, i.e. non-motile cocci, 0.4–0.8 µm in diameter. The colony characteristics were also similar to those of strain YIM 70085^T; light yellow, circular, lubricous and opaque. Some physiological and biochemical characteristics of strain YIM 70178^T are presented in Table 1.

Chemotaxonomic properties, including peptidoglycan type, polar lipids, menaquinones and whole-cell fatty acid pattern, were analysed as described previously (Chen *et al.*, 2004; W.-J. Li *et al.*, 2004a, b). The DNA G+C content was determined by reverse-phase HPLC according to Mesbah *et al.* (1989). The cellular fatty acid profile contained anteiso-C_{15:0} (58.20%), iso-C_{14:0} (12.52%), iso-C_{15:1} (9.29%), iso-C_{16:0} (8.03%), anteiso-C_{17:0}

Table 1. Differential characteristics between strain YIM 70178^T and *Yania halotolerans* YIM 70085^T

The following characteristics are the same for both strains: Gram-positive, non-motile cocci. The optimum pH for growth is 7.0–8.0. Catalase-positive and oxidase-negative. Methyl red and Voges-Proskauer tests, melanin production, H₂S and indole production are negative; Tweens 20 and 80, casein and starch are not decomposed. Positive for lysine decarboxylase, arginine dihydrolase and lipase activity. Negative for ornithine decarboxylase, L-aspartic arylamidase, α-galactosidase activities and growth on cellulose. The following substrates are utilized as sole carbon sources for growth in both strains: maltose, glucose, mannose, fructose, salicin, acetamide and galactose, while mannitol, adonitol, arabinose, arabitol, inositol and sorbitol are not utilized. +, Positive; –, negative; DPG, diphosphatidylglycerol; PG, phosphatidylglycerol; PI, phosphatidylinositol; PL, unidentified phospholipid; GL, unidentified glycolipid.

| Characteristic | YIM 70178 ^T | YIM 70085 ^T |
|---|---------------------------|---|
| Optimal concentration of KCl for growth (% w/v) | 10–15 | 10 |
| Range of salt concentrations for growth (% w/v) | | |
| NaCl | 0.5–25 | 0–25 |
| KCl | 0.5–30 | 0–20 |
| MgCl ₂ ·6H ₂ O | 0.5–30 | 0–15 |
| pH range for growth | 6.0–9.0 | 6.5–8.5 |
| Utilization of sucrose | – | + |
| Enzyme activities | | |
| Urease | – | + |
| β-Glucosidase | – | + |
| N-Acetyl-glucosaminidase | + | – |
| β-Galactosidase | + | – |
| α-Maltosidase | – | + |
| Major menaquinones | MK-8 and MK-9 | MK-8 |
| Polar lipids | DPG, PG, PL and GL | DPG, PG, PI, PL and GL |
| Major fatty acids (> 10%) | anteiso-C _{15:0} | anteiso-C _{15:0} , iso-C _{15:0} |
| G+C content (mol%) | 57.9 | 53.5 |

Table 2. Differential chemotaxonomic characteristics of genera of the families *Yaniaceae* and *Micrococcaceae*

Data for reference taxa were taken from Stackebrandt & Schumann (2000); Reddy *et al.* (2000); Liu *et al.* (2000); Reddy *et al.* (2002); Fan *et al.* (2002); Altenburger *et al.* (2002); Wieser *et al.* (2002); Y. Li *et al.* (2004); Gupta *et al.* (2004); W.-J. Li *et al.* (2004a, b, 2005); Margesin *et al.* (2004) and this study. MCA_{var}, Variable monocarboxylic amino acid; DCA_{var}, variable dicarboxylic amino acid; DPG, diphosphatidylglycerol; PG, phosphatidylglycerol; PI, phosphatidylinositol; PE, phosphatidylethanolamine; DMDG, dimannosyldiacylglycerol; PL, unidentified phospholipid(s); GL, unidentified glycolipid(s). Abbreviations for menaquinones exemplified by MK-8(H₂), partially saturated menaquinone with one of eight isoprene units hydrogenated and MK-9, unsaturated menaquinone with nine isoprene units. All taxa contain L-Lys as diamino acid of the cell-wall peptidoglycan.

| Characteristic | <i>Yaniaceae</i> | | <i>Micrococcaceae</i> | | | | | | |
|----------------------------|--|---|---|---------------------------------------|---|---|---|---|---|
| | <i>Yania</i> | <i>Micrococcus</i> | <i>Arthrobacter, globiformis</i> group | <i>Arthrobacter, nicotianae</i> group | <i>Citricoccus</i> | <i>Kocuria</i> | <i>Nesterenkonia</i> | <i>Renibacterium</i> | <i>Rothia</i> |
| Interpeptide bridge | Gly-L-Glu | Peptide subunit or D-Asp | MCA _{var} | Ala-Glu, Ser-Glu, Asp or Glu | Gly-Glu | L-Ala ₃₋₄ | Gly-L-Glu or L-Glu or Gly-Asp | L-Ala-Gly | L-Ala ₃ , L-Ala, L-Ser, Gly or Gly-Ala |
| Predominant menaquinone(s) | MK-8, -9 | MK-8, -8(H ₂) | MK-9(H ₂), -8(H ₂) | MK-8, -9, -10 | MK-9(H ₂) | MK-7(H ₂), -8(H ₂) | MK-8, -9, -7 | MK-9, -10 | MK-7, -6(H ₂) |
| Polar lipids | DPG, PG, PL, GL | DPG, PI, PG, PL, GL | DPG, PI, PG, DMDG, PE | DPG, PI, PG, DMDG | DPG, PG, PI, GL, PL | DPG, PG, PI, PL, GL | DPG, PI, PG, PL, GL | DPG, GL | DPG, PG |
| Major fatty acids | anteiso-C _{15:0} , iso-C _{15:0} , or anteiso-C _{15:0} | anteiso-C _{15:0} , iso-C _{15:0} | anteiso-C _{15:0} , iso-C _{15:0} , iso-C _{16:0} , anteiso-C _{17:0} | anteiso-C _{15:0} | anteiso-C _{15:0} , anteiso-C _{17:0} , iso-C _{15:0} | anteiso-C _{15:0} , anteiso-C _{17:0} , iso-C _{16:0} , anteiso-C _{17:1} | anteiso-C _{15:0} , anteiso-C _{17:0} , iso-C _{16:0} | anteiso-C _{15:0} , anteiso-C _{17:0} | anteiso-C _{15:0} , anteiso-C _{17:0} , C _{16:0} |
| G + C content (mol%) | 53–58 | 66–76 | 61–69 | 58–69 | 64–68 | 60–75 | 64–72 | 52–54 | 49–58 |

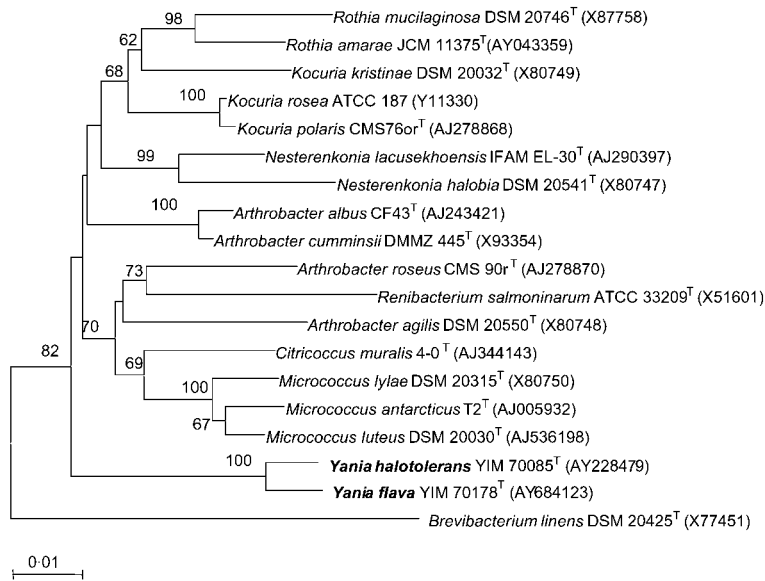


Fig. 1. Phylogenetic dendrogram obtained by distance matrix analysis of 16S rRNA gene sequences, showing the position of strain YIM 70178^T among phylogenetic neighbours. The sequence of *Streptomyces megasporus* DSM 41476^T (Z68100) was used as the outgroup (not shown). Numbers on branch nodes are bootstrap percentages (1000 resamplings, only values over 50% are given). Bar, 1% sequence divergence.

(5.79%), iso-C_{13:0} (2.08%), iso-C_{17:0} (1.88%), C_{16:0} (0.59%), anteiso-C_{15:1} (0.51%), anteiso-C_{13:0} (0.38%), C_{16:1ω7c} (0.35%), C_{18:1ω9c} (0.25%) and C_{14:0} (0.13%). Additional chemotaxonomic data are shown in Tables 1 and 2.

Extraction of genomic DNA and amplification of the 16S rRNA gene were performed according to Xu *et al.* (2003). Phylogenetic analysis was conducted using the MEGA version 2.1 software package (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) and clustering were performed using the neighbour-joining (Saitou & Nei, 1987) and maximum-likelihood (Felsenstein, 1981) methods. Bootstrap analysis was used to evaluate the tree topology of the neighbour-joining data by performing 1000 resamplings (Felsenstein, 1985).

The almost-complete 16S rRNA gene sequence of strain YIM 70178^T (1493 bp) was determined. Phylogenetic analysis, based on a dataset consisting of 1376 unambiguous nucleotides between positions 42 and 1417, showed that the novel isolate was most closely related to *Yania halotolerans* YIM 70085^T. A dendrogram (Fig. 1) confirmed that strains YIM 70178^T and YIM 70085^T are phylogenetic neighbours with 98.4% 16S rRNA gene sequence similarity and a bootstrap value of 100%. Similarity values with sequences of neighbouring taxa were significantly lower (89.2–94.0%). A detailed phylogenetic tree displaying the phylogenetic position of strain YIM 70178^T, *Yania halotolerans* YIM 70085^T and related taxa in the suborder *Micrococccineae* is available as Supplementary Fig. S1 in IJSEM Online.

The isolate YIM 70178^T was different from *Yania halotolerans* YIM 70085^T in some physiological, biochemical and chemotaxonomic characteristics (Table 1). DNA–DNA relatedness tests were performed between strain YIM 70178^T and *Yania halotolerans* YIM 70085^T using the optical

renaturation method (De Ley *et al.*, 1970; Huß *et al.*, 1983; Jahnke, 1992) and DNA–DNA relatedness between the strains was 35.4%. DNA–DNA relatedness provided decisive evidence that strain YIM 70178^T and *Yania halotolerans* YIM 70085^T are members of different genomic species (Wayne *et al.*, 1987). Therefore, based on the above phenotypic and genotypic results, we consider strain YIM 70178^T to represent a novel species of the genus *Yania*, for which we propose the name *Yania flava* sp. nov.

The results of 16S rRNA gene sequence comparisons clearly demonstrated that strains YIM 70178^T and YIM 70085^T are members of the suborder *Micrococccineae*. Both strains have some unique 16S rRNA gene signature nucleotides compared with other families of the suborder *Micrococccineae*, such as 140–223 (A–G), 142–221 (C–A), 615–625 (G–U), 839–847 (A–A) and 1134–1140 (A–U) (this study and W.-J. Li *et al.*, 2004a). Strain YIM 70178^T was isolated from Qinghai Province, north-west China and strain YIM 70085^T originated from Xinjiang Province, in the north of China. Since the two strains were isolated from independent and geographically distant sources, they provide a first estimation of the phenotypic diversity of the genus *Yania*. As discussed previously (W.-J. Li *et al.*, 2004a), the unique set of 16S rRNA gene signature nucleotides and chemotaxonomic markers distinguish the genus *Yania* from members of the closest family *Micrococccaceae* in the suborder *Micrococccineae* (Table 2). Thus, the family *Yaniaceae* fam. nov. is proposed.

Description of *Yaniaceae* fam. nov.

Yaniaceae (Ya'ni.a'ce.ae. N.L. fem. n. *Yania* type genus of the family; -aceae ending to denote a family; N.L. fem. pl. n. *Yaniaceae* the *Yania* family).

The pattern of 16S rRNA gene sequence signatures consists of nucleotides at positions 140–223 (A–G), 142–221 (C–A),

615–625 (G–U), 839–847 (A–A) and 1134–1140 (A–U) (W.-J. Li *et al.*, 2004a). The type genus is *Yania*.

Emended description of the genus *Yania* Li *et al.* 2004

The description of the genus *Yania* (W.-J. Li *et al.*, 2004) is emended as follows. Moderately halophilic or halotolerant. The polar lipids contain diphosphatidylglycerol, phosphatidylglycerol, an unknown phospholipid and an unknown glycolipid. The predominant menaquinone(s) are MK-8 and MK-9 or MK-8. The major cellular fatty acids are anteiso-C_{15:0} and iso-C_{15:0} or anteiso-C_{15:0}. The DNA G+C content is 53–58 mol%.

Description of *Yania flava* sp. nov.

Yania flava (fla'va. L. fem. adj. *flava* golden yellow, referring to the colour of the colonies).

Morphological, chemotaxonomic and general characteristics are as described for the genus. Colonies are light yellow, circular, lubricous and opaque. Acid is produced from glucose, maltose and fructose. It is negative for milk peptonization, milk coagulation, urease and nitrate reduction, gelatin liquefaction, growth in cellulose, H₂S and melanin production. Some other physiological and biochemical characteristics are listed in Table 1. The cell-wall peptidoglycan type is A4 α , L-Lys–Gly–L-Glu. The polar lipids contain diphosphatidylglycerol, phosphatidylglycerol, an unknown phospholipid and an unknown glycolipid. The predominant menaquinones are MK-8 and MK-9. The major cellular fatty acid is anteiso-C_{15:0}. The DNA G+C content is 57.9 mol% (HPLC method).

The type strain, YIM 70178^T (=DSM 16377^T=KCTC 19047^T), was isolated from a saline soil collected from Qinghai Province, north-west China.

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