Stackebrandtia albiflava sp. nov. and emended description of the genus *Stackebrandtia*

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A novel actinomycete, designated YIM 45751^T, was isolated from a soil sample collected from the Xishuang Banna tropical rainforest, Yunnan province, south-west China, and was subjected to a polyphasic taxonomic study. The cell-wall peptidoglycan contained *meso*-diaminopimelic acid as the diagnostic diamino acid. Whole-cell hydrolysates contained ribose, xylose and glucose. The predominant menaquinones were MK-10(H₄), MK-10(H₆), MK-11(H₄) and MK-11(H₆). The polar lipids were diphosphatidylglycerol, phosphatidylmethylethanolamine, phosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol and three unknown phospholipids. 16S rRNA gene sequence analysis showed that *Stackebrandtia nassauensis* DSM 44728^T was the closest phylogenetic neighbour (95.8% 16S rRNA gene sequence similarity). On the basis of phylogenetic and some common chemotaxonomic data, the isolate should belong to the genus *Stackebrandtia*. However, phylogenetic analysis and comparison of physiological and chemotaxonomic characteristics demonstrated that the isolate is distinct from *S. nassauensis* DSM 44728^T. Strain YIM 45751^T represents a separate species of the genus *Stackebrandtia*, for which the name *Stackebrandtia albiflava* sp. nov. is proposed. The type strain is YIM 45751^T (=DSM 45044^T=CCTCC AA 206003^T). Furthermore, the description of the genus *Stackebrandtia* is also required to be emended.

The family *Glycomycetaceae* of the order *Actinomycetales* was proposed by Rainey *et al.* 1997 (in Stackebrandt *et al.*, 1997) and the description was later emended by Labeda & Kroppenstedt (2005). Members of this family contain *meso*-diaminopimelic acid (*meso*-DAP) and *N*-glycolylmuramic acid in the cell wall, but lack mycolic acids. At present, the family contains two recognized genera: the type genus *Glycomyces* (Labeda *et al.*, 1985; Labeda & Kroppenstedt, 2004) and the genus *Stackebrandtia* (Labeda & Kroppenstedt, 2005). Currently, the genus *Stackebrandtia* comprises just one member, *Stackebrandtia nassauensis*. This species is a Gram-positive, strictly aerobic, filamentous actinomycete. It possesses MK-10(H₄), MK-10(H₆), MK-11(H₄) and

MK-11(H₆) as predominant menaquinones. Its fatty acid profile is rich in branched-chain and saturated components, including 10-methyl-branched heptadecanoic acid and iso-branched 2-hydroxy fatty acids. The present study focuses on the description of strain YIM 45751^T, which was isolated from a soil sample. Based on the taxonomic data collected in this study, we propose that strain YIM 45751^T represents a novel species within the genus *Stackebrandtia*.

During our investigation of the isolation methods of rare actinomycetes, a novel actinomycete strain, designated YIM 45751^T, was isolated from a soil sample collected from the Xishuang Banna tropical rainforest, Yunnan province, south-west China, using raffinose–histidine agar (pH 7.0–7.2; Vickers *et al.*, 1984) that was supplemented with cycloheximide and nystatin at 50 and 25 µg ml⁻¹, respectively. The plates were incubated at 28 °C for 20–30 days and single colonies on the plates were purified by transferring them onto ISP 2 (Shirling & Gottlieb, 1966) agar. Strain YIM 45751^T was selected and cultured on ISP 2 agar at 28 °C and maintained as a glycerol suspension (20%, w/v) at –70 °C.

Abbreviation: *meso*-DAP, *meso*-diaminopimelic acid.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain YIM 45751^T is DQ985165.

A scanning electron micrograph and a phylogenetic tree of strain YIM 45751^T are available as supplementary figures with the online version of this paper.

Morphological observations were made on the media of Shirling & Gottlieb (1966), Czapek's agar (Pridham & Lyons, 1980) and DSMZ medium no. 554 (<http://www.dsmz.de/microorganisms/html/media/medium000554.html>). Culture colours were determined by referring to the colour chips from the ISCC-NBS colour charts standard samples no. 2106 (Kelly, 1964). Physiological tests, including production of acid from carbohydrates, utilization of organic acids and carbohydrate, hydrolysis or decomposition of adenine, hypoxanthine, tyrosine, xanthine, casein, aesculin and urea, were evaluated as described previously (Gordon *et al.*, 1974) after 15 days. Growth at different temperatures (4, 10, 15, 20, 28, 30, 37 and 45 °C), NaCl concentrations (1, 3, 5, 7 and 10 %) and pH values (5.0–11.0) was assessed after incubation for 7 and 15 days on ISP 2 agar medium. Nitrate reduction, gelatin liquefaction and degradation of starch were examined by using previously described methods (MacFaddin, 1980). Catalase activity was determined by assessing bubble production in 3 % (v/v) H₂O₂, and oxidase activity was determined by using a 1 % (w/v) solution of tetramethyl-*p*-phenylenediamine (Kovacs, 1956).

Strain YIM 45751^T formed yellow–white to white colonies on ISP 2, DSMZ medium no. 554, ISP 5, ISP 3 and Czapek's agar. Substrate mycelia showed extensive branching and were non-fragmenting (see Supplementary Fig. S1, available in IJSEM Online). Aerial hyphae and diffusion pigment were not observed on the media tested (listed above). The phenotypic characteristics are summarized in the species description, and the differences between strain YIM 45751^T and *S. nassauensis* NRRL B-16338^T are listed in Table 1.

Biomass for molecular analysis and chemotaxonomic studies was obtained after incubation at 28 °C for 7 days by growing in shake flasks of ISP 2 liquid medium. Procedures used to identify the cell-wall amino acids and sugars in whole-cell hydrolysates were as described by Stanek & Roberts (1974). Menaquinones were extracted by using the method of Collins *et al.* (1977) and analysed by HPLC as described by Tamaoka *et al.* (1983). Polar lipids were extracted as described by Minnikin *et al.* (1979) and identified by using two-dimensional TLC and spraying with specific reagents (Collins & Jones, 1980). Analysis of the whole-cell fatty acid pattern followed described methods using the MIDI system (Microbial ID, Inc.) (Kroppenstedt, 1985; Meier *et al.*, 1993). The G+C content of the genomic DNA was determined by HPLC according to Mesbah *et al.* (1989).

The cell-wall peptidoglycan of strain YIM 45751^T contained *meso*-DAP; the whole-cell hydrolysate contained ribose, xylose and glucose. Strain YIM 45751^T is readily differentiated from the type strain of *S. nassauensis* DSM 44728^T by the absence of inositol, arabinose and mannose and the presence of xylose and glucose (Labeda & Kroppenstedt, 2005). In order to demonstrate whether all of the sugars reported by Labeda & Kroppenstedt (2005) are really components of the whole-cell hydrolysates, the whole-cell hydrolysate patterns of strain YIM 45751^T and *S.*

Table 1. Differentiating characteristics between strain YIM 45751^T and its closest phylogenetic neighbour, *Stackebrandtia nassauensis* NRRL B-16338^T

Strains: 1, YIM 45751^T; 2, *S. nassauensis* DSM 44728^T. For both strains, acid is produced from galactose, mannose, arabinose and cellobiose, but not from inositol, adonitol, dulcitol, melezitose or erythritol. Both strains are positive for starch, casein and gelatin hydrolysis, but negative for adenine degradation. Neither strain assimilates tartrate, benzoate or mucate. +, Positive; –, negative; w, weak reaction. Data are from this study or Labeda & Kroppenstedt (2005).

Characteristic	1	2
Hydrolysis of:		
Aesculin	–	+
Hypoxanthine	–	+
Tyrosine	–	+
Xanthine	+	–
Utilization of:		
Citrate	w	–
Propionate	+	–
Acetate	–	w
Malate	–	w
Acid from:		
Mannitol	+	–
Glycerol	–	+
Raffinose	–	+
Glucose	–	+
Trehalose	–	+
Sucrose	–	+

nassauensis DSM 44728^T were reanalysed as described by Stanek & Roberts (1974). In this study, ribose, xylose, mannose and glucose were detected in *S. nassauensis* DSM 44728^T, and the predominant menaquinones of strains YIM 45751^T and *S. nassauensis* DSM 44728^T were MK-10(H₄), MK-10(H₆), MK-11(H₄) and MK-11(H₆), confirming the observation stated to be characteristic of *S. nassauensis* DSM 44728^T of the genus *Stackebrandtia* (Labeda & Kroppenstedt, 2005). However, in this study, the polar lipid profiles of strain YIM 45751^T and *S. nassauensis* DSM 44728^T contained diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylmethylethanolamine, phosphatidylglycerol and three additional unknown phospholipids (Fig. 1), which is not consistent with the results reported by Labeda & Kroppenstedt (2005) for *S. nassauensis* DSM 44728^T. In addition, strain YIM 45751^T also contained phosphatidylinositol and an unknown aminophospholipid as species-specific polar lipids. The cellular fatty acid profile of strain YIM 45751^T mainly contained anteiso-C_{17:0} (18.87 %), iso-C_{17:0} (15.12 %) and iso-C_{15:0} (11.60 %). The G+C content of the genomic DNA was 69.4 mol%.

Genomic DNA extraction, PCR amplification and 16S rRNA gene sequencing were carried out as described previously (Cui *et al.*, 2001). The nearly complete 16S rRNA gene sequence (1515 bp) of strain YIM 45751^T was

Fig. 1. Two-dimensional TLC of polar lipids of (a) strain YIM 45751^T and (b) *S. nassauensis* DSM 44728^T. Abbreviations: PI, phosphatidylinositol; PG, phosphatidylglycerol; DPG, diphosphatidylglycerol; PE, phosphatidylethanolamine; PME, phosphatidylmethylethanolamine; APL, unknown aminophospholipid; PL1, 2, 3, unknown phospholipids.

obtained and compared with the related sequences of type strains of species in the order *Actinomycetales* (downloaded from GenBank). Phylogenetic analysis was performed by using the software package MEGA version 2.1 (Kumar *et al.*, 2001) after multiple alignment of the data using CLUSTAL_X (Thompson *et al.*, 1997). Distances (using 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 (1000 resamplings) was used to evaluate the tree topology of the neighbour-joining data (Felsenstein, 1985).

Phylogenetic analysis showed that strain YIM 45751^T had highest 16S rRNA gene sequence similarity (95.8%) to the only recognized species of the genus *Stackebrandtia*, *S. nassauensis* DSM 44728^T, and both formed a distinct cluster in the phylogenetic tree (see Supplementary Fig. S2, available in IJSEM Online). It clearly represents a distinct genomic species (Stackebrandt & Goebel, 1994).

Its phylogenetic position and chemotaxonomic analysis (e.g. peptidoglycan structure and predominant menaquinones) indicate that the isolate should be assigned to the

genus *Stackebrandtia*. However, based on the big differences in other chemotaxonomic data, such as polar lipids, fatty acid profiles and DNA G+C contents, and other phenotypic data listed in Table 1, strain YIM 45751^T should represent a novel species of the genus *Stackebrandtia*, for which the name *Stackebrandtia albiflava* sp. nov. is proposed. Additionally, as a consequence of newly reported characteristics not included in the original description of *S. nassauensis* DSM 44728^T, the description of the genus *Stackebrandtia* Labeda and Kroppenstedt 2005 needs to be emended.

Emended description of the genus *Stackebrandtia* Labeda and Kroppenstedt 2005

Aerobic, Gram-positive, non-motile, filamentous actinomycete. Branched and non-fragmenting substrate mycelia are produced. Aerial mycelia do or do not occur, depending on the strain. Catalase-positive. The cell-wall peptidoglycan contains *meso*-diaminopimelic acid. The whole-cell sugar pattern consists of ribose, xylose and glucose. Arabinose and mannose are variable among the species. The phospholipid pattern consists of diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylmethylethanolamine, phosphatidylglycerol and three additional unknown phospholipids. The predominant menaquinones are MK-10(H₄), MK-10(H₆), MK-11(H₄) and MK-11(H₆). The major fatty acids are saturated, iso- and anteiso-branched fatty acids. The G+C content of the genomic DNA is 69–73 mol%.

Phylogenetically, the genus is a member of the suborder *Glycomycineae*, order *Actinomycetales*. The type species is *Stackebrandtia nassauensis*.

Description of *Stackebrandtia albiflava* sp. nov.

Stackebrandtia albiflava (al.bi.fl'a'va. L. adj. *albus* -a -um white; L. adj. *flavus* -a -um yellow; N.L. fem. adj. *albiflava* white-yellow, referring to the vegetative mycelium).

Colonies that develop on ISP 5, ISP 3 and Czapek's sucrose agar are white and those that develop on ISP 2 and DSMZ medium no. 554 are yellow-white; no growth is observed on ISP 4 medium. No aerial hyphae or diffusion pigments are observed on any media tested. Grows well at 28–37 °C and pH 7.0, but not at or below 15 °C or above 37 °C. Growth on ISP 2 agar occurs in the absence of NaCl. Catalase-positive and oxidase-negative. Nitrate is reduced to nitrite. H₂S is not produced. The strain can degrade xanthine, casein, starch, gelatin, aesculin, Tweens 20, 60, 80 and urea, but not adenine, hypoxanthine, tyrosine, aesculin or DNA. Utilizes glucose, sucrose, rhamnose, raffinose, sorbitol, melibiose, dextrin, fructose, lactose, xylose, ribose, adonitol, dulcitol, raffinose and propionate as sole carbon sources; citrate is utilized weakly, but inositol, melezitose, erythritol, acetate, tartrate, benzoate, mucate and malate are not utilized. Acid is produced from galactose, mannose, mannitol, arabinose and cellobiose, but not from glucose,

sucrose, raffinose, fucose, glycerol, inositol, adonitol, dulcitol, melezitose or erythritol. The polar lipids are diphosphatidylglycerol, phosphatidylmethylethanolamine, phosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol and three unknown phospholipids. The major fatty acids are anteiso-C_{17:0}, iso-C_{17:0}, iso-C_{15:0} and other minor components, including C_{17:1}ω8c, C_{17:0}, C_{18:1}ω9c, iso-C_{17:1}ω9c, C_{16:0}, iso-C_{16:0}, C_{16:1}ω7c, anteiso-C_{16:0}, anteiso-C_{15:0}, anteiso-C_{18:0}, C_{14:0}, C_{17:1}ω6c, 10-methyl C_{17:0}, C_{15:0}, C_{19:0}, anteiso-C_{17:1}ω9c, C_{19:1}ω9c, C_{13:0}, 10-methyl-C_{18:0}. The DNA G + C content is 69.4 mol%.

The type strain is YIM 45751^T (=DSM 45044^T=CCTCC AA 206003^T), isolated from a soil sample collected from the Xishuang Banna tropical rainforest, Yunnan province, China.

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