Monashia flava gen. nov., sp. nov., an actinobacterium of the family *Intrasporangiaceae*

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A novel actinobacterial strain, MUSC 78^T, was isolated from a mangrove soil collected from Peninsular Malaysia. The taxonomic status of this strain was determined using a polyphasic approach. Comparative 16S rRNA gene sequence analysis revealed that strain MUSC 78^T represented a novel lineage within the class *Actinobacteria*. Strain MUSC 78^T formed a distinct clade in the family *Intrasporangiaceae* and was related most closely to members of the genera *Terrabacter* (98.3–96.8 % 16S rRNA gene sequence similarity), *Intrasporangium* (98.2–96.8 %), *Humibacillus* (97.2 %), *Janibacter* (97.0–95.3 %), *Terracoccus* (96.8 %), *Kribbia* (96.6 %), *Phycicoccus* (96.2–94.7 %), *Knoellia* (96.1–94.8 %), *Tetrasphaera* (96.0–94.9 %) and *Lapillicoccus* (95.9 %). Cells were irregular rod-shaped or cocci and stained Gram-positive. The cell-wall peptidoglycan type was A3γ, with LL-diaminopimelic acid as the diagnostic diamino acid. The main cell-wall sugar was mannose and lower amounts of galactose and rhamnose were present. The predominant menaquinone was MK-8(H_4). The polar lipid profile consisted of phosphatidylglycerol, phosphatidylinositol, phosphatidylethanolamine, diphosphatidylglycerol and phosphoglycolipid. The predominant fatty acids were iso-$C_{15:0}$, anteiso-$C_{15:0}$ and iso-$C_{16:0}$.The DNA G+C content was 73.1 mol%. Based on this polyphasic study, MUSC 78^T exhibited phylogenetic and phenotypic differences from members of the genera of the family *Intrasporangiaceae*, and therefore a novel species of a new genus, *Monashia flava* gen. nov., sp. nov., is proposed. The type strain of *Monashia flava* is MUSC 78^T (=DSM 29621^T=MCCC 1K00454^T=NBRC 110749^T).

The family *Intrasporangiaceae* (Stackebrandt *et al.*, 1997; Stackebrandt & Schumann, 2000) was redescribed by Zhi *et al.* (2009) and the family contains an array of actinobacteria, in addition to the type genus *Intrasporangium* (Kalakoutskii *et al.*, 1967), and has MK-8(H_4) or MK-8 as the major menaquinones and LL-diaminopimelic acid (LL-DAP), *meso*-DAP or L-ornithine (L-Orn) in the cell-wall peptidoglycan (Martin *et al.*, 1997; Maszenan *et al.*, 2000; Groth *et al.*, 2001, 2002; Hanada *et al.*, 2002; Kageyama *et al.*, 2005, 2007, 2008a, b; Jung *et al.*, 2006; Lee & Lee, 2007). At the time of writing, the family *Intrasporangiaceae* contained 19 genera, including 16 genera summarized by Zhi *et al.* (2009) and the recently described *Marihabitans* (Kageyama *et al.*, 2008b), *Fodinibacter* (Wang *et al.*, 2009) and *Ornithinibacter* (Xiao *et al.*, 2011). It is possible to distinguish these genera from each other on the basis of phenotypic and genotypic characteristics. The present investigation was designed to determine the taxonomic status of a novel actinobacterial strain, MUSC 78^T, that contained LL-DAP in the cell-wall peptidoglycan and MK-8(H_4) as the predominant menaquinone. To determine the taxonomic position of strain MUSC 78^T, a polyphasic approach was used to determine the phylogenetic, chemotaxonomic and phenotypic characteristics of the novel strain. The results indicated that strain MUSC 78^T represents a novel species of a new genus, for which the name *Monashia flavus* gen. nov., sp. nov. is proposed.

Abbreviation: DAP, diaminopimelic acid.

The GenBank/EMBL/DDBJ accession number of the 16S rRNA gene sequence of strain MUSC 78^T is KF682157.
Two supplementary figures are available with the online Supplementary Material.

Strain MUSC 78^T was isolated from a soil sample collected at site MUSC-TLS1 (3° 48′ 3.2″ N 103° 20′ 11.0″ E), located at the mangrove forests of the Tanjung Lumpur in the state of Pahang, Peninsular Malaysia, in December 2012. Samples of the upper 20 cm topsoil layer (after removing the top 2–3 cm) were collected using an aseptic metal trowel, placed in sterile plastic bags and stored in −20 °C. Selective pretreatment of soil samples was performed using wet heat in sterilized water (15 min at 50 °C; Takahashi *et al.*, 1996). Five grams of air-dried soil was mixed with 45 ml sterilized water and ground using a mill and then the suspension was spread onto a selective isolation medium, starch casein agar (SCA; Küster & Williams, 1964) supplemented with cycloheximide (25 μg ml^{-1}) and nystatin (10 μg ml^{-1}), and incubated at 28 °C for 7 days. Isolate MUSC 78^T was maintained on R2A agar medium at 28 °C and as glycerol suspensions (20 %, v/v) at −20 °C.

Cultural characteristics of strain MUSC 78^T were determined following growth on ISP 2 and ISP 7 media (Shirling & Gottlieb, 1966), SCA, *Streptomyces* agar (SA; Atlas 1993), *Actinomycetes* isolation agar (AIA; Atlas 1993) and nutrient agar (MacFaddin, 2000) for 7 days at 28 °C. The ISCC-NBS colour charts were used to determine the names and designations of colony colours (Kelly, 1964). Light microscopy (80i; Nikon) and scanning electron microscopy (JEOL-JSM 6400) were used to observe the morphologies of strains after incubation on R2A agar medium at 28 °C for 7 days. Gram staining was done following the standard Gram reaction and was confirmed by using KOH lysis (Cerny, 1978). Growth was tested at 4–44 °C at intervals of 4 °C on R2A agar medium. NaCl tolerance was tested using trypticase soy broth (TSB) and salt concentrations of 0–14 % (w/v) at intervals of 2 %. Growth was tested between pH 4.0 and 10.0 at intervals of 1 pH unit. The production of melanoid pigments and catalase activity were assessed following the protocols described by Lee *et al.* (2014a). Haemolytic activity was assessed on blood agar medium containing 5 % (w/v) peptone, 3 % (w/v) yeast extract, 5 % (w/v) NaCl and 5 % (v/v) horse blood (Carrillo *et al.*, 1996). Plates were examined for haemolysis after incubation at 32 °C for 7 days. Lipase, amylase, cellulase, chitinase, protease and xylanase activities were determined by growing cells on R2A agar medium and following the protocols described by Meena *et al.* (2013). The presence of clear zones around colonies signifies the potential of isolates for surfactant production. Antibiotic susceptibility tests were performed by the disc diffusion method as described by Shieh *et al.* (2003). Antimicrobials (Oxoid) used were as follows: ampicillin (10 μg), ampicillin sulbactam (30 μg), cefotaxime (30 μg), cefuroxime (30 μg), cephalosporin (30 μg), chloramphenicol (30 μg), ciprofloxacin (10 μg), erythromycin (15 μg), gentamicin (20 μg), nalidixic acid (30 μg), penicillin G (10 μg), streptomycin (10 μg), tetracycline (30 μg) and vancomycin (30 μg). Cells were resistant to nalidixic acid but sensitive to the other antimicrobials used. Carbon-source utilization and chemical sensitivity assays were determined using Biolog GenIII MicroPlates according to the manufacturer's instructions (Biolog).

Biomass for molecular systematic studies and freeze-dried cells for chemotaxonomic studies was obtained after growing in TSB at 28 °C for 5 days on a rotary shaker. Analysis of peptidoglycan amino-acid composition and sugars was carried out by the Identification Service of the DSMZ (Braunschweig, Germany). The analyses were performed according to published protocols (Schumann, 2011). Major diagnostic sugars of strain MUSC 78^T were obtained following the procedure described by Whiton *et al.* (1985) and analysed by TLC on cellulose plates according to Staneck & Roberts (1974). Analysis of respiratory menaquinones and polar lipids was carried out by the Identification Service of the DSMZ. The cellular polar lipids were extracted and analysed by TLC (Kates, 1986). Cellular fatty acid analyses of strain MUSC 78^T were carried out by the Identification Service of the DSMZ. Cell mass of strain MUSC 78^T was harvested from TSB after incubation at 28 °C for 5 days. The fatty acids were extracted and prepared according to the standard protocol of the MIDI Microbial Identification system (Sasser, 1990).

Genomic DNA extractions were carried out as described by Hong *et al.* (2009). PCR amplification of the 16S rRNA gene and sequencing of the purified products were done as described by Lee *et al.* (2014b). The 16S rRNA gene sequence of strain MUSC 78^T was aligned with sequences of closely related type genera classified in the family *Intrasporangiaceae* that had been retrieved from the GenBank/EMBL/DDBJ databases using CLUSTAL X software (Thompson *et al.*, 1997). The alignment was manually verified and adjusted prior to the reconstruction of a phylogenetic tree. Phylogenetic trees were reconstructed using the neighbour-joining (Saitou & Nei, 1987) and maximum-likelihood (Felsenstein, 1981) algorithms with MEGA version 6.0 (Tamura *et al.*, 2013). Calculations of levels of sequence similarity were carried out using the EzTaxon-e server (http://eztaxon-e.ezbiocloud.net/; Kim *et al.*, 2012). The stability of the resultant tree topologies was evaluated by using the bootstrap resampling method of Felsenstein (1985). Evolutionary distances were computed using Kimura's two-parameter model (Kimura, 1980). The genomic DNA of strain MUSC 78^T for the determination of G + C content was extracted according to Cashion *et al.* (1977). The G + C content of the DNA was determined by HPLC (Mesbah *et al.*, 1989).

Strain MUSC 78^T formed Gram-stain-positive, non-motile, aerobic, non-spore-forming cocci or irregular rod-shaped cells (Fig. 1). Cells formed yellowish-white-pigmented colonies on ISP 2 medium and AIA. Good growth was observed on R2A agar medium, ISP 2 medium, ISP 7 medium, Luria–Bertani agar, nutrient agar and SA after 7 days at 28 °C, while cells grew moderately on AIA and SCA. Cells were positive for catalase and haemolytic activities. Strain MUSC 78^T was positive for hydrolysis of chitin, soluble starch, casein and CM-cellulose, but negative for hydrolysis of tributyrin (lipase) and xylan. Using TSB

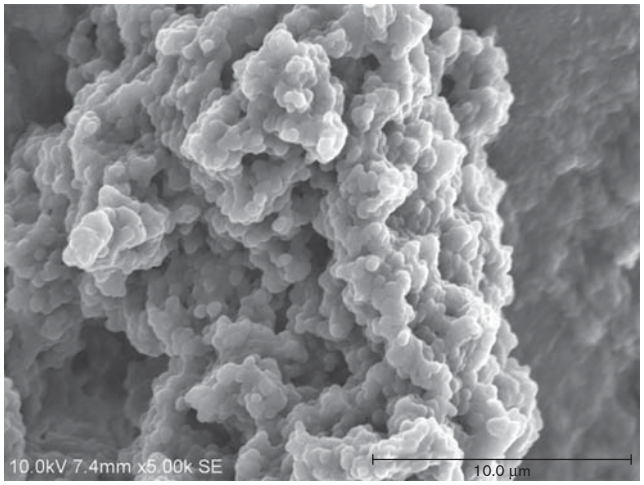

Fig. 1. Scanning electron micrograph of cells from a 5 day old culture of strain MUSC 78^T grown at 28 °C on R2A agar medium. Bar, 10 µm.

(without NaCl), the NaCl tolerance, temperature and pH ranges for growth were 0–4 %, 24–36 °C and pH 6.0–8.0 and optimal growth occurred at 0–2 % NaCl, 28–32 °C and pH 7.0. The morphological, cultural and physiological properties of strain MUSC 78^T are given in the genus and species descriptions. The organism could be distinguished from members of the family *Intrasporangiaceae* based on several chemotaxonomic characteristics (Table 1).

The total hydrolysate (4 M HCl, 16 h, 100 °C) of the peptidoglycan of strain MUSC 78^T contained LL-DAP, glycine (Gly), glutamic acid (Glu), alanine (Ala) and muramic acid (Mur). The molar ratio was 1.2 LL-DAP/3.0 Gly/1.0 Glu/1.5 Ala/0.9 Mur. The identity of these amino acids was confirmed by agreement in the GC retention time with those of authentic standards and by characteristic MS fragment ions of the derivatives. The partial hydrolysate (4 M HCl, 0.75 h, 100 °C) of the peptidoglycan contained the peptides L-Ala–D-Glu, Gly–D-Glu, Gly–D-Ala, Gly3, LL-DAP–D-Ala and LL-DAP–Gly. These data indicated that strain MUSC 78^T contains the peptidoglycan type A3γ (Schleifer & Kandler, 1972). The cell-wall sugars of strain MUSC 78^T were mannose and (at lower amounts) galactose and rhamnose. The menaquinones consisted of MK-8(H_4) (80 %), MK-8 (1 %) and MK-8(H_2) (1 %). The polar lipids consisted of diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol and phosphoglycolipid (Fig. S1, available in the online Supplementary Material). The major fatty acids of strain MUSC 78^T were iso-$C_{15:0}$ (34.8 %), anteiso-$C_{15:0}$ (16.7 %), iso-$C_{16:0}$ (14.5 %), iso-$C_{14:0}$ (8.6 %), anteiso-$C_{17:0}$ (4.6 %), $C_{16:0}$ (2.7 %) and $C_{18:0}$ (2.6 %) (Table 1).

The DNA G+C content of strain MUSC 78^T was 73. 1 mol%, a result that is within the range (68–74.1 %) described for members within the family *Intrasporangiaceae* (Table 1).

The nearly complete 16S rRNA gene sequence was determined for strain MUSC 78^T (1488 bp). Phylogenetic trees were reconstructed based on the 16S rRNA gene sequences (Figs 2 and S2). Comparative 16S rRNA gene sequence analysis showed that strain MUSC 78^T falls within the evolutionary radiation occupied by the family *Intrasporangiaceae* (Figs 2 and S2). The closest phylogenetic neighbours were members of the genera in the family *Intrasporangiaceae* (suborder *Micrococcineae*). Strain MUSC 78^T showed highest 16S rRNA gene sequence similarity to *Terrabacter lapilli* LR-26^T (98.3 %), *Intrasporangium oryzae* NRRL B-24470^T (98.2 %), *Humibacillus xanthopallidus* KV-663^T (97.2 %), *Janibacter anophelis* CCUG 49715^T (97.0 %), *Terracoccus luteus* DSM 44267^T (96.8 %), *Kribbia dieselivorans* N113^T (96.6 %), *Phycicoccus cremeus* V2M29^T (96.2 %), *Knoellia sinensis* DSM 12331^T (96.1 %), *Tetrasphaera japonica* ACM 5116^T (96.0 %) and *Lapillicoccus jejuensis* R-Ac013^T (95.9 %). Strain MUSC 78^T showed the closest evolutionary distance to the type strains of members of the genus *Terrabacter* at a low nucleotide sequence similarity (98.3–96.8 %). However, strain MUSC 78^T could be differentiated from the genus *Terrabacter* by the presence of anteiso-$C_{15:0}$ as a major fatty acid and the presence of phosphatidylglycerol. In addition, the genera *Intrasporangium*, *Humibacillus* and *Terracoccus*, which contain LL-DAP as the major diagnostic diamino acid in the peptidoglycan, do not contain phosphoglycolipid as a diagnostic phospholipid (Table 1). Of the other phylogenetically closely related neighbours, members of the genera *Janibacter*, *Kribbia*, *Phycicoccus*, *Knoellia*, *Tetrasphaera*, *Fodinibacter* and *Oryzihumus* are clearly different from strain MUSC 78^T as these genera contain *meso*-DAP as the diagnostic diamino acid (Table 1). Other genera such as *Ornithinibacter*, *Ornithinicoccus*, *Ornithinimicrobium* and *Serinicoccus* are different from strain MUSC 78^T as they contain L-Orn as the diagnostic diamino acid (Table 1). Differential chemotaxonomic characteristics between strain MUSC 78^T and other genera belonging to the family *Intrasporangiaceae* are summarized in Table 1. Therefore, on the basis of phylogenetic data and differential chemotaxonomic characteristics, strain MUSC 78^T does not belong to any existing genera in the family *Intrasporangiaceae*.

Based on its distinct phylogenetic position within the family *Intrasporangiaceae*, together with characteristic cell morphology, and chemotaxonomic and physiological properties, strain MUSC 78^T should be classified as representing a novel species in a new genus of the family *Intrasporangiaceae*, for which the name *Monashia flavus* gen. nov., sp. nov. is proposed.

Description of *Monashia* gen. nov.

Monashia gen. nov. (Mo.na′shi.a. N.L. fem. n. *Monashia* from Monash University).

Aerobic, non-motile, non-spore-forming, Gram-stain-positive actinobacterium of irregular coccoid to short

Table 1. Differential characteristics between strain MUSC 78^T and genera of the family *Intrasporangiaceae*

Taxa: 1, strain MUSC 78^T; 2, *Terrabacter* (data from Collins *et al.*, 1989, unless otherwise indicated); 3, *Intrasporangium* (Schumann *et al.*, 1997); 4, *Humibacillus* (Kageyama *et al.*, 2008a); 5, *Janibacter* (Martin *et al.*, 1997, unless otherwise indicated); 6, *Terracoccus* (Prauser *et al.*, 1997); 7, *Kribbia* (Jung *et al.*, 2006); 8, *Phycicoccus* (Lee, 2006); 9, *Knoellia* (Groth *et al.*, 2002); 10, *Tetrasphaera* (Maszenan *et al.*, 2000); 11, *Lapillicoccus* (Lee & Lee, 2007); 12, *Arsenicicoccus* (Collins *et al.*, 2004); 13, *Ornithinibacter* (Xiao *et al.*, 2011), 14, *Ornithinicoccus* (Groth *et al.*, 1999); 15, *Ornithinimicrobium* (Groth *et al.*, 2001); 16; *Serinicoccus* (Yi *et al.*, 2004); 17, *Fodinibacter* (Wang *et al.*, 2009); 18, *Marihabitans* (Kageyama *et al.*, 2008b); 19, *Oryzihumus* (Kageyama *et al.*, 2005). ND, No data available.

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
Cell morphology	Irregular rods to cocci	Irregular rods	Hyphae	Irregular rods	Coccoid to rod-shaped	Cocci	Irregular short rods or cocci	Cocci	Coccoid to rod-shaped	Cocci	Cocci	Cocci	Branching hyphal forms	Cocci	Irregular and cocci	Cocci	Irregular short rods	Irregular short rods and cocci	Irregular rods
Wall diamino acid	LL-DAP	LL-DAP	LL-DAP	LL-DAP	*meso*-DAP	LL-DAP	*meso*-DAP	*meso*-DAP	*meso*-DAP	*meso*-DAP	LL-DAP	LL-DAP	L-Orn	L-Orn	L-Orn	L-Orn	*meso*-DAP	*meso*-DAP	*meso*-DAP
DNA G+C content (mol%)	73.1	71–73	68	69–70	70	73	69–70	74	68–69	68–71	74.1	72	69.6	72	70–71	72	72	70	72–73
Major menaquinones(s)	MK-8(H_4)	MK-8(H_4)	MK-8	MK-8(H_4)	MK-8(H_4)	MK-8(H_4)	MK-8(H_4)	MK-8(H_4)	MK-8(H_4)	MK-8(H_4)	MK-8(H_4)	MK-8(H_4)	MK-8(H_4)	MK-8(H_4)	MK-8(H_4)	MK-8(H_4)	MK-8(H_4)	MK-8(H_4)	MK-8(H_4)
Major fatty acids*	i-$C_{15:0}$, ai-$C_{15:0}$, i-$C_{16:0}$	i-$C_{15:0}$, i-$C_{14:0}$, i-$C_{16:0}$†	i-$C_{15:0}$, ai-$C_{15:0}$, i-$C_{16:0}$	i-$C_{15:0}$, iso-$C_{16:0}$, i-$C_{14:0}$, $C_{17:0}\omega8c$	i-$C_{16:0}$, $C_{17:1}\omega8c$, $C_{18:1}\omega9c$, $C_{17:0}$‡	i-$C_{15:0}$, ai-$C_{15:0}$, $C_{16:0}$	10-Me $C_{18:0}$, i-$C_{16:0}$, $C_{18:1}\omega9c$, $C_{16:0}$, $C_{18:0}$	$C_{17:1}\omega8c$, i-$C_{16:0}$, i-$C_{15:0}$	i-$C_{15:0}$, i-$C_{17:0}$, i-$C_{16:0}$, ai-$C_{17:0}$	i-$C_{16:0}$, ai-$C_{17:0}$, i-$C_{16:1}$, i-$C_{15:0}$, $C_{16:1}$, i-$C_{14:0}$, i-$C_{16:0}$	i-$C_{16:0}$, $C_{17:1}\omega8c$, i-$C_{15:0}$	i-$C_{16:0}$, $C_{18:1}\omega9c$, i-$C_{15:0}$, ai-$C_{15:0}$	i-$C_{18:1}\omega9c$, i-$C_{16:0}$, i-$C_{15:0}$, $C_{17:0}$	i-$C_{15:0}$, ai-$C_{15:0}$, i-$C_{17:1}$, $C_{15:0}$	i-$C_{16:0}$, i-$C_{15:0}$, ai-$C_{15:0}$	i-$C_{15:0}$, i-$C_{16:0}$, i-$C_{17:1}$, ai-$C_{15:0}$, ai-$C_{17:0}$	$C_{18:1}\omega9c$, $C_{16:0}$	i-$C_{17:0}$, $C_{17:1}\omega8c$, $C_{18:1}\omega9c$	i-$C_{16:0}$, i-$C_{15:0}$, i-$C_{14:0}$
Polar lipid(s)§	DPG, PE, PG, PGL, PI	DPG, PE, PI, GL	ND	PE	DPG, PG, PI	DPG, PG, PI, PL	ND	DPG, PE, PI	DPG, PG, PE	DPG, PG, PI, APL, PL	ND	ND	DPG, PG, PE, PI, GL	DPG, PG, PI, PS, PL	DPG, PG, PI, PL, GL	DPG, PG, PI, GL	DPG, PE, PG, PI, 2PL	DPG, PG, PL, PIMs	DPG, PG, PGL, PL

*Major fatty acids are defined as constituting >10 % of the total fatty acid content: i, iso ; ai, anteiso; Me, methyl.

†Data from Montero-Barrientos *et al.* (2005).

‡Data from Kämpfer *et al.* (2006).

§APL, unknown aminophospholipid; DPG, diphosphatidylglycerol; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PIMs, phosphatidylinositol mannosides; PL, unknown phospholipid; PS, phosphatidylserine; PGL, unknown phosphoglycolipid; GL, unknown glycolipid.

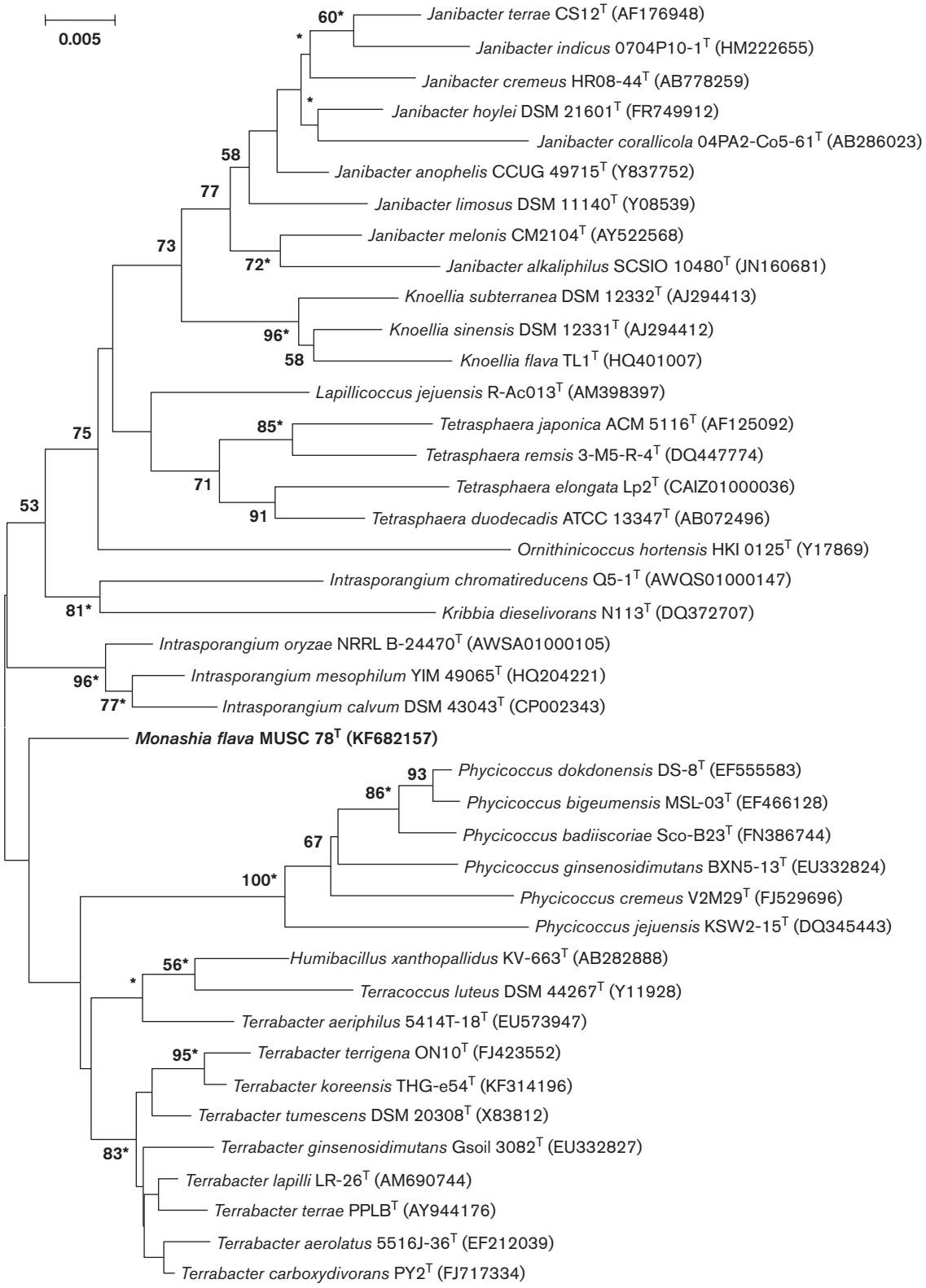

Fig. 2. Phylogenetic neighbour-joining tree (Saitou & Nei, 1987) based on 16S rRNA gene sequences of strain MUSC 78^T and representatives of the family *Intrasporangiaceae*. Asterisks indicate that the corresponding nodes were also recovered using the maximum-likelihood tree-making algorithm. Bootstrap values (based on 1000 replicates) are shown as percentages at each node for values above 50 %. Bar, 0.005 substitutions per nucleotide position.

rod-shaped cells. The predominant menaquinone is MK-8(H_4). The polar lipids are phosphatidylglycerol, phosphatidylinositol, phosphatidylethanolamine, diphosphatidylglycerol and phosphoglycolipid. The major cellular fatty acids are iso-$C_{15:0}$, anteiso-$C_{15:0}$ and iso-$C_{16:0}$. The peptidoglycan contains LL-DAP as the diagnostic diamino acid and the peptidoglycan type is A3γ. The cell-wall sugars are mannose and (at lower amounts) galactose and rhamnose.

Description of *Monashia flava* sp. nov.

Monashia flava (fla′va. l. fem. adj. *flava* yellow, referring to the colour of the colonies).

Has the following properties in addition to those given for the genus. Cells form yellowish-white-pigmented colonies on ISP 2 medium and AIA. Good growth is observed on R2A agar medium, ISP 2 medium, ISP 7 medium, Luria-Bertani agar, nutrient agar and SA after 7 days at 28 °C, while cells grow moderately well on AIA and SCA. Using TSB (without NaCl), the NaCl tolerance, temperature and pH ranges for growth are 0–4 %, 24–36 °C and pH 6.0–8.0 and optimal growth occurs at 0–2 % NaCl, 28–32 °C and pH 7.0. Cells are positive for catalase and haemolytic activities. Positive for hydrolysis of chitin, soluble starch, casein and CM-cellulose, but negative for hydrolysis of tributyrin (lipase) and xylan. The following compounds are utilized as sole carbon sources: dextrin, maltose, trehalose, cellobiose, gentiobiose, sucrose, turanose, stachyose, raffinose, α-lactose, methyl β-D-glucoside, D-salicin, *N*-acetyl-D-glucosamine, *N*-acetyl-β-D-mannosamine, *N*-acetyl-D-galactosamine, *N*-acetyl-neuraminic acid, α-D-glucose, D-mannose, D-fructose, D-galactose, L-fucose, L-rhamnose, inosine, D-sorbitol, D-arabitol, D-glucose 6-phosphate, D-fructose 6-phosphate, gelatin, glycyl L-proline, D-gluconic acid, D-glucuronic acid, D-galacturonic acid, glucuronamide, mucic acid, quinic acid, D-saccharic acid, *p*-hydroxyphenylacetic acid, methyl pyruvate, D-lactic acid methyl ester, citric acid, D-malic acid, L-malic acid, bromosuccinic acid, Tween 40, γ-aminobutyric acid, α-hydroxybutyric acid, α-ketobutyric acid, acetoacetic acid, acetic acid and formic acid. The following compounds are not utilized as sole carbon sources: melibiose, 3-methyl glucose, D-fucose, D-mannitol, *myo*-inositol, glycerol, D-aspartic acid, D-serine, pectin, L-galactonic acid lactone, L-lactic acid, α-ketoglutaric acid, β-hydroxy-DL-butyric acid and propionic acid. Sole nitrogen sources such as L-alanine, L-arginine, L-aspartic acid, L-histidine, L-pyroglutamic acid and L-serine are utilized. L-Glutamic acid is not utilized as sole nitrogen source. In chemical sensitivity assays, cells are resistant towards chemicals including fusidic acid, D-serine, troleandomycin, minocycline, lincomycin, guanine HCl, niaproof 4, vancomycin, tetrazolium violet, tetrazolium blue and sodium bromate, 1 % sodium lactate, rifamycin RV, nalidixic acid, lithium chloride, potassium tellurite, aztreonam and sodium butyrate.

The type strain is MUSC 78^T (=DSM 29621^T=MCCC 1K00454^T=NBRC 110749^T), which was isolated from mangrove soil collected from the Kuantan, the city of Pahang State in Peninsular Malaysia. The G+C content of the genomic DNA of the type strain is 73.1 mol%.

Acknowledgements

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