Mumia flava gen. nov., sp. nov., an actinobacterium of the family Nocardioidaceae

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A novel actinobacterial strain, designated MUSC 201T, was isolated from a mangrove soil collected from Kuantan, the capital city of Pahang State in Malaysia. The taxonomic status of this strain was determined using a polyphasic approach. Comparative 16S rRNA gene sequence analysis revealed that strain MUSC 201T represented a novel lineage within the class Actinobacteria. Strain MUSC 201T formed a distinct clade in the family Nocardioidaceae and was most closely related to the members of the genera Nocardioides (16S rRNA gene sequence similarity, 91.9–95.1%), Aeromicrobium (92.7–94.6%), Marmoricola (92.5–93.1%) and Kribbella (91.5–92.4%). The cells of this strain were irregular coccoid to short rod shaped. The peptidoglycan contained Ll-diaminopimelic acid as diagnostic diamino acid and the peptidoglycan type was A3γ. The peptidoglycan cell wall contained Ll-diaminopimelic acid, glycine, glutamic acid and alanine in a molar ratio of 1.5:0.9:1.0:1.5. The cell-wall sugars were galactose and rhamnose. The predominant menaquinone was MK-9(H4). The polar lipids consisted of diphosphatidylglycerol, phosphatidylglycerol, phosphoglycolipid, glycolipid and four unknown phospholipids. The major cellular fatty acids were C18:1ω9c (30.8%), C16:0 (24.1%), and 10-methyl C18:0 (13.9%). The DNA G+C content was 72.0±0.1 mol%. On the basis of phylogenetic and phenotypic differences from members of the genera of the family Nocardioidaceae, a novel genus and species, Mumia flava gen. nov., sp. nov. are proposed. The type strain of Mumia flava is MUSC 201T (DSM 27763T=MCCC 1A00646T=NBRC 109973T).

The family Nocardioidaceae was first proposed by Nesterenko et al. (1985) and the name was validly published in 1990 (Nesterenko et al., 1990). The description of the family was revised by Zhi et al. (2009). At the time of writing, the family Nocardioidaceae comprises seven genera: Nocardioides (Prauser, 1976), Aeromicrobium (Miller et al., 1991), Kribbella (Park et al., 1999; Sohn et al., 2003), Marmoricola (Urzı`, 2000), Actinopolymorpha (Wang et al., 2001), Thermasporomyces (Yabe et al., 2011) and Flindersiella (Kaewkla & Franco, 2011). The present investigation was designed to determine the taxonomic status of a novel actinobacterial strain belonging to the family Nocardioidaceae, designated MUSC 201T, which was isolated from a mangrove soil sample collected from Kuantan, the capital city of Pahang State, Peninsula of Malaysia. In order to determine the taxonomic and phylogenetic position of strain MUSC 201T, the morphology, physiological and biochemical characteristics, chemotaxonomic markers and 16S rRNA gene sequence of the novel strain were examined and analysed. The results indicated that strain MUSC 201T represented a novel species of a new genus, for which the name Mumia flava gen. nov., sp. nov. is proposed.

A soil sample was collected in December 2012. Topsoil samples of the upper 20 cm layer (after removing the top 2–3 cm) were collected and sampled into sterile plastic bags using an aseptic metal trowel, and stored at −20°C.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain MUSC 201T is KC907394.

One supplementary figure and one supplementary table are available with the online version of this paper.

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Five grams of air-dried soil sas mix with 45 ml sterilized water and mill-ground, then spread onto selective isolation medium; yeast malt agar [International Streptomyces Project (ISP) 2 medium; Shirling & Gottlieb, 1966] supplemented with cycloheximide (25 μg ml⁻¹) and nystatin (10 μg ml⁻¹) and incubated at 28 °C for 7 days. The strain MUSC 201ᵀ was maintained on ISP2 medium at 28 °C and as glycerol suspensions (20 %, v/v) at −20 °C.

Cultural characteristics of strain MUSC 201ᵀ were determined following growth on ISP2 and ISP 7 media (Shirling & Gottlieb, 1966), starch casein agar (SCA; Kuster & Williams 1964), Streptomyces agar (SA; Atlas 1993), actinomycete 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 (JSM 6400, JEOL) were used to observe the morphologies of strains after incubation on ISP2 medium at 28 °C for 7 days. Gram staining was performed by the standard Gram reaction and was confirmed by using KOH lysis (Cerny, 1978). The growth temperature was tested at 12–52 °C at intervals of 4 °C on ISP2 medium. NaCl tolerance was tested using tryptic soy broth (TSB) (casein, 17 g; soybean meal, 3 g; dextrose, 2.5 g; dipotassium hydrogen phosphate, 2.5 g; distilled water, 1 L; pH 7.3) and salt concentrations ranging from 0–18 % (w/v) at intervals of 2 %. The pH range for growth was tested between pH 4.0 and 10.0 at intervals of 1 pH unit. Carbon-source utilization and chemical sensitivity assays were determined using Biolog GenIII MicroPlates (Microbial Identification) system (Sasser, 1990).

Genomic DNA extractions, PCR amplification and sequencing of the 16S rRNA gene of strain MUSC 201ᵀ were carried out as described by Hong et al. (2009). The 16S rRNA gene sequence of strain MUSC 201ᵀ was aligned manually with sequences from the most closely related genera classified in the family Nocardioidaceae that had been retrieved from the GenBank/EMBL/DDBJ databases using MEGA version 5.2 (Tamura et al., 2011). Calculations of sequence similarity level 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). Terrabacter tumescens DSM 20308ᵀ was used as an outgroup. The genomic DNA of strain MUSC 201ᵀ for the determination of G + C content was extracted according to the method of Cashion et al. (1977). The G + C content of the DNA was determined by HPLC (Mesbah et al., 1989).

An almost complete 16S rRNA gene sequence was determined for strain MUSC 201ᵀ (1486 bp). A phylogenetic tree was reconstructed based on the 16S rRNA gene sequences (Fig. 1). The comparative 16S rRNA gene sequence analysis showed that strain MUSC 201ᵀ fell within the evolutionary radiation occupied by the family Nocardioidaceae (Fig. 1). The closest phylogenetic neighbours were members of the genera of the family Nocardioidaceae. Strain MUSC 201ᵀ showed 16S rRNA gene sequence similarities of 95.1, 94.8, 94.6, 93.1, 92.4, 90.1, 89.9, and 89.7 % to the type strains of Nocardioides panacisoli GSoil 346ᵀ, Nocardioides aquiterra GW-9ᵀ, Aeroc Microbium erythreum NRRL B-3381ᵀ, Marmoricola aurantiaca BC 361ᵀ, Kribbella flavida DSM 17836ᵀ, Actinopolymorpha singaporensis IM 7744ᵀ, Flindersiella

Biomass for molecular systematic studies and freeze-dried cells for chemotaxonomic studies were obtained after growing cells in TSB at 28 °C for 7 days on a rotary shaker. The analysis of peptidoglycan amino-acid composition and sugars of strain MUSC 201ᵀ was carried out by the Identification Service of the DSMZ, Braunschweig, Germany. The analyses were carried out according to published protocols (Schumann, 2011). Major diagnostic whole-organism sugars of strain MUSC 201ᵀ were obtained following a 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 analysis of strain MUSC 201ᵀ and closely related type strains was carried out by the Identification Service of the DSMZ. The cell mass of strain MUSC 201ᵀ 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).
Fig. 1. Neighbour-joining tree based on an almost complete 16S rRNA sequence (1486 nt) showing the relationship between strain MUSC201\(^T\) and representatives of the family Nocardioidaceae. Bootstrap values (>50%) based on 1000 resampled datasets are shown at branch nodes. Bar, 5 substitutions per 1000 nucleotide positions. Asterisks indicate that the corresponding nodes were also recovered using maximum-likelihood and maximum-parsimony tree-making algorithms.
endophytica EUM 378\textsuperscript{T} and Thermasporomyces composti I3\textsuperscript{T}, respectively. Strain MUSC 201\textsuperscript{T} formed a distinct clade from the type strains of the genus Nocardioides at a low nucleotide sequence similarity (91.9–95.1\%); this association was supported by all of the different tree-making algorithms used in this study.

The total hydrolysate (4 M HCl, 100 °C, 16 h) of peptidoglycan of strain MUSC 201\textsuperscript{T} contained ll-diaminopimelic acid, glycine, glutamic acid and alanine in a molar ratio of 1.5:0.9:1.0:1.5. The partial hydrolysate (4 M HCl, 100 °C, 45 min) contained peptides l-Ala-d-D-Glu, Glu-d-Ala, ll-Dpm-d-Ala and ll-Dpm-Gly (Schleifer & Kandler, 1972; Schleifer, 1985). From these analytical data, it was concluded that strain MUSC 201\textsuperscript{T} contained the peptidoglycan type A3\textsubscript{γ}, ll-Dpm-Gly. The cell-wall sugars were galactose and rhamnose. The menaquinones detected were MK-9(H\textsubscript{4}) (89\%), MK-9 (1\%), MK-8(H\textsubscript{4}) (1\%), MK-9(H\textsubscript{2}) (1\%), MK-9(H\textsubscript{4}) (1\%) and MK-10(H\textsubscript{4}) (traces). The polar lipids were diphosphatidylglycerol, phosphatidylglycerol, phosphoglycolipid, glycolipid and four unknown phospholipids (Fig. S1, available in the online Supplementary Material). The major cellular fatty acids (>5\%) were C\textsubscript{18:1}\textsuperscript{v\textsubscript{9c}} (30.8\%), C\textsubscript{16:0} (24.1\%), 10-methyl C\textsubscript{18:0} (13.9\%), C\textsubscript{16:0} 2-OH (7.6\%), C\textsubscript{18:0} (5.5\%) and C\textsubscript{17:0} (5.4\%) (Table S1). The G + C content of the DNA was 72.0 ± 0.1 mol\%, as determined by HPLC analysis.

Differential chemotaxonomic characteristics between strain MUSC 201\textsuperscript{T} and other genera belonging to the family Nocardioidaceae are summarized in Table 1.

Cells were Gram-stain-positive, non-motile, aerobic, non-spore-forming and irregular cocci or rod-shaped (Fig. 2). Cells could occur singly, in pairs, in short chains or in small irregular clusters. Good growth was observed on ISP2 medium and nutrient agar after 7 days at 28 °C; cells grew moderately on SA, whereas cells grew poorly on AIA, SCA and Luria–Bertani agar. Colonies were yellowish white on most media tested. No aerial mycelia or diffusible pigments were observed on any of the media. Cells were positive for catalase but negative for haemolytic activity. Growth occurred at pH 5.0–10.0 (optimum pH 7.0–8.0), with 0–8\% NaCl (optimum 0–4\%) and at 20–36 °C (optimum 28–32 °C). Hydrolysis of soluble starch, CM-cellulose and chitin was positive, but hydrolysis of tributyrin (lipase), casein and xylan was negative. The morphological, cultural and physiological properties of strain MUSC 201\textsuperscript{T} are given in the genus and species descriptions. The organism can be distinguished from members of the family Nocardioidaceae using different chemotaxonomic characteristics (Table 1).

Strain MUSC 201\textsuperscript{T} was similar to members of the genera of the family Nocardioidaceae (Nocardioides, Aeromicrobium, Marmoricola, Kribbella, Actinopolymorpha, Flindersiella and Thermasporomyces), which contain ll-diaminopimelic acid as the diagnostic diamino acid. Based on the phylogenetic tree generated using the neighbour-joining algorithm, strain MUSC 201\textsuperscript{T} could also be assigned to the family Nocardioidaceae as it formed a distinct clade with the type strains of the genera Nocardioides, Aeromicrobium and Marmoricola (Fig. 1). The DNA G + C content of 72 ± 0.1 mol\% also fell within the range of DNA G + C contents within the family Nocardioidaceae that range from 69.2 to 73\% (Table 1). Strain MUSC 201\textsuperscript{T} was similar to members of genera such as Aeromicrobium, Kribbella and Thermasporomyces in containing the same predominant menaquinone MK-9(H\textsubscript{4}), but differed from members of genera such as Nocardioides and Marmoricola in that it contained MK-8(H\textsubscript{4}) as a minor menaquinone. Furthermore strain MUSC 201\textsuperscript{T} could be differentiated from members of the genus Nocardioides by many phylogenetic, chemotaxonomic and phenotypic properties (Table 1), e.g. strain MUSC 201\textsuperscript{T} had low 16S rRNA gene sequence similarities with members of the genus Nocardioides (91.9–95.1\%) and was separated from them by a long evolutionary distance in the phylogenetic tree (Fig. 1). Furthermore strain MUSC 201\textsuperscript{T} was significantly different from members of the genus Nocardioides and other genera of the family Nocardioidaceae in the fatty acid and polar lipid profiles, e.g. the polar lipid profile of strain MUSC 201\textsuperscript{T} contained a phosphoglycolipid and a glycolipid that was not detected in any of the other genera. For the fatty acids profile, strain MUSC 201\textsuperscript{T} was significantly different from

Table 1. Phenotypic and chemotaxonomic properties of strain MUSC201\textsuperscript{T} and members of the family Nocardioidaceae

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Major menaquinone</td>
<td>MK-9(H\textsubscript{4})</td>
<td>MK-8(H\textsubscript{4})</td>
<td>MK-9(H\textsubscript{4})</td>
<td>MK-8(H\textsubscript{4})</td>
</tr>
<tr>
<td>Major fatty acids</td>
<td>C\textsubscript{18:1}\textsuperscript{v\textsubscript{9c}}, C\textsubscript{16:0} 10-methyl C\textsubscript{18:0}</td>
<td>C\textsubscript{16:0} iso-C\textsubscript{16:0}</td>
<td>C\textsubscript{16:0} 2-OH, 10-methyl C\textsubscript{18:0}</td>
<td>C\textsubscript{16:0} C\textsubscript{18:1}\textsuperscript{v\textsubscript{9c}} (iso-C\textsubscript{16:0})*</td>
</tr>
<tr>
<td>Polar lipids</td>
<td>DPG, PG, PL, PGL, GL</td>
<td>PG</td>
<td>PG</td>
<td>PG</td>
</tr>
</tbody>
</table>

*Major fatty acid of Marmoricola bigeumensis MSL-05\textsuperscript{T} is C\textsubscript{16:0}.*
The polar lipids are diphosphatidylglycerol, phosphoglycolipid, glycolipid and four unknown phospholipids. The major cellular fatty acids are C<sub>18:1</sub>ω9c, C<sub>16:0</sub> and 10-methyl C<sub>18:0</sub>. The peptidoglycan contains L-L-diaminopimelic acid as diagnostic diamin acid and the peptidoglycan type is A<sub>3</sub>γ. The peptidoglycan cell wall contains L-L-diaminopimelic acid, glycine, glutamic acid and alanine. The cell-wall sugars are galactose and rhamnose.

Cells are positive for catalase and negative for haemolytic activities. Good growth is observed on ISP2 medium and nutrient agar. Colonies are yellowish white on most media tested. No aerial mycelia or diffusible pigments are observed on any of the media. Grows at pH 5.0–10.0 (optimum pH 7.0–8.0) with 0–8 % NaCl (optimum 0–4%) and at 20–36 °C (optimum 28–32 °C). Hydrolysis of soluble starch, CM-cellulose and chitin is positive; negative for hydrolysis of tributyrin (lipase), casein and xylan. With Biolog GEN III MicroPlates, the following compounds are utilized as sole carbon sources: dextrin, maltose, trehalose, cellobiose, gentiobiose, sucrose, turanose, melibiose, α-D-glucose, D-mannose, D-fructose, D-galactose, 3-methyl glucose, D-fucose, L-fucos, L-rhamnose, D-glucose 6-phosphate, D-fructose 6-phosphate, D-galacturonic acid, D-glucuronic acid, glucuronamid, L-lactic acid, citric acid, Tween 40, α-hydroxybutyric acid, hydroxyl β-DL-butiric acid, acetoacetic acid, propionic acid and acetic acid. The following compounds are not utilized as sole carbon sources: 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, inosine, D-sorbitol, D-mannitol, D-arabitol, myo-inositol, glycerol, D-aspartic acid, D-serine, gelatin, glycyl L-proline, pectin, L-galactonic acid lactone, D-gluconic acid, mucic acid, quinic acid, D-saccharic acid, p-hydroxyphenylactic acid, methyl pyruvate, L-lactic acid methyl ester, α-ketoglutaric acid, D-malic acid, L-malic acid, bromosuccinic acid, γ-aminobutyric acid, α-ketobutyric acid and formic acid. Sole nitrogen sources such as L-alanine, L-arginine, L-aspartic acid, L-glutamic acid, L-histidine, L- pyroglytamic acid and L-serine are not utilized. In chemical sensitivity assays, cells are sensitive towards 1 % sodium lactate, tetracyromycin, niaproof 4, vancomycin and sodium bromate, while cells are resistant to fusidic acid, D-serine, rifamycin RV, minocycline, lincomycin, guanine hydrochloride, tetrazolium violet, tetrazolium blue, hydroxybutyric acid, hydroxyl DL-butyric acid, 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, inosine, D-sorbitol, D-mannitol, D-arabitol, myo-inositol, glycerol, D-aspartic acid, D-serine, gelatin, glycyl L-proline, pectin, L-galactonic acid lactone, D-gluconic acid, mucic acid, quinic acid, D-saccharic acid, p-hydroxyphenylactic acid, methyl pyruvate, L-lactic acid methyl ester, α-ketoglutaric acid, D-malic acid, L-malic acid, bromosuccinic acid, γ-aminobutyric acid, α-ketobutyric acid and formic acid. Sole nitrogen sources such as L-alanine, L-arginine, L-aspartic acid, L-glutamic acid, L-histidine, L-pyroglytamic acid and L-serine are not utilized. In chemical sensitivity assays, cells are sensitive towards 1 % sodium lactate, tetracyromycin, niaproof 4, vancomycin and sodium bromate, while cells are resistant to fusidic acid, D-serine, rifamycin RV, minocycline, lincomycin, guanine hydrochloride, tetrazolium violet, tetrazolium blue, sodium lactate, tetracyromycin, niaproof 4, vancomycin and sodium bromate. Cells are resistant to (per disc) erythromycin (15 μg), but sensitive to ampicillin (10 μg), ampicillin sulbactam (30 μg), cefoxime (30 μg), cefuroxime (30 μg), cephalosporin (30 μg), chloramphenicol (30 μg), ciprofloxacin (10 μg), gentamicin (20 μg), nalidixic acid (30 μg), penicillin G (10 μg), streptomycin (10 μg), tetracycline (30 μg) and vancomycin (30 μg).

The type strain is MUSC 201T (=DSM 27763T=MCCC 1A00646T=NBRC 109977T), which was isolated from mangrove soil collected from Kuantan, the capital city of Pahang State in the Peninsular of Malaysia. The G+C content of the genomic DNA of strain MUSC 201T is 72 ± 0.1 mol%.}

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References


