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**Abstract**

A Gram-stain-positive actinobacterium, designated strain YIM DR4008T, was isolated from the root sample of *Psammosilene tunicoides* collected from Lijiang, Yunnan, China. Strain YIM DR4008T could grow at temperatures ranging from 10 to 50°C (optimum 28–30°C), at pH 5.0–11.0 (optimum pH 7.0) and in the presence of up to 4% (w/v) NaCl. Sequence analysis of the 16S ribosomal RNA gene revealed that strain YIM DR4008T shared highest similarity (95.0%) with *Streptomyces griseoplanus* NBRC 12779T and <95% similarity with other known members of the genera *Streptomyces*, *Kitasatospora* and *Streptacidiphilus*. The diagnostic cell-wall diamino acid of strain YIM DR4008T was found to be L-ll-diaminopimelic acid. The whole-cell hydrolysates contained a major amount of galactose and mannose along with a small proportion of fucose, glucose, rhamnose and ribose. The polar lipids consisted of diphosphatidylglycerol, phosphatidylinositol mannosides and three unidentified phospholipids. The respiratory menaquinones were MK-9(H4) and MK-9(H6), while the major cellular fatty acids (>10%) were anteiso-C15:0, C16:0, iso-C16:0, iso-C15:0 and anteiso-C17:0. The genomic DNA G+C content was determined to be 75.3 mol%. Based on the phenotypic, chemotaxonomic and molecular characteristics, strain YIM DR4008T is proposed to be recognized as a novel species of a new genus in the family *Streptomycetaceae*, with the name *Allostreptomyces psammosilenae* gen. nov., sp. nov. The type strain of the type species is YIM DR4008T (=DSM 42178T=CGMCC 4.7247T). An emended description of the family *Streptomycetaceae* is also provided.

The family *Streptomycetaceae* was established in 1943 by Waksman and Henrici to accommodate aerobic, Gram-positive, non-acid–alcohol-fast actinomycetes bearing extensively branched substrate mycelium that are rarely fragmented [1]. At the time of writing, the family consists of three genera namely *Streptomyces* [1], *Kitasatospora* [2] and *Streptacidiphilus* [3]. Members of this family are a hub for industrially important bioactive compounds [4], and have been isolated from various niches such as soil [5, 6], rhizosphere [7], forest soil [8], sand dune soil [9], lake [10] and marine sediment [11]. Of late, much emphasis has been given to isolate microbes residing within plant tissues due to the fact that they are a naturally rich source of genetic diversity and remain relatively unexplored [12]. During the last few years, our laboratory has been actively engaged in exploring novel endophytic bacteria including those belonging to the family *Streptomycetaceae* from economically important plants [13–15]. This paper reports a novel endophytic actinobacterium isolated from the roots of *Psammosilene tunicoides* collected from Lijiang, Yunnan, China. The strain showed phenotypic characters similar to those of the genus *Streptomyces*, however with low sequence similarities.

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**Keywords:** Allostreptomyces psammosilenae, gen. nov., sp. nov.; Psammosilene tunicoides; family Streptomycetaceae.

**Abbreviations:** ISP, International Streptomyces Project; ML, maximum-likelihood; MP, maximum-parsimony; NJ, neighbour-joining.

†These authors contributed equally to this work.

NOTE

The GenBank/EMBL/DDJB accession number for the 16S rRNA gene sequence of strain YIM DR4008T is KX689228.

Three supplementary figures and three supplementary tables are available with the online Supplementary Material.
The strain was therefore selected for detailed polyphasic characterization.

Isolation of actinobacteria from the roots of *Psammosilene tunicoides* was performed as described earlier [16, 17]. The surface-sterilized roots were then placed in a flask containing sterile silica gel and desiccated in an incubator at 30°C for 3 days. Dried plant roots were powdered in a sterilized blender and spread on YED medium [containing (g l⁻¹): yeast, 0.3; casein, 0.3; glucose, 0.3; bone meal, 0.3; agar 1.3; pH 7.2] supplemented with nalidixic acid (25 mg l⁻¹) and nystatin (50 mg l⁻¹). The isolation plates were incubated for 30 days at 28°C. Strain YIM DR4008 was purified on International *Streptomyces* Project (ISP) 2 medium [18] at 28°C. The purified strain was maintained on ISP 2 slants at 4°C and as glycerol suspensions (20 %, v/v) at −80°C. Biomass of strain YIM DR4008 for molecular and chemotaxonomic investigations was harvested from cultures grown in ISP 2 broth (28°C, 7 days). For all studies unless otherwise mentioned, ISP 2 agar was used as the basal growth medium with 28°C as the incubation temperature.

Genomic DNA and PCR amplification of the 16S rRNA gene of strain YIM DR4008 was performed as described by Li et al. [19]. Amplicons were cloned and sequenced by Sangon Biotech (Shanghai). The sequence obtained was compared with available 16S rRNA gene sequences of cultured species from the EzTaxon-e server (http://eztaxon-e.ezbiocloud.net/) [20]. Phylogenetic analysis was performed using the MEGA 5.0 software package [21] after multiple alignment of the sequences using the CLUSTAL X program [22]. Three tree-making algorithms, the neighbour-joining (NJ) [23], maximum-likelihood (ML) [24] and maximum-parsimony (MP) [25] methods, were used to generate phylogenetic dendograms. Phylogenetic distances in the neighbour-joining tree were calculated using Kimura's two-parameter model [26] with 1000 bootstrap replicates [27].

BLAST analysis of the almost-complete 16S rRNA gene sequence (1510 bp) of strain YIM DR4008 showed the novel isolate shared ≤95% sequence similarities with recognized members of the family *Streptomycetaceae*; the highest 16S rRNA gene sequence similarity being with that of strain *Streptomyces griseoplanus* NBRC 12779T (95.0%), while less than 95% with other species of the genus *Streptomyces* and members of the genera *Kitasatospora* and *Streptacidiphilus*. In the NJ phylogenetic dendrogram (Fig. 1), strain YIM DR4008 formed a separate lineage, distinct from related members of the three genera *Streptomyces*, *Kitasatospora* and *Streptacidiphilus* while forming a cluster within the family *Streptomycetaceae* of the class *Actinobacteria*. This finding indicates that this isolate could represent a novel genus within the family *Streptomycetaceae*. The stability of NJ tree was further confirmed by the ML and MP trees (Figs S1 and S2, available in the online Supplementary Material).

Chemotaxonomic characteristics of strain YIM DR4008 were determined following standard procedures. The isomer of diaminopimelic acid of the cell wall and sugars of whole-cell hydrolysates were analysed as described by Hasegawa et al. [28], Staneck and Roberts [29] and Tang et al. [30]. Polar lipids were extracted, separated by two-dimensional thin-layer chromatography (TLC) and identified using described procedures [31, 32]. Menaquinones were extracted from lyophilized cells [33, 34] and analysed by HPLC [35, 36]. For analysis of cellular fatty acids, strain YIM DR4008 was cultured in ISP 2 medium for 5 days at 28°C. The cellular fatty acids were extracted, methylated and analysed by using the protocol of the Sherlock Microbial Identification System (MIDI) (Sherlock Version 6.1; MIDI database: TSBA6) [37]. The G+C content of the genomic DNA was determined by HPLC [38] using *Escherichia coli* JM-109 as the reference strain.

Strain YIM DR4008 was found to have LL-diaminopimelic acid as the diagnostic cell-wall diamino acid, while galactose and mannose were detected as major sugars in whole-cell hydrolysates along with a small amount of fucose, glucose, rhamnose and ribose. The polar lipids detected comprised diphosphatidyglycerol, phosphatidylglycinol mannosides and three unidentified phospholipids (Fig. S3). The respiratory menaquinones of strain YIM DR4008 were MK-9(H₄) and MK-9(H₈). The major fatty acid methyl ester profile (>10 %) contained anteiso-C₁₅:₀ (34.5 %), C₁₆:₀ (15.6 %), iso-C₁₆:₀ (13.2 %), iso-C₁₅:₀ (12.6 %) and anteiso-C₁₇:₀ (10.3 %). The detailed fatty acid profile of strain YIM DR4008 was listed in Table S1. The genomic DNA G+C content of the strain YIM DR4008 was determined to be 75.3 mol%.

The Gram reaction was determined by Solarbio’s Gram staining kit as per the manufacturer’s instructions. The morphology of spores and mycelia of strain YIM DR4008 were observed under a scanning electron microscope (ESEM; XL30 ESEM TMP, Philips) after growth for 10 days on ISP 2 agar. Cultural characteristics were observed on ISP 2, ISP 3, ISP 4 and ISP 5 [18], potato-dextrose agar (PDA), Czapek’s and nutrient agar (NA) [39]. Colony colours were determined by using the ISCC-NBS colour charts [40]. Growth at different temperatures (4, 10, 15, 20, 28, 30, 37, 40, 45, 50, 55 and 60°C) and in the presence of NaCl (0–12 %, w/v, at intervals of 1%) was examined on ISP 2 agar plates. The pH range for growth (pH 4.0–12.0, at intervals of 1 pH unit maintained by using the buffer system as described by Xu et al. [41]) was assessed in ISP 2 broth. Catalase, oxidase, urease, gelatin liquefaction, milk peptonization and coagulation, nitrate reduction, H₂S production, and hydrolysis of cellulose, starch and Tween 20, 40, 60 and 80 were investigated according to the procedures described by Gordon et al. [42] and Williams et al. [43]. Carbon-source utilization tests were performed according to the methods described by Shirling and Gottlieb [18] and Athalye et al. [44] in the modified basal medium recommended by Pridham et al. [45]. Utilization of nitrogen sources was observed according to Nie et al. [46]. The antibiotic susceptibility test was performed using antibiotic discs containing (µg per disc, unless indicated): amikacin (30), cefuroxime sodium (30),
Strain YIM DR4008 was evaluated for antimicrobial activity against a set of bacterial and fungal test pathogens including *Escherichia coli*, *Pseudomonas aeruginosa*, *Alternaria alternata*, *Alternaria brassicaceae*, *Candida albicans* and *Colletotrichum micotianae* by dual-culture antagonistic bioassay method as described below. Sterile discs (8 mm diameter) were impregnated with cultures of strain YIM DR4008 grown on ISP 2 agar (5 days, 28°C). The culture discs were then placed at the centre of test plates previously spread with the test pathogens, incubated at 28°C for 5 days and observed for inhibition zone around the discs. The assay was done on PDA plates for *Alternaria alternata*, *Alternaria brassicaceae*, *Candida albicans*, *Colletotrichum micotianae*, and LB agar for *Escherichia coli*. All the assays were performed in triplicate.

Strain YIM DR4008 was observed to Gram-stain-positive. It grew well on ISP 2, ISP 4, Czapek’s and PDA, moderately on ISP 3 and ISP 5, and weakly on NA. The strain formed extensively branched substrate and aerial mycelia. Colours of the substrate mycelium varied from deep orange yellow to black, while aerial mycelia had white or light yellow green colours on tested media (Table S2). Aerial hyphae carried straight chains of smooth surfaced spores (0.55–0.75 × 0.9–1.2 µm, Fig. 2). Interestingly, no aerial mycelium was observed on ISP 3 and NA media. Diffusible pigments were produced on all tested media and colours ranged from brilliant greenish yellow to brilliant orange, strong yellowish brown, greyish brown and brownish black (Table S2). Growth was observed at 10–50°C (optimum 23–30°C), pH 5.0–11.0 (optimum pH 7.0) and in the presence of up to 4% (w/v) NaCl (optimum 1%). The strain was positive for catalase, oxidase, nitrate reduction, and milk coagulation and peptonization, but negative for H₂S production and urease activity. Strain YIM DR4008 was able to hydrolyse cellulose, gelatin, starch and Tweens 40, 60 and 80, but not Tween 20. Detailed physiological and biochemical characteristics of strain YIM DR4008 are listed in Table S3 or given in the species description. Strain YIM DR4008 was susceptible to amikacin, cefuroxime sodium, chloramphenicol, ciprofloxacin, erythromycin, gentamicin, novobiocin, penicillin, piperacillin, polymyxin B and tetracycline while resistant to ethylhydrocupreine, norfloxacin, oxacillin, chloramphenicol (30), ciprofloxacin (5), erythromycin (15), ethylhydrocupreine (5), gentamicin (10), norfloxacin (10), novobiocin (30), oxacillin (1), penicillin (10 IU), piperacillin (100), polymyxin B (300 IU) sulfamethoxazole (300), tetracycline (30) and vancomycin (30). Formation of halo zones around the discs was evaluated for antimi-
sulfamethoxazole and vancomycin. The strain exhibited antagonistic activity against Escherichia coli, Staphylococcus aureus, Alternaria brassicae and Colletotrichum micotianae, but not against Alternaria alternata, Candida albicans or Pseudomonas aeruginosa.

Besides low sequence similarities, strain YIM DR4008^T is determined to represent a separate lineage within the family Streptomycetaceae in the phylogenetic dendrograms (Figs 1, S1 and S2). A comparative analysis of the 16S rRNA signature nucleotides demonstrated that strain YIM DR4008^T contained the signature nucleotides defined for the family Streptomycetaceae, 234 (C), 449 (A), 672:734 (C–G), 950:1231 (U–G), 952:1229 (U–A), 955:1225 (C–G), 965 (C), 986:1219 (A–U) and 1362 (C), except for 127 (G) [47, 48]. The position 127 being inconsistent even among the other members of the family as given below in the emended description of the family. In addition, strain YIM DR4008^T possesses a 16S rRNA nucleotide different from other members of the family Streptomycetaceae (Streptomyces, Kitasatospora and Streptacidiphilus) at the positions 443:825 (U–G), 849 (C), 875:1004 (C–U), 1029:1031 (U–C), 1039:1122 [(vacant)–A], 1151:1231 (U–A), and 1278 (U), indicating that the isolate is a possible representation of a novel genus within the family Streptomycetaceae. The respiratory menaquinones of the novel strain are consistent with those reported for the members of the family Streptomycetaceae, but the novel strain differs from the other three genera in having galactose and mannose as the major whole-cell sugars. Phosphatidylethanolamine and phosphatidylglycerol are not present as predominant phospholipids in the novel isolate unlike members of the other three genera. Comparative chemotaxonomic, morphological and physiological characteristics of strain YIM DR4008^T along with those reported for the genera Streptomyces, Kitasatospora and Streptacidiphilus are given in Table 1. Based on the above observations, strain YIM DR4008^T merits recognition as a novel species of a new genus within the family Streptomycetaceae, for which the name Allostreptomyces psammosilenae gen. nov., sp. nov. is proposed.

**DESCRIPTION OF ALLOSTREPTOMYCES GEN. NOV.**

Allostreptomyces (Al.lo.strep.to.my'ces. Gr. adj. allos another, the other; N.L. masc. n. Streptomyces an actinobacterial genus name; N.L. masc. n. Allostreptomyces the other Streptomyces referring to the fact that the genus is phylogenetically close to Streptomyces).

Gram-stain-positive, catalase- and oxidase-positive. Forms extensively branched substrate and aerial mycelia. The diagnostic diaminopimelic acid of cell wall is L-l-diaminopimelic acid. Galactose is the diagnostic whole-cell sugar, although galactose may be present in a major amount. The polar lipids comprise diphosphatidylglycerol, phosphatidylinositol mannosides and three unidentified phospholipids. The respiratory menaquinones are MK-9(H_4) and MK-9(H_8). The major cellular fatty acids (>10%) are anteiso-C_{15:0}, C_{16:0}, iso-C_{16:0}, iso-C_{15:0} and anteiso-C_{17:0}.

The type species is Allostreptomyces psammosilenae.

**DESCRIPTION OF ALLOSTREPTOMYCES PSAMMOSILENAE SP. NOV.**

Allostreptomyces psammosilenae (psam.mo.si.le'nae. N.L. gen. n. psammosilenae of the plant Psammosilene tunicoides).

The species contains the following characteristics in addition to those listed for the genus. Aerial hyphae carry straight or spiral chains of smooth-surfaced spores (0.55–0.75×0.9–1.2 μm). Growth occurs at 10–50°C, pH 5.0–11.0 and in the presence of up to 4% (w/v) NaCl. Utilizes
**Table 1.** Morphological, physiological and chemotaxonomic characteristics of strain YIM DR4008T and members of the genera classified in the family Streptomyctaceae

Genera: 1, *Allostreptomyces* gen. nov. (strain YIM DR4008); 2, *Streptomyces*; 3, *Kitasatospora*; 4, *Streptacidiphilus*. Data for taxa 2–4 (except for signature nucleotides) are from Kim et al. [3]. All taxa contained long chains of spores formed on aerial hyphae, and MK-9(H8) as major menaquinones.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
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<td>pH range for growth</td>
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<td>5.0–11.5</td>
<td>5.5–9.0</td>
<td>3.5–6.0</td>
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<tr>
<td>Diaminopimelic acid isomer(s) in cell wall</td>
<td>Ll-DAP</td>
<td>Ll-DAP</td>
<td>Ll- and meso-DAP</td>
<td>Ll-DAP</td>
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<tr>
<td>Diagnostic sugars in whole-cell hydrolysates</td>
<td>Galactose</td>
<td>None</td>
<td>Galactose</td>
<td>Galactose and rhannose</td>
</tr>
<tr>
<td>Predominant phospholipids*</td>
<td>DPG, PIMs</td>
<td>DPG, PE, PI, PIMs</td>
<td>DPG, PE, PI, PIMs</td>
<td>DPG, PE, PI, PIMs</td>
</tr>
<tr>
<td>DNA G+C content (mol%)</td>
<td>75.3</td>
<td>66–73</td>
<td>70–74</td>
<td>70–72</td>
</tr>
<tr>
<td>16S rRNA signature nucleotides</td>
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<td>C</td>
<td>C</td>
</tr>
<tr>
<td></td>
<td>825</td>
<td>G</td>
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<td>A</td>
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<tr>
<td></td>
<td>849</td>
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<td>C</td>
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<td></td>
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<td>U</td>
<td>G</td>
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<td></td>
<td>1122:1231</td>
<td>A</td>
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</table>

*DPG, diphostidylglycerol; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PIMs, phosphatidylinositol mannosides.

The type strain YIM DR4008T (=DSM 42178=CGMCC 4.7247T) was isolated from the roots of *Psammisilene tuniroides*. The DNA G+C content of the type strain is 75.3 mol%.


The pattern of 16S rRNA signatures consist of nucleotides at position 234 (C), 449 (A), 672:734 (C-G), 950:1231 (U-G), 952:1229 (U-A), 955:1225 (C-G), 965 (C), 986:1219 (A-U) and 1362 (C). Whole-organism sugar profiles may contain major amounts of galactose (*Kitasatospora*), galactose and rhannose (*Streptacidiphilus*) or galactose and mannoce (*Allostreptomyces*). The G+C content of the DNA is generally between 66 and 75 mol%.

The type genus is *Streptomyces* Waksman and Henrici (1943)T.

**Funding information**
This research was supported by Natural Science Foundation of China (No. 31670009). W.-J. Li is supported by a project funded by Guangdong Province Higher Vocational Colleges & Schools Pearl River Scholar Funded Scheme (2014).

**Acknowledgements**
The authors are grateful to Professor Aharon Oren, the Hebrew University of Jerusalem, for suggesting the Latin name of the novel strain.

**Conflicts of interest**
The authors declare that there are no conflicts of interest.

**References**


