**Abstract**

A Gram-stain-positive actinobacterium, designated strain YIM DR4008\(^{T}\), was isolated from the root sample of *Psammosilene tunicoides* collected from Lijiang, Yunnan, China. Strain YIM DR4008\(^{T}\) 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 DR4008\(^{T}\) shared highest similarity (95.0%) with *Streptomyces griseoplanus* NBRC 12779\(^{T}\) and <95% similarity with other known members of the genera *Streptomyces*, *Kitasatospora* and *Streptacidiphilus*. The diagnostic cell-wall diamino acid of strain YIM DR4008\(^{T}\) was found to be \(\pm\)-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-C\(_{15:0}\), C\(_{16:0}\), iso-C\(_{16:0}\),iso-C\(_{15:0}\) and anteiso-C\(_{17:0}\). The genomic DNA G+C content was determined to be 75.3 mol%. Based on the phenotypic, chemotaxonomic and molecular characteristics, strain YIM DR4008\(^{T}\) 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 DR4008\(^{T}\) (=DSM 42178\(^{T}\)=CGMCC 4,7247\(^{T}\)). An emended description of the family *Streptomycetaceae* is also provided.
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 YECD 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 DR4008T 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 DR4008T 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 DR4008T 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 dendrograms. 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 DR4008T 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 DR4008T 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 DR4008T were determined following standard procedures. The isomer of dianaminopimelic acid of the cell wall and sugars of whole-cell hydrolysates were analysed as described by Hasegawa *et al.* [28], Stanek 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 DR4008T 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 DR4008T was found to have l-l-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 diphosphatidylglycerol, phosphatidylinositol mannosides and three unidentified phospholipids (Fig. S3). The respiratory menaquinones of strain YIM DR4008T were MK-9(H₄) and MK-9(H₄). The major fatty acid methyl ester profile (>10%) contained anteiso-C₁₅:0 (34.5%), C₁₆:0 (15.6%), iso-C₁₆:0 (13.2%), iso-C₁₇:0 (12.6%) and anteiso-C₁₇:0 (10.3%). The detailed fatty acid profile of strain YIM DR4008T is listed in Table S1. The genomic DNA G+C content of the strain YIM DR4008T 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 DR4008T 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, 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),
chloramphenicol (30), ciprofloxacin (5), erythromycin (15),
elthyldihydrocupreine (5), gentamicin (10), norfloxacin (10),
novobiocin (30), oxacillin (1), penicillin (10 IU), piperacillin
(100), polymyxin B (300 IU) sulfamethoxazole (300), tetra-
cycline (30) and vancomycin (30). Formation of halo zones
around the discs was considered sensitive. Strain YIM DR4008
was resistant to ethylhydrocupreine, norfloxacin, oxacillin,
penicillin, piperacillin, polymyxin B and tetracycline while
susceptible to amikacin, cefuroxime sodium, chlorampheni-
col, ciprofloxacin, erythromycin, gentamicin, novobiocin,
penicillin, piperacillin, polymyxin B and tetracycline while
resistant to ethylhydrocupreine, norfloxacin, oxacillin,

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 (0.55–1.2 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 bril-
liant greenish yellow to brilliant orange, strong yellowish
brown, greyish brown and brownish black (Table S2).
Growth was observed at 10–50 °C (optimum 28–30 °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 H₂O₂ 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 charac-
teristics of strain YIM DR4008 are listed in Table S3 or
given in the species description. Strain YIM DR4008 was
susceptible to amikacin, cefuroxime sodium, chlorampheni-
col, ciprofloxacin, erythromycin, gentamicin, novobiocin,
penicillin, piperacillin, polymyxin B and tetracycline while
resistant to ethylhydrocupreine, norfloxacin, oxacillin,
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<sup>T</sup> 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<sup>T</sup> 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 species *Streptomyces* (G replaced by C in some species) is therefore proposed to be removed from the signature nucleotides of the family as given below in the emended description of the family. In addition, strain YIM DR4008<sup>T</sup> 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<sup>T</sup> along with those reported for the genera *Streptomyces*, *Kitasatospora* and *Streptacidiphilus* are given in Table 1. Based on the above observations, strain YIM DR4008<sup>T</sup> 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 Ldiaminopimelic acid. Galactose is the diagnostic whole-cell sugar, although mannose may be present in a major amount. The polar lipids comprise diphosphatidylglycerol, phosphatidylglycerol mannosides and three unidentified phospholipids. The respiratory menaquinones are MK-9(H<sub>6</sub>) and MK-9(H<sub>8</sub>). The major cellular fatty acids (>10%) are anteiso-C<sub>15:0</sub>, C<sub>16:0</sub>, iso-C<sub>16:0</sub>, iso-C<sub>15:0</sub> and anteiso-C<sub>17:0</sub>.

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 DR4008^T^ and members of the genera classified in the family Streptomyctaceae

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1 (except for signature nucleotides) are from Kim et al. [3]. All taxa contained long chains of spores formed on aerial hyphae, and MK-9(H_{4}) as major menaquinones.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genera: 1, <em>Allostreptomyces</em> gen. nov. (strain YIM DR4008^T^); 2, <em>Streptomyces</em>; 3, <em>Kitasatospora</em>; 4, <em>Streptacidiphilus</em>. 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(H_{4}) as major menaquinones.</td>
<td></td>
</tr>
<tr>
<td><strong>pH range for growth</strong></td>
<td>5.0–11.0</td>
</tr>
<tr>
<td>Diaminopimelic acid isomer(s) in cell wall</td>
<td>LL-DAP</td>
</tr>
<tr>
<td>Diagnostic sugars in whole-cell hydrolysates</td>
<td>Galactose</td>
</tr>
<tr>
<td>Predominant phospholipids*</td>
<td>DPG, PIMs</td>
</tr>
<tr>
<td>DNA G+C content (mol%)</td>
<td>75.3</td>
</tr>
<tr>
<td>16S rRNA signature nucleotides</td>
<td>443:1029:1151</td>
</tr>
<tr>
<td>825</td>
<td>G</td>
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<tr>
<td>849</td>
<td>C</td>
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<td>875:1031</td>
<td>C</td>
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<td>1004:1278</td>
<td>U</td>
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<td>1039</td>
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<tr>
<td>1122:1231</td>
<td>A</td>
</tr>
</tbody>
</table>

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


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 rhamnose (*Streptacidiphilus*) or galactose and mannos (*Allostreptomyces*). The G+C content of the DNA is generally between 66 and 75 mol%.

The type genus is *Streptomyces* Waksman and Henrici (1943)^{46}. The type strain is *Streptomyces* sp. nov., isolated from soil in South China.

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**Conflicts of interest**

The authors declare that there are no conflicts of interest.

**References**


