Streptacidiphilus anmyonensis sp. nov.,
Streptacidiphilus rugosus sp. nov. and
Streptacidiphilus melanogenes sp. nov., acidophilic
actinobacteria isolated from Pinus soils

Sung-Heun Cho, Ji-Hye Han, Hye-Young Ko and Seung Bum Kim

The taxonomic positions of 22 spore-forming, extensively branched actinobacteria isolated from Pinus soils were examined using a polyphasic approach. Analysis of the 16S rRNA gene sequences indicated that all of the isolates fell into three distinctive phylogenetic clusters within the genus Streptacidiphilus of the family Streptomycetaceae, and also that Streptacidiphilus jiangxiensis was the species closest to the three phyloclusters, with 16S rRNA gene sequence similarities ranging from 98.0 to 99.2%. However, the low DNA–DNA relatedness values between representatives of the three clusters and S. jiangxiensis clearly differentiated them from one another. Representative isolates were also found to have chemotaxonomic features typical of the genus Streptacidiphilus and were distinguishable from all established species of Streptacidiphilus on the basis of a combination of phenotypic properties. It is evident from this study that each of the three phyloclusters should be equated with three novel Streptacidiphilus species, for which the following names are proposed: Streptacidiphilus anmyonensis sp. nov. (type strain AM11T = NBRC 103185T = KCTC 19278T), Streptacidiphilus rugosus sp. nov. (type strain AM16T = NBRC 103186T = KCTC 19279T) and Streptacidiphilus melanogenes sp. nov. (type strain SB-B34T = NBRC 103184T = KCTC 19280T).

Acidophilic actinobacteria with streptomycete-like features are common in terrestrial habitats such as acidic forest and mine-drainage soils, where they form a major constituent of the actinobacterial community (Williams et al., 1971; Khan & Williams, 1975; Hagedorn, 1976; Goodfellow & Dawson, 1978; Lonsdale, 1985; Seong, 1992; Cho et al., 2006). These actinobacteria are potential sources of commercially significant antifungal compounds and acid-stable enzymes (Williams & Khan, 1974; Williams & Flowers, 1978) and are likely to have a major role in the decomposition of fungal biomass in acidic litter and soils (Williams & Robinson, 1981). It has been shown that acidophilic actinobacteria consistently form two distinct aggregate taxa (namely, the neutrotolerant acidophilic and strictly acidophilic cluster-groups) on the basis of numerical phenetic data (Khan & Williams, 1975; Lonsdale, 1985; Seong et al., 1993) and genotypic data for the 16S rRNA gene (Kim et al., 2003, 2004; Xu et al., 2006). Streptacidiphilus are aerobic, Gram-positive, acidophilic (i.e. optimal growth around pH 4.5), chemo-organotrophic actinobacteria that form extensively branched substrate mycelium and aerial hyphae that differentiate into long chains of smooth-surfaced spores. The major chemotaxonomic features include the following: LL-diaminopimelic acid as the major diamino acid in the cell wall, galactose and rhamnose as the diagnostic sugars in whole-cell hydrolysates, diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol and phosphatidylinositol mannosides as the main polar lipids, hexahydrogenated and octahydrogenated menaquinoines [MK-9(H₈)] with nine isoprene units as the major respiratory quinones and a mixture of saturated, iso- and anteiso-branched fatty acids (Lonsdale, 1985; Seong, 1992). Strict acidophiles were found to constitute a novel genus within the family Streptomycetaceae, and three species, Streptacidiphilus albus, Streptacidiphilus carbonis and Streptacidiphilus neutrinimicus were initially described (Kim et al., 2003). Two additional species, Streptacidiphilus jiangxiensis (Huang et al., 2004) and Streptacidiphilus oryzae (Wang et al., 2006), were described subsequently.
Following a previous report of the isolation of acidophilic actinobacteria from soils associated with *Pinus thunbergii* (Cho et al., 2006), the taxonomic status of representative isolates belonging to the genus *Streptacidiphilus* was examined in this study by using a polyphasic approach.

All of the strains were maintained and examined on agar media adjusted to pH 4.5–5.0. They were kept on acidified modified Bennett’s agar and starch-casein agar plates (Seong, 1992) at room temperature and as suspensions of mycelial fragments and spores in glycerol (20 %, v/v). Biomass for the diaminopimelic acid, fatty acid, menaquinone and molecular systematic analyses was obtained from shake-flask cultures and test tubes containing modified Bennett’s broth (pH 5.0) grown at 30 °C for 7 days. Biomass for chemical and molecular systematic studies was washed twice in distilled water and then freeze-dried; biomass for preservation was stored at −20 and −70 °C.

The strains were inoculated onto acidified modified Bennett’s and modified inorganic salts-starch (ISP 4 medium; Shirling & Gottlieb, 1966) agar plates and incubated at 30 °C for 14 days. The plates were examined visually to determine the colour of the aerial spore mass, substrate mycelial pigments and soluble pigments. Yeast extract-malt extract (ISP 2 medium; Shirling & Gottlieb, 1966) agar was used to determine the production of soluble pigments. Spore-chain morphology and spore-surface ornamentation were examined using scanning electron microscopy, as described previously (O’Donnell et al., 1993). Phenotypic properties of the strains were examined using established procedures (Seong et al., 1993; Williams et al., 1983), but with acidified media.

Isoprenoid quinones and diaminopimelic acid were investigated using reversed-phase HPLC procedures as described by Komaga & Suzuki (1987) and El-Waziry et al. (1996), respectively. Fatty acids were extracted, methylated and analysed by GC using the standard Microbial Identification System (MIDI) (Sasser, 1990; Kämpfer & Kroppenstedt, 1996).

Isolation of chromosomal DNA, PCR amplification and direct sequencing of the PCR products were carried out as described previously (Park et al., 2005). The resultant 16S rRNA gene sequences were aligned together with corresponding sequences from representative species of the genera *Kitasatospora*, *Streptacidiphilus* and *Streptomyces* by using the PHYLIP program (version 3.1; available at http://plaza.snu.ac.kr/~jchun/phydit/). Phylogenetic trees were inferred using the Fitch–Margoliash, maximum-likelihood, maximum-parsimony and neighbour-joining methods according to previously described procedures (Kim et al., 2003; Park et al., 2006).

Levels of DNA–DNA relatedness were determined using the procedure described by Cho & Giovannoni (2004). The hybridization temperature was 55 °C. Densitometric analyses were carried out using a Personal Densitometer with ImageQuant imaging software (Bio-Rad). All of the novel isolates were Gram-positive, aerobic, acidophilic actinobacteria. All formed extensively branched substrate mycelia and aerial hyphae that differentiated into long, straight to flexuous chains of smooth-surfaced spores. Brown or cream-coloured colonies carrying moderate to abundant white or greyish-white aerial hyphae were formed on acidified oatmeal agar, ISP 4 agar, ISP 2 agar and modified Bennett’s agar.

The major diaminoc acid of the peptidoglycan was LL-diaminopimelic acid, although minor amounts of the meso isomer were detected. Representative isolates AM-11T, AM-16T and SB-B34T contained 13-methyltetradecanoic acid (iso-C15:0; 15–20 % of the total fatty acid composition), 12-methyltetradecanoic acid (anteiso-C15:0; 7–15 %), 14-methylpentadecanoic acid (iso-C16:0; 15–25 %), n-hexadecanoic acid (C16:0; 15–16 %), 15-methylhexadecanoic acid (iso-C17:0; 3–7 %) and 14-methylhexadecanoic acid (anteiso-C17:0; 4–7 %) (see Supplementary Table S1, available in IJSEM Online). All of the three strains contained hexahydrogenated and octahydrogenated menaquinones with nine isoprene units [MK-9(H8) and MK-9(H6), comprising 18–24 and 59–69 % of the total, respectively] as the predominant isoprenologues.

From the phylogenetic analysis of 1358 nucleotide positions from the 16S rRNA gene sequences, the isolates formed three distinctive phylogenetic lines within the taxonomic variation encompassed by the genus *Streptacidiphilus* (Fig. 1). The expanded phylogenetic tree including all 22 isolates tested also showed three independent phyloclusters (Supplementary Fig. S1). It was notable that the isolates from the Anmyeon area (AM prefixes) were split into two separate phyloclusters, whereas isolates from the Sambong area (SB prefixes) formed an independent phylocluster. The strains belonging to each phylocluster shared the same morphological and cultural characteristics.

The DNA–DNA relatedness data for the representative strains and the type strain of *S. jiangxiensis* confirmed that all of the taxa could be clearly separated from one another (Table 1). Strains AM-16T and *S. jiangxiensis* 33214T shared 34 % DNA–DNA relatedness. Strain SB-B34T exhibited 30 % DNA–DNA relatedness with respect to *S. jiangxiensis* 33214T, 42 % with respect to strain AM-11T and 23 % with respect to strain AM-16T. No obvious correlation was observed between the 16S rRNA gene sequence similarity and DNA–DNA relatedness data (Table 1).

The representative isolates could be separated from other *Streptacidiphilus* species with validly published names and also from one another by using a combination of phenotypic tests (Table 2). It is evident from the results of the polyphasic study, including the phenotypic data, the diaminoc acid present in the peptidoglycan, the fatty acid profiles, the DNA–DNA hybridization data and the phylogenetic results for the 16S rRNA gene, that each of the three novel phyloclusters of *Streptacidiphilus* should be recognized as an independent species. The names proposed
for the three novel species *Streptacidiphilus anmyonensis* sp. nov. (type strain AM-11\(^{\text{T}}\)), *Streptacidiphilus rugosus* sp. nov. (type strain AM-16\(^{\text{T}}\)) and *Streptacidiphilus melanogenes* sp. nov. (type strain SB-34\(^{\text{T}}\)).

**Description of Streptacidiphilus anmyonensis** sp. nov.

*Streptacidiphilus anmyonensis* (an.myon.en’sis. N.L. masc. adj. *anmyonensis* of Anmyon, where the first strains were isolated).

Forms cream-coloured colonies that carry moderate to abundant, white to greyish-white aerial hyphae on acidified oatmeal, ISP 4, ISP 2 and modified Bennett’s agar plates. Substrate mycelium is brownish grey or brown on acidified oatmeal agar and ISP 2 agar, but cream on ISP 4 agar and modified Bennett’s agar. Aerial hyphae differentiate into long flexuous chains of spores (0.6–0.9 µm); the spore surface is smooth. Soluble pigments are not produced on any of the above-mentioned media. Growth occurs at pH 3.0–8.0 and 28–35°C. Starch and Tween 80 are degraded, but xanthine and Tweens 20 and 40 are not. Glycerol, D-glucuronic acid, D(+) glucosamine hydrochloride, *myo*-inositol, melibiose, D-sorbitol, sucrose and D(+) xylose (each at 1 %, w/v) and l-arginine (at 0.1 %, w/v) are used as sole carbon sources for energy and growth, but L-aspartic acid and sodium oxalate (each at 0.1 %, w/v) are not. L-Isoleucine is used as a sole nitrogen source. Chemotaxonomic properties are typical of those for the genus *Streptacidiphilus*. The major fatty acids are iso-C\(_{15}:0\) (18.4 % of the total fatty acid composition), anteiso-C\(_{15}:0\) (11.4 %), iso-C\(_{16}:0\) (19.1 %), C\(_{16}:0\) (14.7 %), iso-C\(_{17}:0\) (7.4 %) and anteiso-C\(_{17}:0\) (7.5 %). Contains hexahydroge

![Neighbour-joining phylogenetic tree](image_url)

**Table 1.** DNA–DNA relatedness and 16S rRNA gene sequence similarity among strains AM-11\(^{\text{T}}\), AM-16\(^{\text{T}}\), SB-34\(^{\text{T}}\) and *S. jiangxiensis* 33214\(^{\text{T}}\)

<table>
<thead>
<tr>
<th>Strain</th>
<th>33214(^{\text{T}})</th>
<th>AM-11(^{\text{T}})</th>
<th>AM-16(^{\text{T}})</th>
<th>SB-34(^{\text{T}})</th>
</tr>
</thead>
<tbody>
<tr>
<td>33214(^{\text{T}})</td>
<td>–</td>
<td>99.3</td>
<td>98.1</td>
<td>98.8</td>
</tr>
<tr>
<td>AM-11(^{\text{T}})</td>
<td>35</td>
<td>–</td>
<td>98.5</td>
<td>99.3</td>
</tr>
<tr>
<td>AM-16(^{\text{T}})</td>
<td>34</td>
<td>29</td>
<td>–</td>
<td>99.2</td>
</tr>
<tr>
<td>SB-34(^{\text{T}})</td>
<td>30</td>
<td>42</td>
<td>28</td>
<td>–</td>
</tr>
</tbody>
</table>

Values above the diagonal are 16S rRNA gene sequence similarity values (%). Values below the diagonal are mean percentages from three reciprocal DNA–DNA hybridization reactions.

**Description of Streptacidiphilus rugosus** sp. nov.


Forms cream-coloured, rugose colonies that carry moderate to abundant white aerial hyphae on acidified oatmeal, ISP 4, ISP 2 and modified Bennett’s agar plates. Substrate mycelium is cream on acidified ISP 4, ISP 2 and modified Bennett’s agar, but yellowish brown or brown on oatmeal...
Three novel Streptacidiphilus species

Table 2. Phenotypic properties that serve to distinguish strains AM-11<sup>T</sup>, AM-16<sup>T</sup> and SB-B34<sup>T</sup> from type strains of Streptacidiphilus species with validly published names

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
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<tr>
<td>Starch</td>
<td>+</td>
<td>+</td>
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<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
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<td>Tween 20</td>
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<td>-</td>
<td>-</td>
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<td>+</td>
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<td>+</td>
<td>+</td>
<td>-</td>
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<td>Xanthine</td>
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<td>+</td>
<td>+</td>
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<td>Growth on sole carbon sources at 1 % (w/v)</td>
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<tr>
<td>d-Glucosamine hydrochloride</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>d-Glucosamine hydrochloride</td>
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<td>+</td>
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<td>-</td>
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<td>+</td>
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<td>D-Sorbitol</td>
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<td>+</td>
<td>+</td>
<td>-</td>
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<td>+</td>
<td>+</td>
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<tr>
<td>D-Xylose</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Growth on sole carbon sources at 0.1 % (w/v)</td>
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<tr>
<td>l-Arginine</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
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<tr>
<td>l-Aspartic acid</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Sodium oxalate</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Growth on L-isoleucine (0.1 %, w/v) as sole nitrogen source</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Growth at pH 3</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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</tr>
</tbody>
</table>

Strains: 1, AM-11<sup>T</sup>; 2, AM-16<sup>T</sup>; 3, SB-B34<sup>T</sup>; 4, S. jiangxiensis 33214<sup>T</sup>; 5, S. albus JI83<sup>T</sup>; 6, S. carbonis JLA15<sup>T</sup>; 7, S. neutrinimicus JLI06<sup>T</sup>; 8, S. oryzae TH49<sup>T</sup>. All strains were positive for the utilization of glycerol and sucrose and also for growth at pH 4, 5 and 6.

The species currently includes the type strain AM-16<sup>T</sup> (NBRC 103186<sup>T</sup> = KCTC 19279<sup>T</sup>) and strains AM-20, AM-21, AM-22, AM-26, AM-27, AM-28 and AM-29, isolated from Pinus-associated soils from Anmyeon, near the coastal areas of Tae-An, Chungnam, Republic of Korea.

Description of Streptacidiphilus melanogenes sp. nov.

Streptacidiphilus melanogenes [me.la.no’gen.es. Gr. n. melas -anos black; Gr. v. suff. -genes producing; N.L. part. adj. melanogenes producing black (pigment)].

Forms cream-coloured colonies that carry moderate to abundant white aerial hyphae on acidified oatmeal, ISP 4, ISP 2 and modified Bennett’s agar plates. Substrate mycelium is brownish grey or brown on acidified ISP 4 agar and ISP 2 agar, but cream on oatmeal agar and modified Bennett’s agar. Aerial hyphae differentiate into long flexuous chains of spores (0.6 x 1.2 μm); the spore surface is smooth. Brownish-grey diffusible pigments are formed on acidified oatmeal agar and ISP 2 agar. Soluble pigments are not produced on ISP 4 agar or modified Bennett’s agar. Growth occurs at pH 3.0—8.0 and 28–35 °C. Starch and Tween 80 are degraded, but xanthine and Tweens 20 and 40 are not. Glycerol, d-glucosic acid, myo-inositol, D-sorbitol and sucrose (each at 1 %, w/v), L-arginine and sodium oxalate (each at 0.1 %, w/v) are used as sole carbon sources for energy and growth, but D(+)-glucosamine hydrochloride, melibiose and D(+)-xylose (each at 1 %, w/v) and L-aspartic acid (at 0.1 %, w/v) are not. L-Isolucine is used as a sole nitrogen source. Chemotaxonomic properties are typical of those for the genus Streptacidiphilus. The major fatty acids are iso-C<sub>15:0</sub> (15.7 %), iso-C<sub>16:0</sub> (15.6 %) and anteiso-C<sub>17:0</sub> (14.7 %). Contains hexahydrogenated and octahydrogenated menaquinones with nine isoprene units [MK-9(H<sub>6</sub>) and MK-9(H<sub>8</sub>)); 19.8 and 59.6 % of the total, respectively] as the predominant isoprenologues. The diamino acid of the peptidoglycan is L-diaminopimelic acid (94 % of the total diaminopimelic acid composition), although a minor amount of the meso isomer (6 %) is also detected.

The species currently includes the type strain AM-16<sup>T</sup> (NBRC 103186<sup>T</sup> = KCTC 19279<sup>T</sup>) and strains AM-20, AM-21, AM-22, AM-26, AM-27, AM-28 and AM-29, isolated from Pinus-associated soils from Anmyeon, near the coastal areas of Tae-An, Chungnam, Republic of Korea.

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menaquinones with nine isoprene units [MK-9(H6) and MK-9(H8); 18.1 and 68.6 % of the total, respectively] as the predominant isoprenologues. The diamino acid of the peptidoglycan is L-diaminopimelic acid (100 % of the total dianminopolamic acid composition).

The species currently includes the type strain SB-B34^T (=NBRC 103184^T =KCTC 19280^T) and strains SB-B33 and SB-B35, isolated from Pinus-associated soils from Sambong, near the coastal areas of Taean, Chungnam, Republic of Korea.

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References


