**Amycolatopsis acidiphila** sp. nov., a moderately acidophilic species isolated from coal mine soil

Bilguun Oyuntsetseg, Sung-Heun Cho, Sun Jeong Jeon, Hyang Burm Lee, Kee-Sun Shin, In-Seop Kim and Seung Bum Kim

**Abstract**

Little is known on members of the genus *Amycolatopsis* inhabiting acidic habitats. In this study, a moderately acidophilic *Amycolatopsis* strain, designated 2-5, was isolated from coal mine soil, and subjected to a polyphasic taxonomic characterization. Analysis based on 16S rRNA gene sequences indicated that the strain was most closely related to the type strain of *Amycolatopsis bartoniae*, sharing 99.30 % similarity, while similarity to all other *Amycolatopsis* species was less than 97 %. The DNA–DNA relatedness between the new isolate and the type strain of *A. bartoniae* was 56.5±0.7 %. The optimal pH range of the isolate for growth was 5.5–6.0, but growth also occurred at pH 4.5 and 7.5. The isolate tolerated up to 6 % (w/v) NaCl (optimum, 0 %), and the temperature range for growth was 15–40 °C (optimum, 30 °C). The isolate was able to utilize most substrates tested for sole carbon sources, showing its metabolic versatility. The isolate exhibited antimicrobial activity against *Serratia marcescens* and weak antifungal activity against *Fusarium proliferatum*. The chemotaxonomic profiles of strain 2-5 included polar lipids containing phosphatidyethanolamine, phosphatidylymethylethanolamine, phosphatidylglycerol, phosphatidylinositol and phosphatidylinositol dimannosides, fatty acids containing C17:0ω6c and iso-C15:0 as the major components, MK-9(H2) as the predominant menaquinone, and meso-diaminopimelic acid and arabinose, galactose, glucose and ribose as the diagnostic diamino acid and sugars in the cell wall. The combined phenotypic, chemotaxonomic and genotypic analyses clearly indicated that the isolate merits recognition as representing a novel species of *Amycolatopsis*, for which the name *Amycolatopsis acidiphila* sp. nov. is proposed. The type strain is 2-5 (=KCTC 39523=JCM 30562).

**TAXONOMIC DESCRIPTION**


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Acidophilic actinobacteria are an important soil constituent and are filamentous, aerobic, non-motile, Gram-positive and chemo-organotrophic, usually producing long chains of arthrospores [1–4]. Acidophilic sporocactinobacteria can be assigned to two groups: neutrotolerant acidophilic strains that grow at pH 4.5–7.5, with an optimum between pH 5.0 and 5.5; and strictly acidophilic strains that grow at pH 3.5–6.5, with an optimum around pH 4.5 [3–5]. Strict acidophiles were found to form a new genus, designated *Streptacidiphilus*, within the family *Streptomycetaceae* [2]. In contrast, neutrotolerant acidophiles were classified into *Streptomyces* [6–8]. In a separate study by Cho et al. [1], a high level of diversity of both strictly acidophilic and neutrotolerant acidophilic actinobacteria, encompassing *Streptomyces* and *Streptacidiphilus*, was reported from the soil environment.

However, later studies revealed more diverse groups of acidophilic actinobacteria other than *Streptomyces*. The genus *Actinospica* was proposed to accommodate a new group of strictly acidophilic soil actinobacteria [9], *Ferrimicrobium* to accommodate extremely acidophilic iron-oxidizing, non-spore-forming actinobacteria [10], and *Catenulispora* to accommodate soil sporocactinobacteria exhibiting growth pH profiles similar to those for acidophilic members of *Streptomyces* [11]. In a survey on the diversity of acidophilic actinobacteria in soil and leaf litter samples, the genera *Actinoallomurus*, *Actinospica*, *Kitasatospora* and *Nocardia* were recovered in addition to *Streptomycetes* and *Streptacidiphilus* [12]. In a more recent study, members of the genera *Allokutzneria*, *Amycolatopsis*, *Mycobacterium*, *Nocardia*, *Nonomuraea*, *Saccharopolyspora* and *Verrucospora* growing at low pH were recovered from rhizosphere soil [13].

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**Keywords:** acidophilic Amycolatopsis; antimicrobial potential; coal mine soil; Mongolia.

The GenBank/EMBL/DBJ accession number for the 16S rRNA gene sequence of strain 2-5 is GU132436.

Two supplementary tables and two supplementary figures are available with the online Supplementary Material.
Amycolatopsis is known as an important source of various bioactive compounds such as antibacterial, antifungal, antiviral, immunosuppressant and antitumor compounds, notably *Amycolatopsis azurea* (azureomycins and octacosamicins), *Amycolatopsis mediterranei* (rifamycins, dethymicin, etc.), *Amycolatopsis orientalis* (vancomycin, muraceins, orienticin, quartromicin, etc.) and *Amycolatopsis lurida* (benzathrins, ristocetin) [14]. Poomthongdee et al. [13] reported a high level of antimicrobial potential of acidophilic actinobacteria including *Amycolatopsis* species isolated from rhizosphere soil.

The genus *Amycolatopsis* currently encompasses 67 validly described species as of May 2017 (http://www.bacterio.net/amycolatopsis.html). In this study, an actinobacterial strain belonging to *Amycolatopsis* was isolated from soil of a coal mine site in Mongolia during an examination of the diversity of acidophilic actinobacteria in various Mongolian soils. The isolate was subjected to phenotypic and phylogenetic characterization, and its antimicrobial potential was also examined.

The soil sample was collected from the Nalaikh coal mine site near Ulaanbataar, Mongolia. The pH of the sample was 5.9. The sample was subjected to heat treatment following previously described procedures [4]. For isolation, 10 ml of sterile 1/4 strength Ringer solution (0.12 g CaCl$_2$, 0.105 g KCl, 0.05 g NaHCO$_3$ and 2.25 g NaCl per litre of distilled water) was added to 1 g of soil sample. The mixture was shaken on a reciprocal shaker for 20 min. Aliquots (0.2 ml) of dilutions were spread onto acidified (pH adjusted to 4.5) starch casein agar (SCA, soluble starch 10 g, casein 0.3 g, KNO$_3$ 2 g, NaCl 2 g, K$_2$HPO$_4$ 2 g, MgSO$_4$ 7H$_2$O 0.05 g, CaCO$_3$ 0.02 g, FeSO$_4$ 7H$_2$O 0.01 g and agar 15 g per litre of distilled water) supplemented with cycloheximide and nystatin to avoid fungal growth, each at a final concentration of 50 µg ml$^{-1}$. The inoculated plates were incubated at 30°C for at least 1 week, and colonies were selected and subcultured using the same medium, and also using modified Bennett’s agar (MBA; Difco), yeasts on Sabouraud dextrose agar (SDA, Difco) and mitosporic fungi on potato dextrose agar (PDA; Difco). For mitosporic fungi and oomycetes, the agar plugs of the fungi were placed 2.0 cm apart on each side of the streaked micro-organisms growing on PDA plates. For bacteria and yeasts, the strains were streaked on PDA 2.0 cm apart on each plate. The plates were incubated at 30°C, and growth inhibition by strain 2-5$^T$ was checked until 2 weeks.

Analysis of the almost-complete 16S rRNA gene sequence (1471 nucleotide positions) indicated that strain 2-5$^T$ was most closely related to *Amycolatopsis bartoniae* SF26$^T$ with 99.30% similarity. The two strains also formed a cluster in the neighbour-joining tree with a high bootstrap support, and the cluster was also recovered in the maximum-likelihood tree (Fig. 1). An extended tree including all validly described species of *Amycolatopsis* also indicated the close relationship between the two strains (Fig. S1). However, the DNA–DNA relatedness between strain 2-5$^T$ and *A. bartoniae* DSM 45807$^T$ was 56.5±0.7%, which was well below the suggested cut-off for species distinction [20]. The other nearest neighbours shared less than 97% 16S rRNA gene sequence similarity with strain 2-5$^T$, *Amycolatopsis halophila* YIM 93223$^T$ being the closest with 96.94% similarity. Note that among species of *Amycolatopsis*, the type strains of *Amycolatopsis pretoriensis* and *Amycolatopsis lewignotenssis* shared 99.85% 16S rRNA gene sequence similarity, but the DNA–DNA relatedness between them was as low as 54% [14]. Similarly, the type strains of *Amycolatopsis*...
rifamycinica and Amycolatopsis kentuckiensis shared 99.63 % 16S rRNA gene sequence similarity, but the DNA–DNA relatedness between them was 67 % [14]. Strain 2-5T grew best on acidified MBA, while good growth was also observed on acidified SCA, and ISP media 2 and 3. The aerial spore mass was white or grey–white, and the substrate mycelium was light yellow. Light brown diffusible pigments were produced during growth. Extensively branching aerial and substrate hyphae were formed. The pH range for growth was pH 4.0–7.5, and the optimum was pH 5.5–6. In broth culture, the pH increased slightly during the first 5 days, then dropped to values around the optimal pH for growth (data not shown). The isolate grew optimally in the absence of NaCl, but growth was also observed in the presence of up to 6 % (w/v) NaCl. The temperature range for growth was 15–40 °C, and the optimum was 30 °C.

Mesophilic strains that can grow below pH 5 have been reported for the genus Amycolatopsis, for example Amycolatopsis circi, Amycolatopsis equina, Amycolatopsis hippodromi, Amycolatopsis plunensis, Amycolatopsis ruanii, Amycolatopsis saalfeldensis, Amycolatopsis thermalba and Amycolatopsis umgeniensis [21–25]. Although there is a case of isolation of Amycolatopsis strains from an acidic environment, i.e. Amycolatopsis saalfeldensis from acidic and heavy-metal-containing rocks of an alum slate mine [21], it is not clear if those strains are acidophilic. The type strain of

![Neighbour-joining tree](image-url)

**Fig. 1.** Neighbour-joining tree based on 16S rRNA gene sequences showing the relationship between strain 2-5T and established species of the genus Amycolatopsis. Numbers at nodes are levels of bootstrap support over 70 %, and asterisks indicate branches also recovered in the maximum-likelihood tree. Bar, 0.01 substitutions per nucleotide position. Prauserella rugosa DSM 43194T was used as an outgroup.
Table 1. Differential properties that separate strain 2-5\textsuperscript{T} from Amycolatopsis bartoniae SF26\textsuperscript{T}, the closest neighbour

All data were obtained in this study using acidified media (pH 5.5) except for polar lipids of A. bartoniae, which were taken from Zucchi et al. [25].

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>2-5\textsuperscript{T}</th>
<th>A. bartoniae SF26\textsuperscript{T}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colour of aerial hyphae</td>
<td>White/grey–white</td>
<td>White*</td>
</tr>
<tr>
<td>Production of diffusible pigment</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Growth range (optimum):</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Temperature (˚C)</td>
<td>15–40 (30)</td>
<td>15–45 (37)*</td>
</tr>
<tr>
<td>pH</td>
<td>4.5–7.5 (5.5–6)</td>
<td>5–8 (7)</td>
</tr>
<tr>
<td>NaCl (% w/v)</td>
<td>0–6 (0)</td>
<td>0–5 (0)</td>
</tr>
<tr>
<td>Degradation of:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Starch</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Enzyme activity (API ZYM):</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alkaline phosphatase</td>
<td>–</td>
<td>w</td>
</tr>
<tr>
<td>α-Chymotrypsin</td>
<td>+</td>
<td>w</td>
</tr>
<tr>
<td>Cystine arylamidase</td>
<td>w</td>
<td>w</td>
</tr>
<tr>
<td>Esterase (C4)</td>
<td>+</td>
<td>w</td>
</tr>
<tr>
<td>Esterase lipase (C8)</td>
<td>+</td>
<td>w</td>
</tr>
<tr>
<td>β-Galactosidase</td>
<td>w</td>
<td>–</td>
</tr>
<tr>
<td>α-Glucosidase</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>β-Glucosidase</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Lipase (C14)</td>
<td>w</td>
<td>–</td>
</tr>
<tr>
<td>α-Mannosidase</td>
<td>w</td>
<td>–</td>
</tr>
<tr>
<td>Naphthol-AS-Bl-phosphohydrolase</td>
<td>+</td>
<td>w</td>
</tr>
<tr>
<td>Main fatty acids (&gt;15%)</td>
<td>C\textsubscript{17:0}ω6c, iso-C\textsubscript{16:0}</td>
<td>iso-C\textsubscript{16:0}, anteiso-C\textsubscript{17:0}, C\textsubscript{16:0}, anteiso-C\textsubscript{18:0}/C\textsubscript{18:2ω6,9c}</td>
</tr>
<tr>
<td>Diagnostic polar lipids†</td>
<td>PE, PG, PI, PIDM, PME</td>
<td>DPG, PE, PG, PI, PME</td>
</tr>
</tbody>
</table>

*According to the original study, aerial hyphae were not produced, and no growth was observed at 10 and 45˚C, while the optimum temperature was 28˚C for A. bartoniae [25].
†DPG, diphasatidglycerol; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PIDM, phosphatidylinositol dimannosides; PME, phosphatidylmethyllethanolamine.

A. saalfeldensis was phylogenetically distant from strain 2-5\textsuperscript{T}, as they shared 96.11 % 16S rRNA gene sequence similarity. The NaCl tolerance range of the isolate was similar to previous observations on the genus [14]. There have been cases of isolation of halophilic or halotolerant Amycolatopsis strains [26–29], and thus salt tolerance can be considered as a common feature among the members of Amycolatopsis.

The carbon source tests indicated that the strain was able to utilize a wide range of sugars and amino acids as sole carbon sources, as all of the sugars and most amino acids were utilized, as listed in the species description. This observation is in line with the general description of Amycolatopsis, which has the ability to utilize diverse substrates and also to grow on a broad range of organic and synthetic media [14]. Yet, the combination of phenotypic and chemotaxonomic properties clearly separated strain 2-5\textsuperscript{T} from A. bartoniae SF26\textsuperscript{T}, the closest related species (Table 1).

Strain 2-5\textsuperscript{T} contained phosphatidylethanolamine, phosphatidylmethyllethanolamine, phosphatidylglycerol, phosphatidylinositol, phosphatidylglycero-l dimannosides and unidentified aminolipids as the main polar lipids (Fig. S2). The main fatty acids (>10 %) were iso-C\textsubscript{16:0}, C\textsubscript{17:1ω6c}, iso-C\textsubscript{15:0} and anteiso-C\textsubscript{15:0}; while C\textsubscript{16:0}, iso-C\textsubscript{17:0}, anteiso-C\textsubscript{17:0}, iso-C\textsubscript{16:1}H and iso-C\textsubscript{14:0} were present as minor components (Table S2). MK-9(H\textsubscript{4}) was found as the predominant menaquinone, and meso-diaminopimelic acid was the diagnostic amino acid in the cell wall. Arabinose, galactose, glucose and ribose were found as the main cell-wall sugars. Mycolic acids were not detected. The DNA G+C content of strain 2-5\textsuperscript{T} was 72.0 %. The chemotaxonomic properties were generally consistent with those of Amycolatopsis [14], but some features were discriminative for strain 2-5\textsuperscript{T} from other related species. Notably, the major presence of C\textsubscript{17:1ω6c} clearly distinguished the novel strain from A. bartoniae (Table S2).

Strain 2-5\textsuperscript{T} exhibited antagonistic activity against the bacterium Serratia marcescens KCTC 12457\textsuperscript{T} and weakly against the fungus Fusarium proliferatum EMLYP2, although no other positive results were obtained against most bacterial and fungal strains tested in this study (Table S1).

Strain 2-5\textsuperscript{T} was distinguishable from other related species using the combination of cultural, physiological and biochemical properties as well as by the genotypic analysis, and thus evidently merits recognition as representing a novel species of Amycolatopsis, for which the name Amycolatopsis acidiphila sp. nov. is proposed.
DESCRIPTION OF AMYCOLATOPSIS ACIDIPHILA SP. NOV.

Amycolatopsis acidiphila (a.c.i.d’phi.ila. N.L. n. acidum acid; Gr. adj. philos loving; N.L. fem. adj. acidiphila acid-loving).

Forms extensively branched aerial and substrate hyphae. Grows on acidified MBA, SCA, and ISP media 2 and 3. The aerial spore mass is white or grey–white, and the substrate mycelium is light yellow. Light brown diffusible pigments are produced. The pH range for growth is pH 4.5–7.5, and the optimum is 5.5–6. Grows optimally in the absence of NaCl, but growth is observed in the presence of up to 6% \((\mathrm{w/v})\) NaCl. The temperature range for growth is 15–40 °C, and the optimum is 30 °C. Nitrate is not reduced. L-Arabinose, D-fructose, D-galactose, D-glucose, myo-inositol, D-mannitol, melibiose, raffinose, L-rhamnose, D-salicin, sarcosine, D-sorbitol, D-xyllose, L-arginine, L-asparagine, L-glutamine, L-histidine and L-isoleucine are utilized as sole carbon sources, but not L-alanine or L-lysine. Casein, gelatin, Tween 20, 40, 60 and 80, and xanthine are degraded, but not CM-cellulose, DNA, starch or tributyrin. Based on API ZYM tests, \(N\)-acetyl-\(\beta\)-glucosaminidase, acid phosphatase, \(\alpha\)-chymotrypsin, esterase (C4), esterase lipase (C8), \(\alpha\)-glucosidase, \(\beta\)-glucosidase, leucine arylamidase and naphthol-AS-Bl-phosphohydrolase activities are positive, cystine arylamidase, \(\beta\)-galactosidase, lipase, \(\alpha\)-mannosidase and valine arylamidase activities are weakly positive, and alkaline phosphatase, \(\alpha\)-fucosidase, \(\alpha\)-galactosidase, \(\beta\)-glucuronidase and trypsin activities are negative. Phosphatidylethanolamine, phosphatidylglycerol, phosphatidylinositol, phosphatidylserine and unidentified aminolipids are the main polar lipids. Arabinose, galactose, glucose and ribose are the main cell-wall sugars. The main membrane fatty acids are iso-C16:0, \(\mathrm{C}_{17}:1\)ω6c, iso-C15:0, anteiso-C15:0 and C16:0. MK-9(H4) is the predominant menaquinone, and \(\text{meso-diaminopimelic acid}\) is the diagnostic amino acid in the cell wall.

The type strain, 2-5T (=KCTC 39523T=JCM 30562T), was isolated from soil of the Nalaikh coal mining site in Nalaikh Province, Mongolia. The DNA G+C content of the type strain is 72 mol%.

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

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