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Published on: 01 Nov 2012 - International Journal of Systematic and Evolutionary Microbiology (Society for General Microbiology)

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Streptomyces atacamensis sp. nov., isolated from an extreme hyper-arid soil of the Atacama Desert, Chile

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The taxonomic position of a *Streptomyces* strain isolated from an extreme hyper-arid soil sample collected from the Atacama Desert was determined using a polyphasic approach. The strain, isolate C60^T, had chemical and morphological features typical of members of the genus *Streptomyces* and formed a distinct phyletic line in the *Streptomyces* 16S rRNA gene tree, together with the type strain of *Streptomyces radiopugnans*. The two strains were distinguished readily using a combination of phenotypic properties and by a DNA–DNA relatedness value of 23.17 (±0.95)%. On the basis of these genotypic and phenotypic data, it is proposed that isolate C60^T (=CGMCC 4.7018^T=KACC 15492^T) be classified in the genus *Streptomyces* as *Streptomyces atacamensis* sp. nov.

Streptomyces strains remain a rich source of commercially significant antibiotics (Goodfellow & Fiedler, 2010). In the search for new therapeutic compounds it is important to screen novel isolates (Antony-Babu & Goodfellow, 2008), such as those isolated recently from hyper-arid Atacama Desert soils, Chile (Bull, 2004; Okoro *et al.*, 2009). We have shown that two *Streptomyces* strains from the hyper-arid salt flat Salar de Atacama produce new ansamycin and 22-membered macrolactones that express a range of anti-bacterial and anti-tumour activities (Nachtigall *et al.*, 2011; Rateb *et al.*, 2011a, 2011b). A second remarkable feature of the genus *Streptomyces* is the huge number of species with validly published names, nearly 600 to date (Euzéby, 2011). The subgeneric classification of the genus, while complex, has been clarified by the application of genotypic and phenotypic procedures (Labeda *et al.*, 2012), which have also been used to delineate novel species which synthesize bioactive compounds (Kumar & Goodfellow, 2008, 2010).

The present study was designed to establish the taxonomic status of a *Streptomyces* strain, isolate C60^T, recovered from an even more extreme hyper-arid region of the Atacama Desert. The sampling site in the Valle de la Luna (23° 02' S 68° 20' W) is defined as extremely hyper-arid or absolute desert on the basis of the ratio of mean rainfall to mean evaporation (Houston, 2006; Okoro *et al.*, 2009) and is devoid of vegetation. Nevertheless, small numbers [100–500 c.f.u. (g soil)⁻¹ depending on the isolation medium used] of putative actinomycetes were recovered, among them isolate C60^T (Okoro *et al.*, 2009). This isolate formed a distinct subclade in the 16S rRNA gene tree based on sequences from members of the genus *Streptomyces* and was shown to contain non-ribosomal peptide synthetase genes. A taxonomic study conducted using a polyphasic approach and based on a combination of genotypic and phenotypic methods, indicated that the isolate should be recognized as a novel species of the genus *Streptomyces*.

Strain C60^T was isolated on raffinose–histidine agar (Vickers *et al.*, 1984), supplemented with cycloheximide (25 µg ml⁻¹) and nystatin (25 µg ml⁻¹), which had been inoculated with a soil suspension and incubated at 28 °C

Abbreviation: ISP, International *Streptomyces* project.

The GenBank/EMBL/DDJB accession number for the 16S rRNA gene sequence of strain C60^T is HE577171.

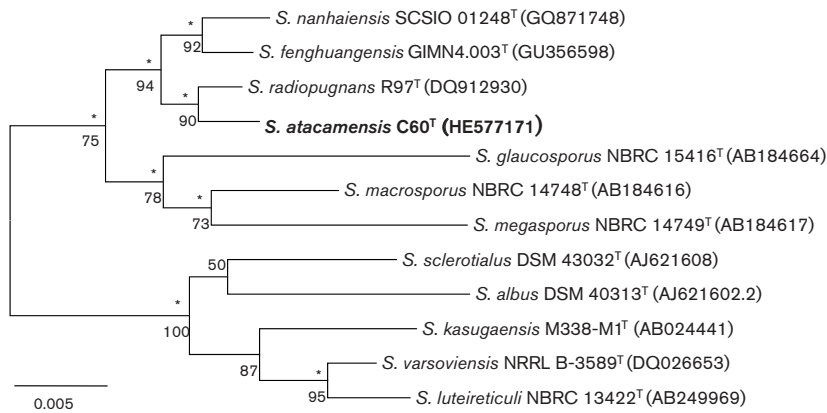


Fig. 1. Neighbour-joining tree based on nearly complete 16S rRNA gene sequences (1457 nt) showing relationships between isolate C60^T and closely related species of the genus *Streptomyces*. Asterisks indicate branches of the tree that were also found using the maximum-parsimony and minimum-evolution tree-making algorithms. Numbers at the nodes are percentage bootstrap values based on 1000 resampled datasets: only values $\geq 50\%$ are given. Bar, 0.005 substitutions per nucleotide position.

for 14 days. The isolate was maintained on modified Bennett's agar slopes (Jones, 1949) at 4 °C and as suspensions of spores and hyphae in 20%, v/v, glycerol at -20 °C. Biomass for the molecular systematic and most of the chemotaxonomic studies was scraped from modified Bennett's agar plates, which had been incubated for 14 days at 28 °C, and was washed twice in distilled water. Biomass for the chemotaxonomic studies was freeze-dried and that for the molecular systematic work stored at -20 °C. Biomass for the fatty acid analysis was harvested from glucose yeast extract-malt extract broth (Shirling & Gottlieb, 1966) after 3 days at 25 °C.

Genomic DNA was extracted from isolate C60^T and amplified by PCR, and 16S rRNA gene sequencing was performed as previously described by Kim & Goodfellow (2002). The resultant, almost complete sequence (1457 nt) was compared with the corresponding sequences of the most closely related type strains of members of the genus *Streptomyces* using the EzTaxon server 2 (Chun *et al.*, 2007); gene sequences were aligned using CLUSTAL W (Thompson *et al.*, 1994). Phylogenetic trees were generated using the maximum-parsimony (Fitch, 1971), minimum-evolution (Rzhetsky & Nei, 1992) and neighbour-joining (Saitou & Nei, 1987) tree-making algorithms from MEGA4 (Tamura *et al.*, 2007); the Jukes & Cantor (1969) model was used to derive an evolutionary distance matrix for the neighbour-joining method. Tree topologies were evaluated by a bootstrap analysis (Felsenstein, 1985) of the neighbour-joining dataset based on 1000 replicates using MEGA4.0.

Comparison of the almost complete 16S rRNA gene sequence of isolate C60^T with the corresponding sequences of the closely related type strains showed that it formed a branch in the *Streptomyces* gene tree with *Streptomyces radiopugnans* CGMCC 4.3519^T, a relationship which was supported by all of the tree-making algorithms and had a bootstrap value of 90% (Fig. 1). The 16S rRNA gene similarity between these organisms was 99.4%, a value which corresponded to 9 nucleotide differences over 1457 locations. The novel strain formed a well supported subclade, together with a corresponding branch composed of the type strains of *Streptomyces sanyensis* and *Streptomyces nanhaiensis* (Fig. 1).

Isolate C60^T was examined for chemotaxonomic and morphological properties considered to be typical of the genus *Streptomyces* (Williams *et al.*, 1989; Manfio *et al.*, 1995). The arrangement of hyphae and spore chains of the organism grown for 14 days at 28 °C on oatmeal agar [International *Streptomyces* project (ISP) medium 3; Shirling & Gottlieb, 1966] were determined using the coverslip technique of Kawato & Shinobu (1959). Spore chain morphology and spore surface ornamentation were detected by examining gold-coated, dehydrated specimens taken from the 14-day oatmeal agar plates, using an electron microscope (Cambridge Stereoscan 240), following the procedure described by O'Donnell *et al.* (1993). The isomers of diaminopimelic acid were analysed using the procedure developed by Hasegawa *et al.* (1983) and menaquinones were extracted and purified according to Collins (1985) and then examined by HPLC (Minnikin *et al.*, 1984). The cellular fatty acids were extracted, methylated and separated by GC (model 6890; Hewlett Packard), according to the protocol of the Sherlock Microbial Identification System (MIDI; Sasser, 1990). The fatty acid methyl esters were identified and quantified

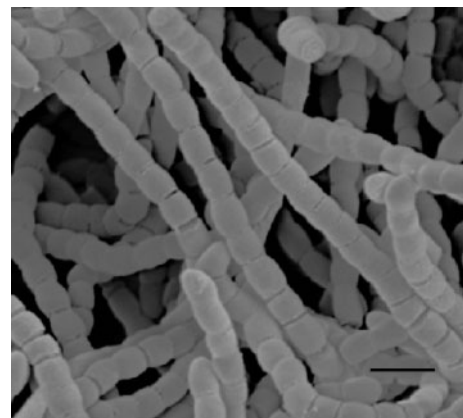


Fig. 2. Scanning electron micrograph of isolate C60^T on oatmeal agar after 14 days at 28 °C showing straight chains of spores. Bar, 1 µm.

Table 1. Growth and cultural characteristics of strain C60^T on standard ISP agar media, following incubation at 28 °C for 14 days
 + + +, Abundant growth; + +, moderate growth. The isolate did not form diffusible pigments on any of the media tested.

Medium	Growth	Substrate mycelium colour	Aerial mycelium colour
Glycerol asparagine agar (ISP 5)	+ +	Light yellow	Light yellow
Inorganic salts starch agar (ISP 4)	+ + +	Brownish white	White
Oatmeal agar (ISP 3)	+ + +	Olive green	Grey
Peptone yeast extract iron agar (ISP 6)	+ + +	Creamy brown	White
Tryptone yeast extract agar (ISP 1)	+ +	Cream	White
Tyrosine agar (ISP 7)	+ +	Cream	White
Glucose yeast extract–malt extract agar (ISP 2)	+ + +	White	Grey

by using the ACTIN1 database (version 6.10) of the Sherlock Microbial Identification System.

The isolate formed an extensively branched substrate mycelium and abundant grey aerial hyphae on oatmeal

agar. At maturity, aerial hyphae differentiated into long, straight chains of cylindrical, smooth-surfaced spores (Fig. 2). The organism contained LL-diaminopimelic acid in whole-organism hydrolysates and hexa- and octa-hydrogenated menaquinones with nine isoprene units [MK-9 (H₆, H₈)] in the

Table 2. Phenotypic properties that distinguish isolate C60^T from the type strain of *Streptomyces radiopugnans*

All of the data were from the present study, unless indicated otherwise. +, Positive; –, negative.

Characteristic	C60 ^T	<i>S. radiopugnans</i> CGMCC 4.3519 ^T
Aerial spore mass colour on oatmeal and glucose yeast extract–malt extract agar media	Grey	Pale grey*
Spore chain morphology	Straight	Spiral*
Spore surface ornamentation	Smooth	Rough to warty*
Biochemical tests		
Arbutin hydrolysis	–	+
Urease production	+	–
Degradation tests		
Cellulose	–	+
Elastin	+	–
Guanine	–	+
Keratin	+	–
Tween 40	+	–
Xanthine	–	+
Growth on sole carbon sources (1 %, w/v)		
Dextran	+	–
D-Galactose	+	–
Inulin	–	+
Lactose	+	–
meso-Inositol	+	–
D-Raffinose	+	–
D-Sucrose	–	+
D-Trehalose	+	–
Sodium azide (0.1 %, w/v)	–	+
Sodium propionate (0.1 %, w/v)	+	–
Growth on sole nitrogen sources (0.1 %, w/v)		
L-Phenylalanine	+	–
L-Threonine	+	–
L-Valine	+	–
Growth in presence of 7 %, w/v, NaCl	+	–

*Data taken from Mao *et al.* (2007).

ratio 5:1. The fatty acid profile contained major amounts of iso-C_{15:0} (11.6%), anteiso-C_{15:0} (12.4%), iso-C_{16:0} (13.5%), iso-C_{17:0} (6.2%) and anteiso-C_{17:0} (13.7%). All of these properties are in line with the classification of the isolate in the genus *Streptomyces* (Williams *et al.*, 1989; Manfio *et al.*, 1995).

DNA–DNA relatedness studies were performed, in triplicate, between strain C60^T and *S. radiopugnans* CGMCC 4.3519^T using the microplate method and biotinylated probe DNA (Ezaki *et al.*, 1989) with modifications as described by Rong & Huang (2010). The two strains shared a mean DNA–DNA relatedness similarity of 23.17 (±0.95)%, far removed from the 70% cut-off point recommended for the delineation of genomic species (Wayne *et al.*, 1987). The isolate had a DNA G + C content of 71.5 mol%.

Isolate C60^T and *S. radiopugnans* CGMCC 4.3519^T were examined for a broad range of phenotypic properties as described by Williams *et al.* (1983); the isolate was also subjected to additional tests drawn from those used by Williams and his colleagues. In addition, cultural characteristics of the isolate were recorded after incubation at 28 °C for 14 days on standard media used in the ISP (Shirling & Gottlieb, 1966). The strain grew well on all of the ISP media, showing a range of aerial spore mass and substrate mycelial pigments (Table 1).

Isolate C60^T and *S. radiopugnans* CGMCC 4.3519^T could be distinguished readily on the basis of a broad range of phenotypic properties as shown in Table 2. Unlike the latter strain, isolate C60^T formed straight chains of smooth-surfaced spores, degraded elastin, keratin and Tween 40, hydrolysed urea and showed a greater propensity to grow on sole carbon and sole nitrogen sources. In contrast, both strains hydrolysed aesculin, produced hydrogen sulphide; degraded hypoxanthine, but not tributryin, uric acid or xylan; grew on L-arabinose, D-mannitol, xylitol and D-xylose as sole carbon sources, grew at 10–35 °C and from pH 4 to 11. The results of the additional phenotypic tests mentioned above are given in the species description.

It is suggested that minimal standards for the delineation of members of the genus *Streptomyces* should be based on an appropriate selection of genotypic and phenotypic properties (Manfio *et al.*, 1995; Kumar & Goodfellow, 2008, 2010). In the present study, isolate C60^T was distinguished readily from the type strain of *S. radiopugnans*, its nearest phylogenetic neighbour, on the basis of 16S rRNA gene sequence similarity, DNA–DNA hybridization and phenotypic data. It is, therefore, proposed that isolate C60^T be recognized as a novel species, *Streptomyces atacamensis* sp. nov.

Description of *Streptomyces atacamensis* sp. nov.

Streptomyces atacamensis (a.ta.cam.en'sis. N.L. masc. adj. *atacamensis* belonging to an extreme hyper-arid site in the Atacama Desert of north-west Chile, the source of the soil from which the strain was isolated).

Aerobic, Gram-positive, non-acid–alcohol-fast actinomycete, which forms an extensively branched substrate

mycelium that carries aerial hyphae that differentiate into straight chains of smooth-surfaced, cylindrical spores (0.7–0.9 µm) on oatmeal agar. Does not form melanin on peptone yeast extract iron or tyrosine agars. Grows at 10 to 45 °C and from pH 4 to 11. Degrades adenine, casein, chitin, gelatin, L-tyrosine and Tween 40, but not DNA or RNA. Amygdalin, D-arabitol, D-fructose, D-glucose, glycerol, glycogen, maltose, D-mannose, *meso*-erythritol, D-salicin, D-sorbitol and L-sorbose are used as sole carbon sources, but not L-arabitol, dulcitol or melezitose (all at 1%, w/v). Similarly, uses butane-1,4-diol and propanol, but not methanol as sole carbon sources (at 1%, v/v). Sodium citrate, sodium malonate and sodium pyruvate, but not sodium acetate, are used as sole carbon sources (at 0.1%, w/v). L-Alanine, L-aminobutyric acid, glycine, L-histidine, L-isoleucine, DL-methionine, DL-norleucine, L-norvaline, L-ornithine, L-proline, L-serine and L-tryptophan are used as sole nitrogen sources. Additional phenotypic features are mentioned in the text and in Tables 1 and 2. Chemotaxonomic properties are typical of the genus *Streptomyces*.

The type and only strain, C60^T (=CGMCC 4.7018^T=KACC 15492^T), was isolated from the Valle de la Luna region of the Atacama Desert, north-west Chile. The species description is based on a single strain and hence serves as the description of the type strain. The DNA G + C content of the type strain is 71.5 mol%.

Acknowledgements

A. T. B. thanks the Leverhulme Trust for an Emeritus Fellowship, and A. T. B. and J. A. S. thank the Royal Society for their International Joint Project grant JP100654; the work was also funded in part by the National Science Foundation of China (grant 31100003).

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