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ORIGINAL PAPER

# Diversity of culturable actinomycetes in hyper-arid soils of the Atacama Desert, Chile

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**Abstract** The Atacama Desert presents one of the most extreme environments on Earth and we report here the first extensive isolations of actinomycetes from soils at various locations within the Desert. The use of selective isolation procedures enabled actinomycetes to be recovered from arid, hyper-arid and even extreme hyper-arid environments in significant numbers and diversity. In some cases actinomycetes were the only culturable bacteria to be isolated under the conditions of this study. Phylogenetic analysis and some phenotypic characterisation revealed that the majority of isolates belonged to members of the genera *Amycolatopsis*, *Lechevalieria* and *Streptomyces*, a high proportion of which represent novel centres of taxonomic variation. The results of this study support the view that arid desert soils constitute a largely unexplored repository of novel bacteria, while the high incidence of non-ribosomal peptide synthase genes in our isolates recommend them as promising material in screening for new bioactive natural products.

**Keywords** Culturable actinomycetes · Atacama Desert · Hyper-arid soils

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## Introduction

In the search for novel natural products that can be developed as a resource for biotechnology we have focussed on the discovery of novel organisms and argued that the latter may be found particularly, although not exclusively, in unusual and under-explored environments. In this context the marine environment is proving to be a major source of new natural products, including antimicrobial and anti-cancer compounds, most notably being expressed by actinomycete bacteria (Bull et al. 2005; Bull and Stach 2007).

Another under researched biome in terms of its microbiota are desert soils. The Atacama Desert in northern Chile is a coastal desert (c. 20°S to 30°S) usually cited as the driest on Earth (Clarke 2006). It is the world's oldest continuously arid desert (Hartley et al. 2005) most of which is classified as hyper-arid (Houston 2006), i.e. the ratio of mean annual rainfall (MAR) to mean annual evaporation (MAE) is less than 0.05, and parts of which are defined as extreme hyper-arid (ratio $< 0.002$). The harshness of this

environment is further emphasised by high UV radiation, frequent presence of inorganic oxidants, very low concentrations of organic carbon, and, in several areas, by high salinity. These conditions may appear inimical to life and it has been suggested that those prevailing in the extreme Yungay area of the Atacama approach the dry limit of microbial survival (Navarro-Gonzalez et al. 2003). However, the few surveys that have been made of microorganisms in Atacama Desert soils reveal a diversity of bacteria and one of the most thorough studies has shown that viable bacteria and amplifiable DNA can be recovered from these soils (Drees et al. 2006).

Drees and his colleagues sampled sub-surface soils across an east-west transect of the Atacama Desert at elevations ranging from 400 m in the barren coastal range to over 4,000 m in the vegetated high Andean steppe. Culturable bacteria were isolated from all but one location with the highest counts occurring in soils sampled from the perennial vegetation zone above 3,500 m. Molecular profiling using denaturing gradient gel electrophoresis (DGGE) revealed a unique community in hyper-arid soils dominated by *Gemmatimonadetes* and *Planctomycetes*. In other recent studies, 16S rRNA gene and phospholipid fatty acid profiles (PLFA) derived from hyper-arid surface and subsurface soils at Yungay sites were used in a broad characterisation of the bacterial community (Connon et al. 2007; Lester et al. 2007). These analyses showed that the major components of the community were members of the class *Actinobacteria* (up to 94%). A very small number of *Actinobacterium* clones have been reported in water taken from sinkholes close to lake Tebenquiche located in the northern part of the Salar de Atacama (Demergasso et al. 2008).

The present investigation was an attempt to isolate members of a targeted bacterial group—the order *Actinomycetales*—from Atacama Desert soils. This emphasis on actinomycetes is based on their pre-eminence as producers of bioactive compounds and the revival of interest in natural product search and discovery (Bull 2004; Bull and Stach 2007). Our strategy for successful biodiscovery campaigns has been based on the premise that extreme environments, such as deep seas and polar soils, are likely to contain novel microorganisms that in turn have the capacity to produce novel metabolites. This view is extended in the present analysis of hyper-arid desert soils. Given that only a minute fraction of bacterial diversity has been characterised and has a log-normal distribution (Curtis et al. 2002), we predict that novel chemodiversity is likely to be found in so-called 'rare' and as-yet-to-be-cultured organisms. In the present study, therefore, we have used selective isolation conditions to recover actinomycetes, and taxon- and gene-specific oligonucleotide probes to screen the resulting culture libraries. Here we report initial findings of actinomycete diversity in three Atacama Desert soils selected on the basis of the degree of aridity, salinity, and elevation.

## Materials and methods

### Soils

Samples were collected from the top 5 cm of soil using a sterile scoop, placed in sterile polycarbonate bottles and sealed with insulating tape (Table 1). The resultant samples were held at ambient temperature for a maximum of 5 days before permanent storage at 4°C.

Bulk pH of the soil samples was determined following the method of Reed and Cummings (1945). Conductivity measurements were made on clear filtrates following shaking 2 g of each soil sample with 20 ml distilled water and filtering using the procedure described by Avery and Bascomb (1982). Triplicate samples (ca. 1 g) were dried to constant weight at 105°C and the moisture contents recorded as the average percentage loss of weight. The dried soil samples were then placed in a muffle furnace (Carbolite, Sheffield, England, UK) and the temperature raised slowly to 700°C and kept constant for 30 min in order to completely combust the organic matter prior to overnight cooling and reweighing; the average percentage weight loss was recorded as the organic matter content.

### Selective isolation, enumeration and maintenance of putative actinomycetes

Soil samples (1 g) were mixed with ¼ strength Ringer's solution to give $10^{-1/2}$ and $10^{-1}$ dilutions, blended (1 min) in an Ultra-Turrax T25 homogeniser (Janke and Kunkel, Staufen, Germany) then shaken (1 h) on a tumble-shaker (TMI Tumbler, Luckham

**Table 1** Atacama desert sampling locations

| Location | Latitude | Longitude | Elevation (m asl) | Date | Biome | MAR/MAE[a] |
|---|---|---|---|---|---|---|
| El Tatio (near middle basin) | 22°19′S | 68°06′W | c.4,300 | 3-10-2004 | Geyser field[b] Arid | 0.077 |
| Salar de Atacama, Laguna de Chaxa | 23°17′S | 68°10′W | 2,300 | 4-10-2004 | Salt flat[b] Hyper-arid | 0.009 |
| Valle de la Luna, Cueva de Sal | 23°02′S | 68°20′W | 2,450 | 5-10-2004 | Absolute desert[b] Extreme hyper-arid | 0.002 |

[a] *MAR* mean average rainfall, *MAE* mean average evaporation. Ratio estimated from data provided in Houston (2006) and Dirección Meteorológica de Chile (http://www.meteochile.cl/)

[b] Zero vegetation cover

Ltd, Sussex, UK). Aliquots (0.1 ml) of each dilution were spread over the surfaces of six selective isolation media (Table 2) that had been dried for 15 min, as recommended by Vickers et al. (1984). Samples used to inoculate the starch–casein and raffinose–histidine agar plates were heated in a water bath at 55°C for 6 min. In all cases four plates per dilution were inoculated and incubated at 28°C for 21 days. Numbers of presumptive actinomycetes and total culturable bacteria were counted and the results expressed as mean colony forming units (cfu) per gram dry weight soil.

The selective isolation plates were examined by eye and by binocular microscopy (Olympus Optical Co. Ltd, Tokyo, Japan). Putative actinomycetes were taken from the various isolation media and inoculated onto modified Bennett's agar plates (Jones 1949) and incubated for 7 days at 28°C. Stock cultures were prepared for each strain by transferring mycelium and spores from each of the purified isolates into cryotubes containing 1.5 ml 20% (w/v) sterile glycerol solution (Wellington and Williams 1978) and stored at −20°C.

## Circumscription of isolates

Putative actinomycetes were inoculated onto oatmeal (Küster 1959) and peptone-yeast extract-iron agar (Shirling and Gottlieb 1966) plates which were incubated at 25°C for 14 and 4 days, respectively. Aerial spore mass colour, reverse substrate mycelium pigmentation and the colour of any diffusible pigments were recorded on the oatmeal agar plates using a National Bureau of Standards (NBS) Colour

**Table 2** Selective media used for the selective isolation of actinomycetes

| Media | Selective agent | Target organism(s) | Reference |
|---|---|---|---|
| Diagnostic sensitivity test agar | Cyclohexamine (25 μg $ml^{-1}$), tetracycline hydrochloride (32 μg $ml^{-1}$) | *Nocardia* | Orchard et al. (1977) |
| Glucose-yeast extract agar | Rifampicin (20 μg $ml^{-1}$) | *Actinomadura* | Gordon and Mihm (1962); Athlaye et al. (1981) |
| Raffinose-histidine agar | Cyclohexamide (25 μg $ml^{-1}$), nystatin (25 μg $ml^{-1}$) | Rare or uncommon streptomycetes, selective against *Streptomyces albidoflavus* | Vickers et al. (1984) |
| Starch-casein agar | Cyclohexamide (25 μg $ml^{-1}$), nysatin (25 μg $ml^{-1}$) | *Streptomyces* | Küster and Williams (1964) |
| SM1 agar | Neomycin (4 μg $ml^{-1}$), nystatin (50 μg $ml^{-1}$), D(+) sorbitol (1%, w/v) | *Amycolatopsis* | Tan et al. (2006) |
| SM2 agar | Neomycin (4 μg $ml^{-1}$), D(+) melezitose (1%, w/v), nystatin (50 μg $ml^{-1}$) | *Amycolatopsis* | Tan et al. (2006) |

Name Chart (Kelly 1958); the peptone-yeast extract-iron agar plates were examined for the production of melanin pigments. The isolates were assigned to colour groups on the basis of the recorded properties thereby following the procedure described by Williams et al. (1969). It has been shown repeatedly that colour groups provide a valuable index of taxonomic diversity of streptomycetes in natural habitats (Sembiring et al. 2000; Antony-Babu and Goodfellow 2008).

### Molecular and phylogenetic methods

Total genomic DNA was extracted from 81 representative isolates that were positive for *LL*- or *meso*-diaminopimelic acid ($A_2$ pm) using Gen Elute™ Bacterial Genomic Kits (Sigma), following the manufacturer's protocol for the analysis of Gram-positive bacteria, and stored at −20°C prior to use. The quality of the extracted DNA preparations was checked by agarose gel electrophoresis. PCR amplification and the direct sequencing of 16S rRNA genes were made as described by Kim et al. (1998). The sequence of the complete lengths of the 16S rRNA genes of the representative isolates was determined in separate cycles using two internal forward primers (27f and MG3f) (Lane 1991; Chun 1995) and one reverse oligonucleotide primer (1525r, Kuroda et al. 2001). The two forward and the single reverse complementary 16S rRNA gene sequences were manually assembled using the PHYDIT programme, version 1 (Chun 1995), and aligned against corresponding sequences of representatives of the relevant genera retrieved from GenBank (NCBI, USA). *Amycolatopsis*-specific 16S rRNA fragment amplification was made using the method of Tan et al. (2006).

Phylogenetic trees and similarity matrices were constructed using the PHYLIP suite of programmes (Felsenstein 1993) and rooted trees were deduced using neighbour-joining (Saitou and Nei 1987), maximum-parsimony (Kluge and Farris 1969) and maximum-likelihood (Felsenstein and Churchill 1996) tree-making algorithms; the *Amycolatopsis*; *Lechevalieria* and *Streptomyces* trees were rooted using the type strains of *Amycolatopsis fastidiosa*, *Actinosynnema mirium* and *Streptomyces albus* as outgroups. The topology of the resultant trees were evaluated by bootstrap analyses (Felsenstein 1985). Phylogenetic trees were prepared using TREECON version 1.3b software (Van de Peer and De Wacther 1994). 16S rRNA gene sequences were compared with those of corresponding type strains and related marker strains of the appropriate genus.

### Phenotypic characterisation

Phenotypic characterisation based on a range of established biochemical, substrate degradation and physiological tests (Gordon et al. 1974; de Boer et al. 1990; Goodfellow et al. 2001) were made on 8 *Amycolatopsis* and 3 *Lechevalieria* isolates that were assigned to these taxa on the basis of 16S rRNA gene sequence data. All tests were carried out in duplicate.

Spore chain morphology (Pridham et al. 1958) and spore surface ornamentation (Tresner et al. 1961; Dietz and Mathews 1971; Locci 2006) were recorded for representative strains of *Amycolatopsis* (2) and *Streptomyces* (6). The micromorphological properties of the *Lechevalieria* isolates was examined on glucose-yeast extract agar (Gordon and Mihm 1962).

Representative members of the colour groups were tested for isomers of diaminopimelic acid (Hasegawa et al. 1983), a standard solution (10 mM) of $A_2$ pm (Sigma) containing a mixture of *DL*- *LL*- and *meso*-$A_2$ pm isomers was used as a reference. This procedure is widely used to distinguish streptomycetes from other spore-forming actinomycetes as whole-organism hydrolysates of the former contain the LL-isomer and the latter the *meso*-isomer of diaminopimelic acid (Williams et al. 1989; Tan et al. 2006).

A selection of *Amycolatopsis*, *Lechevalieria* and *Streptomyces* strains were screened for non-ribosomal peptide synthase (NRPS) genes using the procedures of Ayuso-Sacido and Genilloud (2005).

## Results

### Physicochemical and bacteriological characteristics of soils

The physicochemical properties and total viable bacterial and putative actinomycete counts for the three soils are shown in Tables 3 and 4.

**Table 3** Physico-chemical properties of the soils

| Soil | pH | Moisture content (%) | Total organic matter (%) | Electrical conductivity (dS $m^{-1}$) |
|---|---|---|---|---|
| El Tatio | 7.3 | 0.004 | 0.02 | 0.126 |
| Salar de Atacama | 7.7 | 0.007 | 0.03 | 0.134 |
| Valle de la Luna | 7.6 | 0.004 | 0.03 | 1.540 |

**Table 4** Total viable bacterial counts (cfu $g^{-1}$ soil) and percentage of putative actinomycetes

| Soil | Isolation medium | | |
|---|---|---|---|
| | Starch-casein | Raffinose-histidine | Glucose-yeast extract |
| El Tatio | $3.7 \times 10^3$ (73) | $8.7 \times 10^3$ (11) | $1.4 \times 10^3$ (11) |
| Salar de Atacama | $9.1 \times 10^3$ (37) | $2.6 \times 10^4$ (13) | 0 |
| Valle de la Luna | $1.4 \times 10^3$ (7) | $2.0 \times 10^2$ (100) | $5.0 \times 10^2$ (100) |

Values in parentheses indicate putative actinomycetes as percentage of total viable bacteria

Total viable bacteria and putative actinomycete recoveries on SM1, SM2 and diagnostic sensitivity Test media were poor and zero in the case of the Valle de la Luna soil

## Preliminary circumscription of actinomycetes

Organisms putatively identified as actinomycetes were distinguished on the basis of characteristic colonial morphology, notably the ability to form substrate mycelia and, in many cases, aerial hyphae. No attempts were made to characterise the non-actinomycete bacteria. One hundred and forty-seven actinomycetes representing different colony types were subcultured onto oatmeal and peptone-yeast extract-iron agars and subsequently assigned to 21 multi-membered and 10 single-membered colour groups. Whole-organism hydrolysates of representatives of the colour groups were examined for the presence of isomers of diaminopimelic acid; 35 isolates (76%) contained *LL*-$A_2$ pm and 11 (24%) *meso*-$A_2$ pm.

## Phylogenetic analysis

The 46 strains categorised on the basis of their $A_2$ pm profiles were subject to 16S rRNA gene sequence analyses and were found to be members of the following genera: *Amycolatopsis* (8 strains, *meso*-$A_2$ pm; family *Pseudonocardiaceae*), *Lechevalieria* (3 strains, *meso*-$A_2$ pm; family *Actinosynnemataceae*), and *Streptomyces* (35 strains, *LL*-$A_2$ pm; family *Streptomycetaceae*).

The *Amycolatopsis* isolates had lineages that were distinct from each other and from members of the genus *Amycolatopsis* and formed a distinct subclade the taxonomic integrity of which were supported by all the three tree-making algorithms (Fig. 1). All of the isolates produced a signature amplification product with the *Amycolatopsis*-specific primers and with the exception of isolate C17 (El Tatio), they were isolated from the Salar de Atacama soil. The *Amycolatopsis* isolates are most closely related to the members of the *A. methanolica* subclade, a taxon which is supported by all of the tree-making algorithms. The highest 16S rRNA gene similarity value between members of the two subclades was between isolates C29 and *A. eurytherma* DSM 44348$^T$; these organisms showed a similarity of 98.4%. In contrast, the highest 16S rRNA gene similarity between members of the *A. methanolica* subclade is between the type strains of *A. methnolica* and *A. thermoflava*; these strains have a similarity of 98.8%.

The *Lechevalieria* isolates formed a distinct phyletic line within the *Lechevalieria* 16S rRNA gene tree, a taxon supported by all of the tree-making algorithms (Fig. 2). The isolates shared similarities within the range 99.3–99.4%. The highest 16S rRNA nucleotide similarities between the isolates and the *Lechevalieria* type strains was between isolates C68 and *L. xinjiangensis* DSM 45081$^T$, these organisms shared a similarity of 98.7%. All the *Lechevalieria* isolates were obtained from the Salar de Atacama soil.

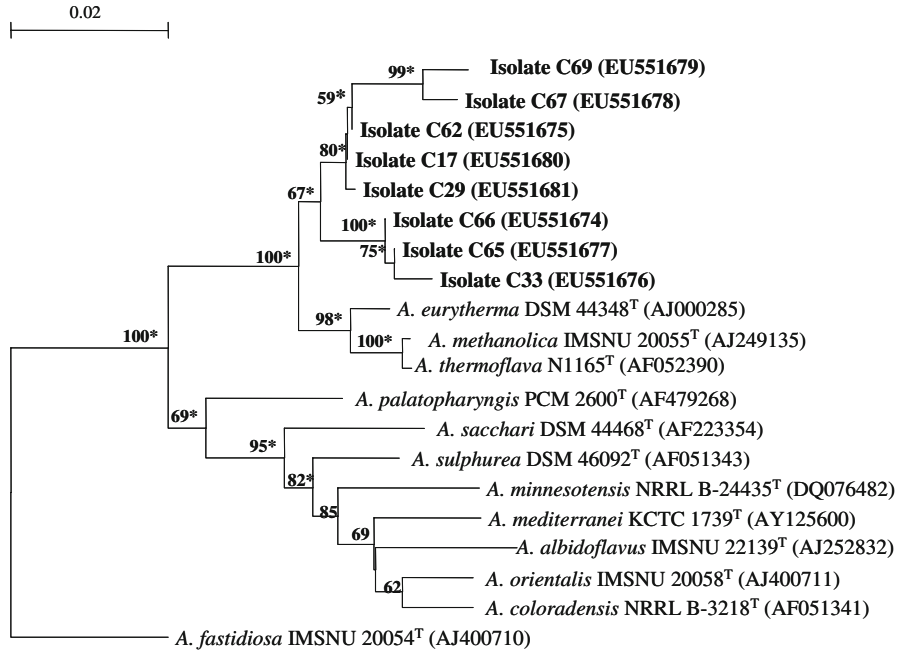



**Fig. 1** Neighbour-joining tree based on nearly complete 16S rRNA gene sequences showing relationships between the *Amycolatopsis* isolates and between them and the most closely related *Amycolatopsis* type strains. *Asterisks* indicate branches of the tree that were also recovered using the maximum-parsimony tree-making algorithms. *Numbers* at the nodes indicate the levels of bootstrap support based on a neighbour-joining analysis of 1,000 re-sampled datasets; only values above 50% are given. The *scale bar* indicates 0.02 substitutions per nucleotide position

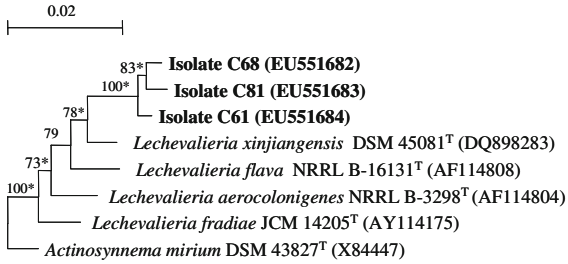
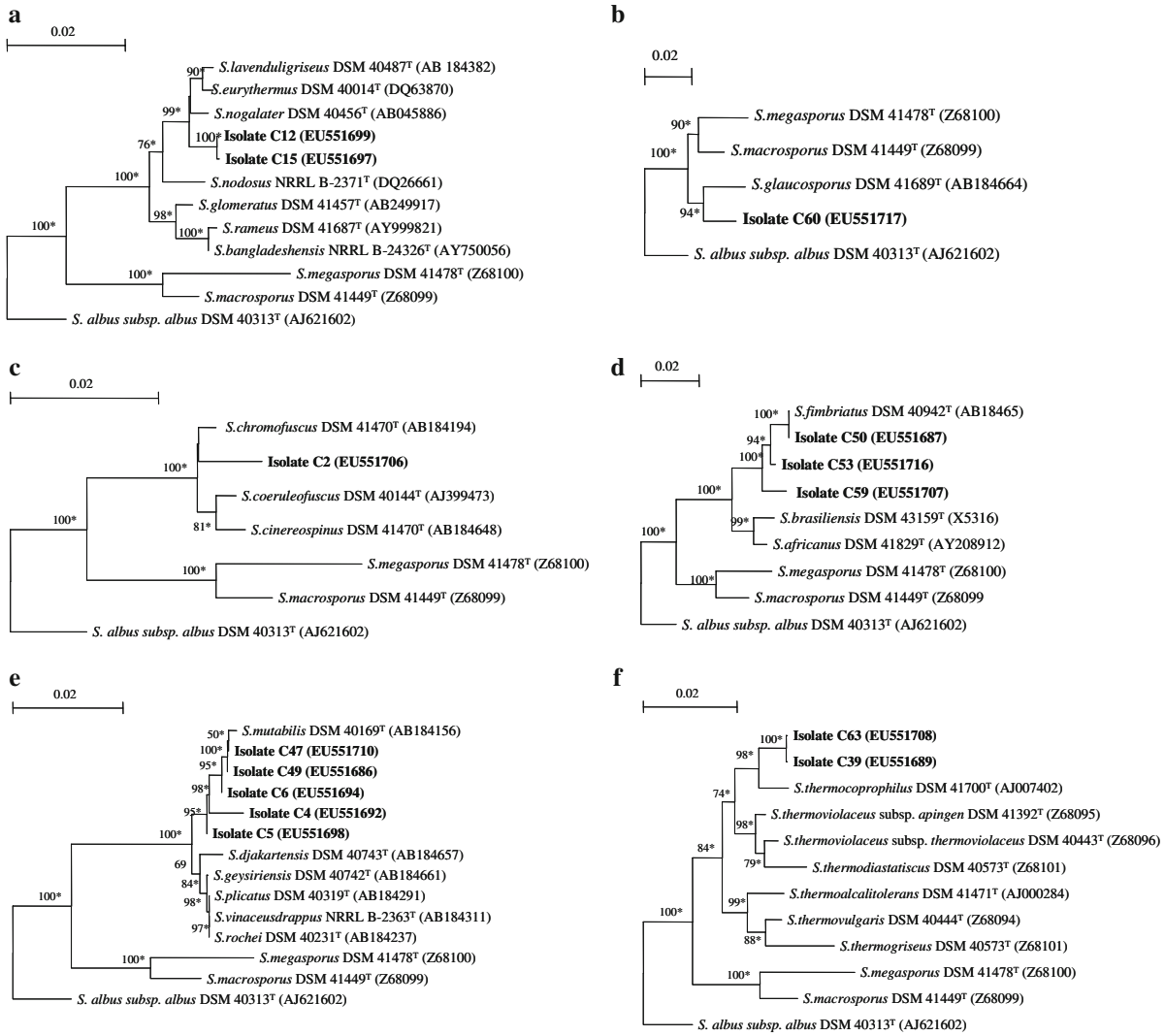



**Fig. 2** Neighbour-joining tree based on nearly complete 16S rRNA gene sequences showing relationships between isolates C61, C68 and C81, and between them and the type strains of *Lechevalieria* species. Other details are given in the legend to Fig. 1

Most of the 16S rRNA sequencing studies were focused on *Streptomyces* strains which were isolated from all three soils but most frequently from the Salar de Atacama. Strains isolated from the El Tatio soil formed a distinct subclade in the *Streptomyces* 16S rRNA gene tree with the type strains of *S. eurythermus*, *S. lavenduligriseus* and *S. nogalater*, an association supported by all the tree-making algorithms (Fig. 3a). The isolates shared a 99.9% rRNA gene similarity and were most closely related to *S. nogalater* DSM 40456$^T$ (99.2 and 99.3% 16S rRNA gene similarities). The 16S rRNA gene sequence of a streptomycete strain, isolate C60, recovered from the extreme hyper-arid soil of the Valle de la Luna formed a distinct subclade with the type strains of *S. glaucosporus, S. macrosporus* and *S. megasporus* (Fig. 3b). This isolate is most closely related to *S. glaucosporus* DSM 41689$^T$ sharing a 16S rRNA gene similarity with the latter of 97.5%. Some of the *Streptomyces* isolates from the Salar de Atacama soil fell into four, relatively small, but distinct taxa, namely, the *S. coeruleofuscus* (isolate C2; Fig. 3c); *S. fimbriatus* (isolates C50, C53, and C59; Fig. 3d), *S. mutabilis* (isolates C4, C5, C6, C47 and C49; Fig. 3e) and *S. thermocoprophilus* (isolates C39 and C63; Fig. 3f) subclades.

Isolate C2 was most closely related to *S. chromofuscus* DSM 41470$^T$; the two strains shared a 16S rRNA gene similarity of 99%. Similarly, isolates C50, C53 and C59 were most closely related to *S. fimbriatus* DSM 40942$^T$. Indeed, isolate C50 and the type strain of *S. fimbriatus* have identical 16S rRNA gene sequences while isolates C53 and C59 shared similarities with this organism of 93.9 and 97.7%, respectively. In turn, isolates C4, C5, C6, C47 and C49 were closely related to *S. mutabilis* DSM 40169$^T$. All of these isolates, apart from C4, showed 16S rRNA similarities with the *S. mutabilis* strain within the range 99.7–99.9%. Isolate C4 showed a much lower similarity, namely 99%, with the *S. mutabilis* strain. Isolates C39 and C63 had an almost identical 16S rRNA gene sequence (a single




**Figs. 3** Neighbour-joining tree based on nearly complete 16S rRNA gene sequences showing relationships between some of the *Streptomyces* isolates and between them and related *Streptomyces* type strains. Other details are given in the legend to Fig. 1

nucleotide difference at 1,395 locations) and were closely related to *S. thermocoprophilus* DSM 41700$^T$; the two isolates shared a similarity with the latter of 98.9%.

It is particularly interesting that 21 out of the 33 (64%) streptomycetes isolated from the Salar de Atacama sample formed a well delineated subclade in the *Streptomyces* 16S rRNA gene tree; the type strains of *S. coeruleoprunus*, *S. fradiae*, *S. griseosporus* and *S. somaliensis* were loosely related to this taxon (Fig. 4). Ten of the isolates shared similarities of over 99.8%, while the two most distantly related strains, isolates C18 and C23 shared a 98.3% 16S rRNA gene similarity. The closest phylogenetic relationships between the isolates and the four *Streptomyces* marker strains were between isolates C25 and C51 and *S. somaliensis* DSM 40778$^T$ (98.5 and 98.6% similarity, respectively). Similarly, these isolates showed relatively high 16S rRNA gene similarities with *S. griseosporus* (98.4 and 98.5%).

## Phenotypic characterisation

The suite of phenotypic tests used in this study enabled all 8 isolates of *Amycolatopsis* to be distinguished from the type strains of *A. eurytherma*,

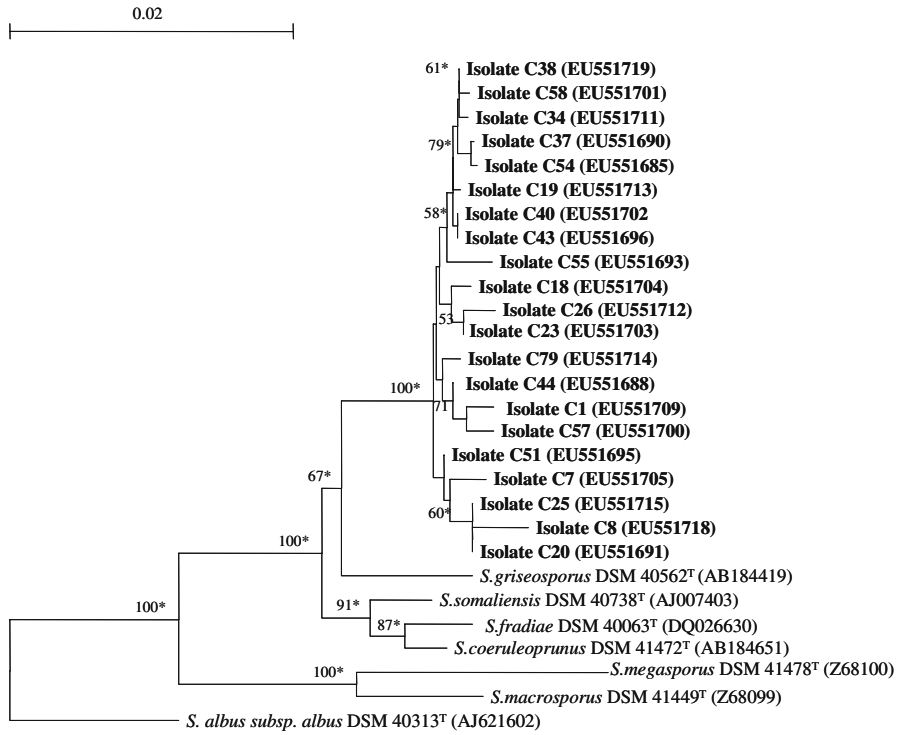



**Fig. 4** Neighbour-joining tree based on nearly complete 16S rRNA gene sequences showing relationships between 21 *Streptomyces* isolates and between them and their closest phylogenetic neighbours. Other details are given in legend to Fig. 1

*A. methanolica* and *A. thermoflava*, while the type strains themselves can be distinguished from one another using a combination of phenotypic properties (de Boer et al. 1990; Chun et al. 1999). Unlike the type strains, the *Amycolatopsis* isolates produced acid from dextrin and *meso*-inositol but were negative for aesculin hydrolysis and tyrosine degradation. Differential properties that can be weighted to distinguish between the *Amycolatopsis* isolates include allantoin hydrolysis, nitrate reduction and the capacity to degrade uric acid, xanthine and xylan. Similarly, the 3 *Lechevalieria* isolates were differentiated from the type strains of *L. aerocolonigenes, L. flava, L. fradiae* and *L. xinjiangensis* on the basis of their phenotypes. Unlike the latter none of the isolates hydrolysed urea, reduced nitrate, grew at 45°C or used sodium malonate or sorbitol as sole carbon sources. The isolates also exhibited distinct phenotypic profiles as shown by their capacity to produce acid from adonitol, hydrolyse aesculin, degrade arbutin and grow in the presence of 4%, w/v NaCl.

Representative isolates were examined for their spore chain morphology and spore surface ornamentation. Thus, strains C65 and C69 formed aerial and substrate hyphae that fragmented into spore-like cylindrical elements that are characteristic of members of the genus *Amycolatopsis*. Strains C68 and C81 formed a substrate mycelium that fragmented into rod-shaped elements, characteristic of members of the genus *Lechevalieria*. The remaining strains presented a range of morphological properties typical of streptomycetes such as spore chains in open spirals and loops. Full details of phenotypic properties will appear in subsequent papers describing the taxonomy of novel strains.

Thirty-five out of the 46 phylotypes tested gave a PCR amplification product of the expected size (~700 bp) with the NRPS primers A3F and A7R. NRPS gene clusters were detected in all of the *Amycolatopsis* and *Lechevalieria* isolates, and in 24 out of the 35 *Streptomyces* strains. Preliminary BLAST searching revealed that the nearest matching sequences for the *Amycolatopsis* isolates included a putative NRPS gene cluster in "*Amycolatopsis lactamadurans*", isolate AAL35, an erythromycin producing organism, *Saccharopolyspora erythraea*, which also produces erythromycin, and a peptide-3 biosynthetic gene cluster in *Streptomyces avermitilis*. Maximum identity values for these matches ranged from 72 to 79%. Of the *Lechevalieria* strains only

strain C81 gave a significant match, namely with *Frankia alni,* a calcimycin-producing actinomycete with a maximum identity value of 70%.

Eight of the *Streptomyces* isolates gave nearest matches either to the *S. avermitilis* peptide-2 biosynthetic gene cluster, the *S. filamentosus* daptomycin biosynthetic gene cluster, the *S. fungicidius* DXP synthases 2 gene cluster and or to the *S. verticillus* bleomycin biosynthetic gene cluster; maximum identity values ranged from 70 to 92%.

## Discussion

Few comprehensive analyses have been attempted on the bacterial diversity of hyper-arid deserts and focussed studies of actinomycetes in these environments have been completely neglected. The present study is the first in which selective isolation procedures have been applied to explore the taxonomic diversity of actinomycetes in soils of "the driest desert in the world" (Clarke 2006). The soils that we surveyed are representative of arid (El Tatio) and hyper-arid (Salar de Atacama and Valle de la Luna) types. El Tatio is exposed to very high solar radiation, including elevated UV-B, while Salar de Atacama is the largest salt flat in Chile some of whose soils contain unusually high concentrations of lithium. The most extreme of these soils, collected in the Cueva de Sal (an ancient high saline deposit within the Valle de la Luna), is defined as extreme hyper-arid on the basis of MAR/MAE data.

In view of very low or zero viable bacterial counts and the inability to recover amplifiable DNA from arid core soils of the Yungay area of the Atacama Desert, it has been postulated that this environment represents the dry limit of microbial life on the planet (McKay et al. 2003; Navarro-Gonzalez et al. 2003). However, evidence of bacteria in such absolute Atacama Desert soils has come from Drees et al. (2006), Connon et al. (2007) and Lester et al. (2007) and is extended by our findings. The major differences between these studies were elevation (1,460 m and hence lower, Lester et al. loc cit), total organic matter (slightly lower in the soil examined by Lester et al. loc cit) and salinity (high EC of 1.540 dS $m^{-1}$ for the Cueva de Sal site, present study) (Table 5). Viable bacteria were recovered from all of the soils in our study although maximum counts were up to 100-fold lower than those reported by Drees et al. (2006), a probable consequence of our decision to use actinomycete-selective media. However, our culturable counts compare very closely with those of Lester et al. (2007) despite using different recovery media. In none of the other Atacama bacteriological studies cited above were actinomycetes isolated although culture-independent analyses have revealed phylotypes belonging to the family *Rubrobacteriaceae* (Drees et al. 2006; Connon et al. 2007; Lester et al. 2007), and actinobacterial clones distantly related to members of the genus *Frankia* (Connon et al. 2007). Actinomycete phylotypes and isolates have been reported from arid soils of the Sahara and Sonoran Deserts (Nagy et al. 2005; Boudjella et al. 2006; Chanal et al. 2006) where they may be the dominant or most common members of the bacterial communities.

In the light of previous surveys of bacteria in Atacama Desert soils the density of actinomycetes that we revealed is remarkably high and reflects the use of a range of actinomycete-selective isolation media and relatively long incubation times. Such densities are all the more remarkable because they relate to all degrees of soil aridity, including the extreme hyper-arid Valle de la Luna from which, under the conditions of this study, the only culturable bacteria were actinomycetes ($1–5 \times 10^2$ cfu $g^{-1}$). The highest actinomycete counts were recorded for the El Tatio (arid) and Salar de Atacama (hyper-arid) soils, but at this stage we do not have sufficient data

**Table 5** Comparison of physico-chemical properties of Atacama absolute desert sites

| Soil character | Drees et al. (2006)[a] | Connon et al. (2007) and Lester et al. (2007)[b] | Present study[b] |
|---|---|---|---|
| pH | 7.01–7.54 | 6.8–7.3 | 7.6–7.7 |
| TOC (%) | 0.02–0.03 | 0.05–0.07 | 0.03 |
| EC (dS $m^{-1}$) | 0.01–0.35 | 0.015–0.036 | 0.134–1.540 |
| TDS[c] (mg $L^{-1}$) | 6.4–224 | 23 | 85.8–985.6 |

[a] Sub-surface (25–30 cm) soil; [b] surface soil; [c] total dissolved salts

to enable correlations to be made between abundance and soil type or location. Single correlations were not found between actinomycete abundance and either moisture, organic matter, pH or salinity, although the lower counts at the Valle de la Luna site may be related at least in part to its very high salinity.

The greatest diversity of actinomycetes was recovered from the hyper-arid soil (Salar de Atacama) and of the taxa identified 88, 100 and 91% of members of the genera *Amycolatopsis*, *Lechevalieria* and *Streptomyces* were from this site. Again, attempts to correlate taxonomic distribution with soil type are premature. Although only members of the above three genera were identified conclusively, it is reasonable to predict that the actinomycete diversity in these soils is substantially higher based on the plurality of colour groups observed.

It has been repeatedly shown that *Amycolatopsis* type strains with almost identical 16S rRNA gene sequences have DNA:DNA relatedness values well below the 70% cut-off point recommended by Wayne et al. (1987) for the delineation of strains that belong to the same genomic species. The type strains of *A. methanolica* and *A. thermoflava*, for instance, have almost identical 16S rRNA gene sequences but were found to have DNA relatedness values of only 21% when the former was used as the probe (Chun et al. 1999). It can be concluded, therefore, that not only do none of the isolates belong to validly described *Amycolatopsis* species but that several of them form the nuclei of novel *Amycolatopsis* species though further comparative studies are needed to establish this. Similarly the *Lechevalieria* isolates are putative new taxa with strains C61, C68 and C81 forming a distinct subclade within the *Lechevalieria* 16S rRNA gene tree. The highest 16S rDNA similarity between the *Lechevalieria* type strains was with *L. flava* NRRL B-16131$^T$ and *L. xinjiangensis* DSM 45081$^T$ but although these organisms have a relatively high sequence similarity (98.8%) they have a DNA:DNA relatedness of only 28% (Wang et al. 2007), a value well below the recommended cut-off point for the delineation of bacterial species (Wayne et al. 1987). It is clear, therefore, that the *Lechevalieria* isolates cannot be assigned to any of the validly described *Lechevalieria* species.

Streptomycetes remain a unique source of new secondary metabolites, notably antibiotics (Watve et al. 2001; Bérdy 2005) but it is becoming increasingly difficult to discover commercially useful metabolites from members of common *Streptomyces* species. Hence the need to isolate, characterise and screen novel streptomycetes. The taxonomic and metabolic diversity encompassed by the genus *Streptomyces* is extraordinary as new and putatively novel *Streptomyces* species are being isolated from un- and under-explored habitats and shown to be a rich source of new and interesting bioactive compounds, including antibiotics (Bull et al. 2005; Fiedler et al. 2005). The 16S rRNA sequence data show that many of the *Streptomyces* strains isolated from the Atacama Desert soils belong to putatively new species as they are separated from their known phylogenetic neighbours by sequence similarities well below those found between members of closely related *Streptomyces* species such as those classified in the *S. griseus* (Lu et al. 2005) and *S.violaceusniger* clades (Goodfellow et al. 2007). Most interesting is the heterogeneous, monophyletic and sharply defined clade that encompasses 21 streptomycetes isolated from the Salar de Atacama soil. Taxonomic descriptions of the new species discovered in this study will be the subject of subsequent publications.

From a potential biotechnology perspective it is notable that all of the *Amycolatopsis* and *Lechevalieria* and a majority of the *Streptomyces* isolates proved positive for the presence of NRPS genes. NRPS gene clusters have been detected recently in *Lechevalieria* species (Ayuso-Sacido and Genilloud 2005) that are involved in the synthesis of antitumor (Onaka 2006; Sanchez et al. 2006) and antifungal (Lee et al. 2004) compounds. Our own preliminary survey based on a comparison of gene sequence identities with those involved in the synthesis of known bioactive compounds promises that among those encoded by Atacama actinomycetes are novel NRP synthases.

## Conclusions

At present, overall conclusions regarding the bacterial diversity of the Atacama Desert must be made with caution due to the different culture dependent and culture-independent methods that have been deployed, the sites that have been surveyed, and, as pointed out by Lester et al. (2007) the "spatial and possibly temporal heterogeneities in microbial

distribution" that undoubtedly occur. Lozupone and Knight (2007) have proposed that the major environmental determinant of bacterial diversity is salinity and, if this hypothesis is borne out, the saline soils of the Atacama Desert could be prime targets for discovering novel micro-biodiversity and natural products. The range of salinity of soils sampled in our investigation was considerable but the effect of salinity per se on actinomycete isolation was outside the scope of the study. On the other hand others, including Connon et al. (2007), state that water availability is the primary controlling factor for microbial activity and diversity in desert soils. It is known that water contents of Atacama desert soils are subject to day-night variations (e.g., mediated by fog) and that even the most arid regions of the Atacama receive intermittent rainfall; moreover, as may be the case at our low lower altitude sites, these soils will be subject to variable moisture contents as a result of high water tables controlled by precipitation in the Andes. On the basis of present data we do not presume that the actinomycetes that we have isolated are indigenous to the Atacama Desert. What is clear, however, is that members of novel actinomycete taxa can be isolated from these soils that present promising biotechnology opportunities.

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