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ORIGINAL PAPER

Hunting for cultivable *Micromonospora* strains in soils of the Atacama Desert

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Abstract Innovative procedures were used to selectively isolate small numbers of *Micromonospora* strains from extreme hyper-arid and high altitude Atacama Desert soils. Micromonosporae were recognised on isolation plates by their ability to produce filamentous microcolonies that were strongly attached to the agar. Most of the isolates formed characteristic orange colonies that lacked aerial hyphae and turned black on spore formation, whereas those from the high altitude soil were dry, blue-green and covered by white aerial hyphae. The isolates were assigned to seven multi- and eleven single-membered groups based on BOX-PCR profiles. Representatives of the groups were assigned to either multi-membered clades that also contained marker strains or formed distinct phyletic lines in the *Micromonospora* 16S rRNA gene tree; many of the isolates were considered to be putatively novel species of *Micromonospora*. Most of the isolates from the high altitude soils showed activity against wild type strains of *Bacillus subtilis* and *Pseudomonas fluorescens* while those from the rhizosphere of *Parastrephia quadrangulares* and from the Lomas Bayas hyper-arid soil showed resistance to UV radiation.

Keywords *Micromonospora* · Atacama Desert · BOX-PCR · Polyphasic taxonomy · UV radiation

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Introduction

Given their importance in biotechnology, bioprospecting and ecology, actinobacteria remain sources of interest to the microbiological community (Goodfellow and Fiedler 2010; Demain 2014; Barka et al. 2016). Actinobacteria were initially isolated from the Atacama Desert over fifty years ago (Cameron et al. 1966; Opfell and Zebal 1967) though the first extensive survey of these organisms in this desert was only reported recently (Okoro et al. 2009). Okoro and her colleagues isolated small numbers of filamentous actinobacteria from arid, hyper-arid and extreme hyper-arid Atacama Desert soils using selective isolation procedures and polyphasic

taxonomic methods, which showed that a high proportion of the isolates belonged to the genera *Amycolatopsis, Lechevaleria* and *Streptomyces*, many of which were assigned to putative new species and contained novel non-ribosomal peptide synthase genes. Subsequent studies have shown that Atacama Desert habitats are not only a rich source of novel streptomycetes but also of rare and poorly studied taxa, some of which produce bioactive compounds and have been given validly published names (Bull et al. 2016; Busarakam et al. 2014, 2016; Goodfellow et al. 2017; Idris et al. 2017a, b; Trujillo et al. 2017). *Streptomyces leeuwenhoekii* strains (Busarakam et al. 2014), for instance, synthesise novel macrolactone and polyketide antibiotics (Nachtigall et al. 2011; Rateb et al. 2011a, b) and chaxapeptin, a new lasso peptide (Elsayed et al. 2015) while the type strain of *Lentzia chajnantorensis* (Idris et al. 2017b) produces novel diene and monoene glycosides, several of which show anti-HIV integrase activity (Wichner et al. 2017). Complementary metagenomic analyses of Atacama Desert habitats have revealed a remarkable actinobacterial diversity most of which has not been detected using culture-dependent methods (Bull et al. 2017; Idris et al. 2017c). Improved selective isolation and cultivation methods are needed to isolate components of this diversity, not least *Micromonospora* strains which are known to be a rich source of new specialist metabolites and have the potential to defend plants against root-infecting fungi (Carro et al. 2018a).

Micromonospora (Ørskov 1923), the type genus of the family Micromonosporaceae (Krasil'nikov 1938; Zhi et al. 2009), currently encompasses 79 species with validly published names (Parte 2014; http://www.bacterio.net/micromonospora.html) many of which have been circumscribed using polyphasic methods (Genilloud 2012; Carro et al. 2018b) though the genus remains under-speciated (Carro et al. 2012a, 2013a). Micromonosporae have been isolated from diverse natural habitats (Genilloud 2012), notably from rhizosphere soil (Carro et al. 2013b; Thawai et al. 2016) from desert locations in China (Ding et al. 2013) and from tissues of a broad range of plants, such as *Triticum aestivum* (Coombs and Franco 2003), *Zea mays* (Shen et al. 2014), and *Parathelypteris beddomei* (Zhao et al. 2017); as well as from nitrogen-fixing root nodules of actinorhizal plants (Trujillo et al. 2006; Carro et al. 2013a) and legumes (Trujillo et al. 2007; Garcia et al. 2010; Trujillo et al. 2010; Carro et al. 2018b).

It is apparent from culture-independent studies that micromonosporae form a small, but integral part of actinobacterial communities in Atacama Desert habitats (Bull et al. 2017; Idris et al. 2017c). The failure to isolate them from such habitats may reflect the use of isolation media mainly designed to be selective for streptomycetes (Busarakam 2014; Idris 2016). The primary aims of the present study were to isolate micromonosporae from diverse Atacama Desert soils using procedures designed to be selective for members of this taxon and to determine whether any such isolates represented putatively novel taxa.

Materials and methods

Sampling sites

Soil samples (Table 1) were collected from several locations in the Atacama Desert (Fig. 1) between 2010 and 2016 (ATB, MG) and an additional one in 2012 (Professor Luis Cáceres, University of Antofagasta). The samples were collected aseptically using spatulas sterilised in the field with ethanol and contained in sterile polycarbonate bottles. Following transport to the UK the samples were stored at 4 °C. Further details of the sampling sites can be found elsewhere (Bull et al. 2017; Idris et al. 2017c).

Selective isolation

Three isolation procedures were used: a) Phenol-heat protocol (P–H): a gram of each of the soil samples was diluted in 0.85% sodium chloride solution containing 1.5% phenol (Hayakawa et al. 1991), the preparations shaken on a rotary shaker for an hour, incubated at 70 °C for 40 min in a water bath (Nonomura and Ohara, 1969), shaken again at room temperature for 2 h, then serial dilutions prepared in the saline solution, and the resultant preparations shaken at room temperature prior to spreading over chitin-vitamin (CHV) (Zhang 2011), Gause number 1 (G nº 1) (Gause et al. 1983), humic acid-vitamin (HV) (Hayakawa and Nonomura 1987), M65 (DSMZ medium number 65), R2A (Reasoner et al. 1979) and Zhang' starch soil extract agar (ZSSE) (Zhang 2011); all of the media were supplemented with 50 µg/ml of

Table 1 Location, sampling sites and dates of collection of soil samples from the Atacama Desert

Location	Sampling site and code	Collection date	Altitude (masl)	Latitude (°S)	Longitude (°W)	Habitat	No of isolates
Cerro Chajnant or[a]	ALMA 4 ATA, 4G	20.10.12	4000	23°03′31″	67°52′27″	Subsurface soil (30 cm)	25
	ALMA 5 5R2A	20.10.12	5046	23°00′49″	67°45′31″	Surface soil (2 cm)	1
Lomas bayas	LB 3	24.02.14	1500	23°24′27″	69°31′03″	Extreme hyper-arid surface soil (2 cm)	5
Yungay core region	Cerro Aguas Blancas, Y6	13.11.10	1047	24°06′18″	70°01′15″	Extreme hyper-arid subsurface soil (30 cm)	1
Salar de tara	STR1	05.10.1 6	4174	23°03′9 7″	67°18′8 7″	Rhizospher e of *Parastrephia quadrangularis*	13

[a] The Atacama Large Millimeter Array (ALMA) Observatory is situated on Cerro Chajnantor: ALMA is operated as an international partnership which includes the European Southern Observatory (ESO). Permission to collect soil samples from Cerro Chajnantor was given by the Director of the ESO

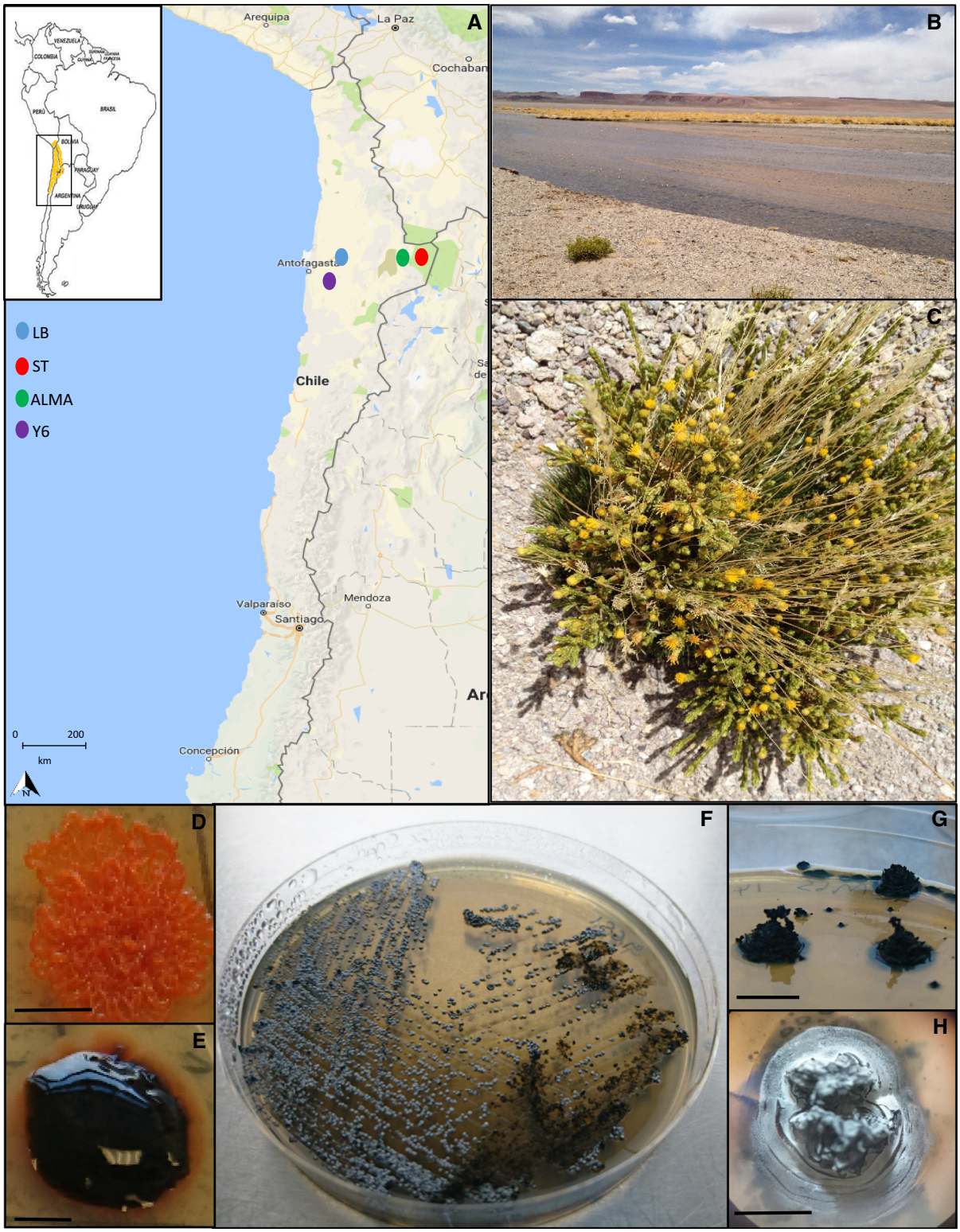
cycloheximide and 50 µg/ml of nalidixic acid; b) sprinkle protocol (S): 0.5 g of soil particles of individual samples which had been preheated at 120 °C for 15 min were sprinkled directly over the isolation media and c) a standard dilution plate protocol (D): dilutions of each soil sample in saline were used to inoculate each of the isolation media following the procedure described by Goodfellow et al. (1967). All of the inoculated plates were incubated at 28 °C and examined weekly for up to 28 days for the presence of *Micromonospora* colonies that were detected, using a stereoscopic microscope, and then transferred to the medium from which they were isolated but without the antibiotics; the resultant plates were incubated at 28 °C for 15 days. The 45 isolated strains were maintained on either M65 or ZSSE agar and in 20% v/v glycerol at − 80 °C for long-term preservation.

Dereplication of isolates

Genomic DNA for BOX-PCR was extracted from all the isolates using a Bacterial DNA Extraction Kit (Sigma) following the instructions of the manufacturer and BOX-PCR fingerprint profiles generated using modifications of the methods described by Versalovic et al. (1994) and Trujillo et al. (2010). To this end, Bioline 2 × MiFi™ mix was used for PCR amplification in a final volume of 25 µl per reaction following the manufacturer's recommendations; the thermal cycling parameters were: 7 minutes at 95 °C, 30 cycles of 1 minute at 94 °C, 1 minute at 52 °C and 3 minutes at 72 °C followed by a final extension at 72 °C for 10 minutes. Five microliters of each PCR product was loaded onto a 2% agarose gel containing 10 µl of GelRed™ (Crisafuli et al. 2015) per 100 ml and electrophoresis run at 70 V for 3 h in freshly prepared 1 × TBE-EDTA buffer at pH 8.0 using a Bio-Rad Pac 300 power supply; a DNA molecular weight marker 1 kb HyperLadder™ (Bioline) was used as the molecular size standard. After electrophoresis, gels were photographed, stored on disk as TIFF files, and manually aligned into 9 multi- and 10 single-membered similarity groups.

Phylogeny

PCR-mediated amplification of genomic DNA of 20 representatives of the BOX-PCR groups was conducted using the universal primers 27F and 1522R (Lane 1991). Bioline 2 × MiFi™ mix was used for PCR amplification in a final volume of 50 µl per reaction following the manufacturer's recommendations. The thermal cycling parameters were: 9 minutes at 95 °C, 30 cycles of 1 minute at 94 °C, 1 minute at 56 °C and 2 minutes at 72 °C followed by a final extension at 72 °C for 10 minutes. The PCR products were purified using a QIAquick® PCR purification kit, according to the manufacturer´s instructions (Qiagen) and sequenced using the EZ-seq Barcode Service (Macrogen). The resultant sequences (around 1400 nucleotides) were manually aligned using MUSCLE (Edgar 2004) and then compared with corresponding sequences of *Micromonospora* type strains retrieved from the EzBioCloud server (Yoon et al. 2016). Phylogenetic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◀**Fig. 1** **a** Sampling sites: Lomas Bayas (LB), Salar de Tara (ST), Atacama Large Millimeter Array observatory (ALMA) and Yungay (Y6); **b** Salar de Tara site; **c** *Parastrephia. quadrangularis* plant growing at the Salar the Tara; **d** Typical orange colony of a *Micromonospora* isolate; **e** Typical black *Micromonospora* colony following spore production; **f–h**. Morphology observed for some Atacama micromonosporae; **f** Sporulating *Micromonospora* colonies; **g**. Mountain-shaped *Micromonospora* isolates; **h** Colony from ALMA4 isolate covered by white aerial hyphae

distances were calculated with the Kimura 2-parameter model (Kimura 1980) and tree topologies inferred using the neighbour-joining (Saitou and Nei 1987) and maximum-likelihood algorithms (Felsenstein 1981) and one thousand bootstrap replications. Tree reconstructions were carried out using MEGA 7 software (Kumar et al. 2016).

Stress tests

All of the strains were examined for their ability to grow in the presence of various concentrations of sodium chloride (1, 3, 5, 7 and 9% w/v) and at a range of temperature (4, 10, 20, 28, 37 and 45 °C) and pH values (4.5, 5.5, 6.5, 8.0 and 9.0) using M65 and ZSSE agar as basal media; pH values were determined using phosphate buffers, as described previously (Carro et al. 2012b). All of the plates, apart from those from the temperature tests, were incubated at 28 °C for 15 days. The strains were also examined for their ability to grow following exposure to 100 mJoules UV light for 30 and for 60 min in a UV chamber (Biorad) using *Geodermatophilus poikilotrophi* DSM 44209^T as the positive control (Montero–Calasanz et al. 2014) set at a wavelength of 254 nm (UVC). The capacity of the strains to grow under anaerobic conditions was tested on M65 and ZSSE agar plates that were incubated for 28 °C for 10 days in anaerobic atmosphere generation bags (Sigma-Aldrich 68,061).

Antimicrobial activity assays

The isolates were examined for their ability to inhibit the growth of wild type strains of *Bacillus subtilis*, *Escherichia coli* and *Pseudomonas fluorescens* using a standard plug assay (Fiedler 2004). Lawns of each of the isolates were prepared on yeast extract-malt extract and oatmeal agar [International *Streptomyces* Project (ISP) media 2 and 3; Shirling and Gottlieb 1966] plates incubated at 28 °C for 14 days. Agar plugs taken from the incubated plates were transferred to sterile square Petri Dishes and overnight Luria broth cultures [OD_{600} of approximately 0.6] of each of the wild type strains in molten nutrient agar added to a final concentration of OD_{600} of 0.0125. The inoculated dishes were incubated at 28 °C and examined for zones of inhibition around the agar plugs after 24 and 48 h.

Results and discussion

Isolation of micromonosporae

The ability to isolate specific fractions of the actinobacterial community from the Atacama Desert biome is of paramount importance especially since metagenomic analyses have revealed a staggering degree of actinobacterial diversity therein, the vast majority of which has gone undetected using culture-dependent procedures (Idris et al. 2017c) which partly reflects the use of isolation media that favour the growth of streptomycetes (Williams et al. 1984). Isolation procedures designed to recover specific actinobacterial genera from environmental samples are many and varied but tend to reflect the biological properties of the target organisms (Goodfellow 2010), as was the case in the present study. It is, for instance, known that *Micromonospora* spores are activated by heat pretreatment regimes, are resistant to phenol and germinate on nutrient rich media such as HV agar (Hoskisson et al. 2000; Shen et al. 2014; Carro et al. 2018b). It is, therefore, encouraging that over half of the *Micromonospora* strains were isolated from the soil samples using the phenol-heat procedure, albeit on several isolation media (Table 2).

Micromonosporae growing on the isolation plates were recognised, under the stereoscopic microscope, by their ability to produce filamentous microcolonies that were strongly attached to agar and these colonies were readily distinguished from those of aerobic, endospore-forming bacilli (data not shown). It was particularly interesting that most of the *Micromonospora* strains came from the ALMA 4 sample as complementary culture-independent studies have shown that soil samples from this location contain micromonosporal propagules (Bull et al.

Table 2 Source and substrate mycelial colour of the isolated *Micromonospora* strains

Isolates	Isolation protocol	Isolation media	Colour of substrate mycelium
ALMA 4			
4G51, 4G53, 4G55, 4G57	D	G no 1	Green
ATA32, ATA34, ATA47	P–H	ZSSE	Orange
ATA48	P–H	CHV	Orange
ATA31, ATA33, ATA35, ATA36a, ATA36b, ATA37, ATA38, ATA39, ATA40, ATA42, ATA43, ATA44, ATA45	P–H	ZSSE	Green
ATA50	P–H	CHV	Green
ATA46, ATA51	P–H	HV	Green
ATA52	P–H	HV	Black
ALMA 5			
5R2A7	D	R2A	Orange
Lomas bayas			
LB 4, LB 19, LB 32, LB 39, LB 41	D	M65	Orange
Yungay core region			
Y6_2	P–H	HV	Orange
Salar de Tara (rhizosphere)			
STR1-41, STR1-74, STR1-85	P–H	HV	Orange
STR 1s-5, STR1 s-6, STR1 s-7, STR 1s-11	S	HV	Orange
STR1-7, STR1-71, STR1-72	P–H	ZSSE	Orange
STR 1s-13a, STR1 s-14, STR1 s-16	S	ZSSE	Orange

2017). The strains isolated from this site proved to be unusual as they formed dry, filamentous blue-green colonies covered by white aerial hyphae following growth for three weeks on M65 and ZSSE media (Fig. 1). The ability of these isolates to form aerial hyphae is notable as some *Micromonospora* type strains have been shown to contain homologous genes to those associated with aerial hyphae formation and spore maturation in streptomycetes (Carro et al. 2018a). Strains isolated from soil samples taken from the three remaining locations showed a typical micromonosporal phenotype, one characterised by the production of filamentous orange colonies that lack aerial hyphae and turn blue-black upon spore formation (Genilloud 2012). Five of these strains were isolated from extreme hyper-arid soil collected from the Lomas Bayas region (Idris et al. 2017c), one of the driest areas in the Atacama Desert; all of these isolates were recovered by plating serial dilutions of the soil onto M65 agar. In turn, the 13 strains isolated from the rhizosphere of *Parastrephia quadrangularis* (*Compositae*, tribe *Asteraceae*) were obtained either by sprinkling mineral particles or spreading serial dilutions of the phenol-heat protocol onto HV or ZSSE agar and incubating for four weeks at 28 °C. *Micromonospora* strains were recovered using all three selective isolation procedures (Table 2).

BOX-PCR profiles

The BOX-PCR profiles of the isolates encompass considerable genetic diversity with fragments ranging from 0.05 to 2.0 kb (Fig. 2) thereby providing further evidence that this method is effective in distinguishing between *Micromonospora* strains (Maldonado et al. 2008; Trujillo et al. 2010). The isolates were assigned to seven multi- and 11 single-membered groups defined at the 60% similarity level (Table 3). Five of the multi-membered groups and five of the singletons were composed of strains isolated from the ALMA 4 soil samples, whilst the largest of these taxa, group XI, contained seven isolates. In turn, the strains isolated from *P. quadrangularis* rhizosphere soil were assigned to a multi-membered group that

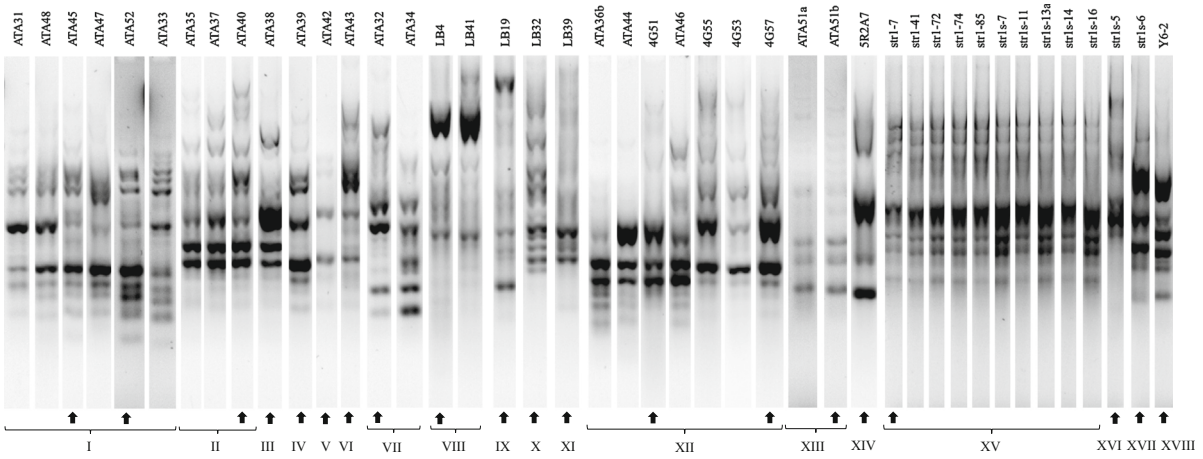

Fig. 2 BOX–PCR fingerprints showing the genetic diversity of *Micromonospora* strains isolated from Atacama Desert soils

encompassed 10 strains and to two single membered groups; the five Lomas Bayas strains formed one multi- and 3 single-membered taxa while the single isolates from ALMA 5 and Yungay Core Region samples gave unique profiles (Fig. 2). It is interesting that the multi-membered groups only contained strains isolated from a single location and that few strains were clones (Fig. 2; Table 3).

Phylogenetic analyses

Almost complete 16S rRNA gene sequences [1372–507 nucleotides (nt)] were generated for the 20 isolates chosen to represent the BOX-PCR groups (Table 3). The generic assignment of all of these strains was confirmed as they were recovered in the *Micromonospora* 16S rRNA gene tree (Fig. 3). The 16S rRNA gene sequence similarities between these isolates and the type strains of their close phylogenetic neighbours fell within the range 98.4–99.9% (Table 3). Several of the isolates were assigned to well-supported clusters in the *Micromonospora* tree though none of these taxa included strains isolated from more than one location. Ten out of the twelve ALMA 4 strains formed a well-supported phyletic line within a weakly supported clade that encompassed the type strains of *Micromonospora costi* (Thawai 2015), their near neighbour, and *Micromonospora fulviviridis* (Kroppenstedt et al. 2005); it is interesting that the isolates of this clade were recovered from different samples of ALMA 4 soil using two of the three isolation protocols and several selective isolation media (Table 2). Isolate 5R2A7, one of the remaining ALMA strains, formed a well-supported clade together with the type strain of *Micromonospora coriariae* (Trujillo et al. 2006), its near phylogenetic neighbour, and with *Micromonospora cremea* (Carro et al. 2012b) whereas isolate ATA32, the remaining ALMA 4 strain, formed an unsupported clade with the type strain of *Micromonospora narathiwatensis* (Thawai et al. 2007) though it is closely related to *Micromonospora eburnea* (Thawai et al. 2005).

It is notable that two out of the three strains recovered from the rhizosphere of *P. quadrangularis,* isolates STR1-7 and STR1s-6, were loosely associated with the type strains of *Micromonospora lupini* (Trujillo et al. 2007), *Micromonospora taraxaci* (Zhao et al. 2014) and *Micromonospora violae* (Zhang et al. 2014) which were isolated from a root nodule of *Lupinus angustifolius* and the roots of *Taraxacum mongolicum* and *Viola philippica*, respectively (Fig. 3). Moreover, the final strain, isolate STR1s-5, is closely related to the type strain of *Micromonospora ureilytica* (Carro et al. 2016b), which was isolated from a root nodule of *Pisum sativum*. In turn, two of the four strains isolated from the extreme hyper-arid Lomas Bayas soil, isolates LB19 and LB32, are phylogenetically close to the type strain of *Micromonospora saelicesensis* (Trujillo et al. 2007), an isolate from a root nodule of *L. angustifolius*. It is also notable that isolate LB39 was recovered in the well supported clade that included the type strains of *M. chokoriensis, M. taraxaci* and

Table 3 Assignment of isolates to BOX groups and determination of their nearest phylogenetic neighbours

BOX group	Isolates	Representative isolates	% Similarity to current closest phylogenetic type strain	
I	ATA31, ATA33, ATA45, ATA47,ATA48, ATA52	ATA 45, ATA52	*M. costi*	99.1
II	ATA35, ATA37, ATA40	ATA40	*M. costi*	99.1
III	ATA38	ATA38	*M. costi*	98.4
IV	ATA39	ATA39	*M. costi*	98.4
V	ATA42	ATA42	*M. costi*	99.1
VI	ATA43	ATA43	*M. terminaliae*	98.8
VII	ATA32, ATA34	ATA32	*M. eburnea*	99.4
VIII	LB4, LB41	LB4	*M. chalcea*	99.6
IX	LB19	LB19	*M. saelicesensis*	99.8
X	LB32	LB32	*M. saelicesensis*	99.8
XI	LB39	LB39	*M. chokoriensis*	99.9
XII	ATA36, ATA44, 4G51, ATA 46, 4G55, 4G53, 4G57	4G51, 4G57	*M. costi*	99.1
XIII	ATA51a, ATA51b	ATA51b	*M. costi*	99.1
XIV	5R2A7	5R2A7	*M. coriariae*	99.8
XV	STR1-7, STR1-41, STR1-72, STR1-74, STR1-85, STR1s-7, STR1s-11, STR1s-13A, STR1s-14, STR1s-16	STR1-7	*M. chokoriensis*	99
XVI	STR1s-5	STR1 s-5	*M. chokoriensis*	99.4
XVII	STR1s-6	STR1 s-6	*M. ureilytica*	99.6
XVIII	Y6-2	Y6-2	*M. pisi*	98.8

M. violae (Fig. 3); the final strain from the Lomas Bayas soil, isolate LB4, formed a well-supported clade with the type strain of *Micromonospora chalcea* (Foulerton 1905; Orskov 1923) which was isolated from air. It can be seen from the Fig. 3 that the sole strain from the extreme hyper-arid Yungay Core soil, isolate Y6-2, forms a well-supported clade with the type strain of *Micromonospora pisi* (Garcia et al. 2010), an isolate from a root nodule of *P. sativum*.

To date, representatives of dereplicated groups of actinobacteria isolated from Atacama Desert soils which show low pairwise 16S rRNA gene sequence similarities, (≤ 99%; Meier–Kolthoff et al. 2013) with the type strains of their close phylogenetic neighbour have been invariably assigned to new species when subject to polyphasic taxonomic analyses, as exemplified by *Lechevalieria*, *Pseudonocardia* and *Streptomyces* species (Okoro et al. 2010; Busarakam et al. 2014; Trujillo et al. 2017). This cut-off point has also proved to be a reliable indicator for the presumptive recognition of novel *Micromonospora* species (Trujillo et al. 2007; Garcia et al. 2010; Carro et al. 2016a) though pairwise 16S rRNA gene sequence similarities very much higher than the 99.0% threshold have been found to be indicative of new *Micromonospora* species, as exemplified by *M. taraxaci* and *M. violae* (Zhang et al. 2014; Zhao et al. 2014). Given these indicators, it seems likely that further comparative taxonomic analyses will show that most, if not all, of the representatives of the BOX-PCR groups will be found to represent novel *Micromonospora* species. Indeed, isolates ATA38, ATA39, and ATA43 from ALMA4, isolate STR1-7 from the rhizosphere of *P. quadrangularis*, and isolate Y6_2 from Yungay are cases in point as they share 16S rRNA gene sequence similarities with their close phylogenetic neighbours at or below the 99.0% threshold (Table 3).

Adaptation to extreme conditions

In general, the pH and temperature profiles of the isolates were typical of *Micromonospora* strains (Genilloud 2012) as they grew from 20 to 37 °C, at pH 6.0 to 8.0, but not at 4, 12 or 45 °C or below pH 5.0. None of the isolates grew under anaerobic conditions, but most of them were able to grow in the presence of 1% w/v sodium chloride; six out of the

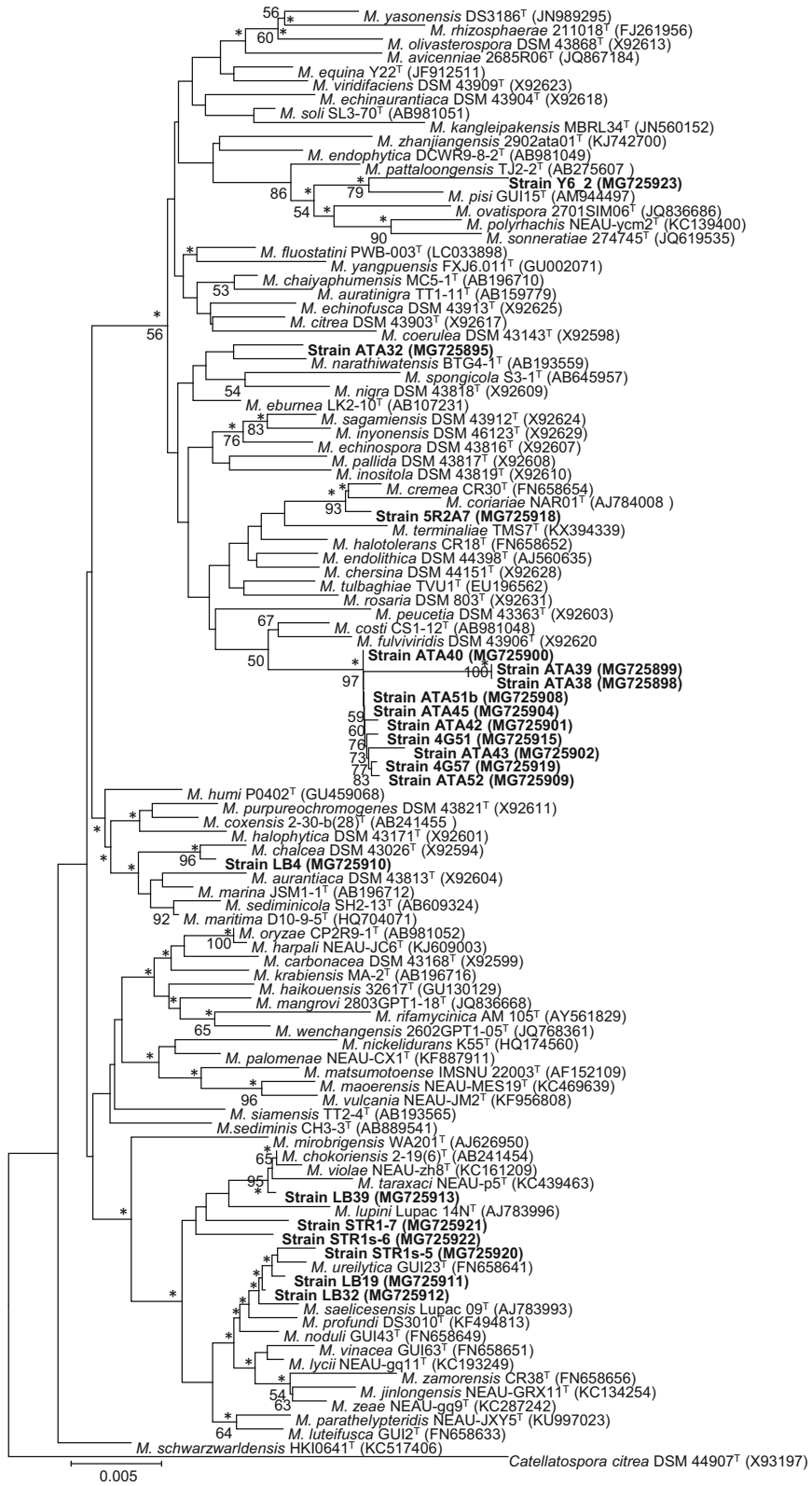

Fig. 3 Neighbour-joining phylogenetic tree based on almost complete 16S rRNA gene sequences showing relationships between the *Micromonospora* strains and between them and *Micromonospora* type strains. Numbers at the nodes indicate the levels of bootstrap support (%), only values above 50% are shown. Asterisks indicate branches of the tree that were also recovered in the maximum-likelihood tree. *Catellatospora citrea* was used as the outgroup. Bar, 0.005 substitutions per nucleotide position

thirteen strains isolated from the rhizosphere of *P. quadrangularis* grew in the presence of 3% w/v sodium chloride. It was also interesting that only 30% of the samples from ALMA soil grew on M65 agar following exposure to UV light (UVC) at 100 mJoules/second for 30 min, although this number increased to 60% on ZSSE agar. Similarly, high percentages were observed for strains isolated from the rhizosphere and Lomas Bayas soils on M65 (64 and 80%) and ZSSE (50 and 60%) agar, respectively. These results are interesting as it has been shown that the type strain of *Modestobacter caceresii*, an isolate recovered from a soil sample from the Yungay Core Region, has the capacity to protect and repair damaged caused by UV radiation (Busarakam et al. 2016). Interestingly, the sole *Micromonospora* strain obtained from this area, isolate Y6-2, grew after an hour exposure to UV light at 100 mJoules/second, whereas only around 20% of the isolates grew under this condition. Although UVC radiation no longer reaches the Earth's surface, it has been used as a selective tool with reference to early life on the planet and high altitude biology. Recently Paulino-Lima et al. (2016) screened desert soils, including Atacama soil, for UVC-resistant bacteria and over 40% of their isolates were members of the phylum *Actinobacteria*; however, *Micromonospora* strains were not recovered by these authors. Consequently, the distinctive radiation resistance of *Micromonospora* strain Y6-2 suggests that it could be a novel subject for detailed radiation-resistant physiological and biochemical researches, that should be extended to include the other radiation stressors naturally present at the Earth surface. The study of these radiations will be of interest for future studies of the strains and to determine if key osmoprotectant proteins are implicated in the process as previously shown in *Rhodobacter* (Pérez et al. 2017).

Approximately half of the isolates showed weak activity in the plug assays against the wild type strains of *B. subtilis* and *P. fluorescens* though no activity was shown against the *E. coli* strain tested. However, it is important that these preliminary antimicrobial activity studies are extended as the genomes of *Micromonospora* type strains have much greater potential to synthesise novel specialised metabolites than previously realised (Carro et al. 2018a). Indeed, novel strains of *Micromonospora* and corresponding strains of other genera classified in the family *Micromonosporaceae* should be given greater prominence in the search for new classes of bioactive compounds, notably antibiotics that are needed to control drug resistant pathogens.

It can be concluded that the isolation procedures used in this study provide an effective way of isolating putatively novel *Micromonospora* species from Atacama Desert soils. Indeed, innovative selective isolation procedures based on the biological properties of target organisms are needed to cultivate elements of the extensive actinobacterial 'dark matter' detected in soils of the Atacama Desert landscape using culture-independent methods (Bull et al. 2017; Idris et al. 2017c).

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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