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

Lorena Carro[1], Valeria Razmilic[1,2], Imen Nouioui[1], Lee Richardson[1], Che Pan[1], Patrycja Golinska[3], Juan A. Asenjo[2], Alan T. Bull[4], Hans-Peter Klenk[1], Michael Goodfellow[1]

[1] School of Natural and Environmental Sciences, Newcastle University, Newcastle-upon-Tyne, UK.

[2] Centre for Biotechnology and Bioengineering (CeBiB), Department of Chemical Engineering and Biotechnology, Universidad de Chile, Beauchef 850, Santiago, Chile.

[3] Department of Microbiology, Nicolaus Copernicus University, Torun, Poland

[4] School of Biosciences, University of Kent, Canterbury, Kent CT2 7NJ, UK

Corresponding author: Lorena Carro lcg@usal.es, Telephone: +44 (0)1912084853.

Abstract (150-250)

Innovative procedures were used to selectively isolate small numbers of *Micromonospora* strains from extreme hyper-arid and high altitude Atacama Desert soils. Micromonosporae were recognized 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.

Introduction

Given their importance in biotechnology, bioprospecting and ecology, actinobacteria remain a source 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; Idris et al. 2017b; Trujillo et al. 2017). *Streptomyces leewenhoekii* strains (Busarakam et al. 2014), for instance, synthesize novel macrolactone and polyketide antibiotics (Nachtigall et al. 2011; Rateb et al. 2011a; Rateb et al. 2011b) 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) many of which have been circumscribed using polyphasic methods (Genilloud 2012; Carro et al. 2018b) though the genus remains underspeciated (Carro et al. 2012a; Carro et al. 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 sterilized in the field with ethanol and contained in sterile polycarbonate bottles. Following transport to the UK the samples were stored at 4ºC. 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 70ºC for 40 minutes in a water bath (Nonomura and Ohara, 1969), shaken again at room temperature for 2 hours, 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 50µg/ml of cycloheximide and 50µg/ml of nalidixic acid; b) sprinkle protocol (S): 0.5g of soil particles of individual samples which had been preheated at 120ºC for 15 minutes 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 28ºC 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 28ºC for 15 days. The 45 isolated strains were maintained on either M65 or ZSSE agar and in 20% v/v glycerol at -80ºC 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 2x MiFi^{TM} 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 hours in freshly prepared 1x TBE-EDTA buffer at pH 8.0 using a Bio-Rad Pac 300 power supply; a DNA molecular weight marker 1kb 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 2x 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 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 ºC) 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 28ºC for 15 days. The strains were also examined for their ability to grow following exposure to 100 mJoules for 30 and for 60 minutes 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 28ºC for 10 days in anaerobic atmosphere generation bags (Sigma-Aldrich 68061).

Antibiotic sensitivity 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 28ºC 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 28ºC and examined for zones of inhibition around the agar plugs after 24 and 48 hours.

Results and Discussion

Isolation of micromonosporae

The ability to isolate specific fractions of actinobacterial communities 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), this 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 humic acid-vitamin 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 recognized, under the stereoscopic microscope, by their ability to produce filamentous microcolonies that were strongly attached to agar, 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., 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 interesting as some *Micromonospora* type strains have been shown to contain putative 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 characterized 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 and ZSSE agar and incubating for four weeks at 28ºC. *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, 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 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 – 1507 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 closest phylogenetic neighbours fell within the range 98.4 to 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 nearest 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 nearest 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 was most closely related to *Micromonospora eburnea* (Thawai et al. 2005).

It is interesting 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, was most 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, were 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 interesting that isolate LB39 was recovered in the well supported clade that included the

type strains of *M. chokoriensis, M. taraxaci* and *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 Figure 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 *Pisum sativum*.

To date, representatives of dereplicated groups of actinobacteria isolated from Atacama Desert soils which show low pairwise 16S rRNA gene sequence similarities, (≤90%; Meier-Kolthoff et al. 2013) with the type strains of their nearest 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 closest 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 37ºC, at pH 6.0 to 8.0, but not at 4, 12 or 45ºC 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 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 minutes, 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, 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 interestingly over 40% of their isolates were members of the phylum *Actinobacteria;*

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 target for detailed radiation-resistant physiological and biochemical researches that should be extended to include the other radiation stressors naturally present ~~on~~ 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. However, it is important that these preliminary antibiotic sensitivity studies are extended as the genomes of *Micromonospora* type strains have much greater potential to synthesize 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 small numbers of 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)

Acknowledgments

This project was partly funded by a UK-Newton Project for UK-Chile collaboration (JIC CA 586) and by support from the Basal Programme of CONICYT for the Centre for Biotechnology and Bioengineering (CeBiB) (project FB0001). IN and LC thank Newcastle University for postdoctoral fellowships and ATB and MG are indebted to the Leverhulme Trust for Emeritus Fellowships.

Conflict of Interest

The authors declare that they have no conflict of interest.

This article does not contain any studies with human participants or animals performed by any of the authors.

References

Barka EA, Vatsa P, Sanchez L et al. (2016) Taxonomy, physiology, and natural products of *Actinobacteria.* Microbiol Mol Biol Rev 80:1-43 doi:10.1128/MMBR.00019-15

Bull AT, Asenjo JA, Goodfellow M, Gomez-Silva B (2016) The Atacama Desert: Technical resources and the growing importance of novel microbial diversity. Annu Rev Microbiol 70:215-234 doi:10.1146/annurev-micro-102215-095236

Bull AT, Idris H, Sanderson R, Asenjo J, Andrews B, Goodfellow M (2017) High altitude, hyper-arid soils of the Central-Andes harbor mega-diverse communities of actinobacteria. Extremophiles doi:10.1007/s00792-017-0976-5

Busarakam K (2014) Novel Actinobacterial Diversity in Arid Atacama Desert Soils as a Source of New Drug Leads. Ph. D. Thesis. Newcastle University, Newcastle upon Tyne

Busarakam K, Bull AT, Girard G, Labeda DP, van Wezel GP, Goodfellow M (2014) *Streptomyces leeuwenhoekii* sp. nov., the producer of chaxalactins and chaxamycins, forms a distinct branch in *Streptomyces* gene trees. Antonie van Leeuwenhoek 105:849-861 doi:10.1007/s10482-014-0139-y

Busarakam K, Bull AT, Trujillo ME, Riesco R, Sangal V, van Wezel GP, Goodfellow M (2016) *Modestobacter caceresii* sp. nov., novel actinobacteria with an insight into their adaptive mechanisms for survival in extreme hyper-arid Atacama Desert soils. Syst Appl Microbiol 39:243-251 doi:10.1016/j.syapm.2016.03.007

Cameron RE, Gensel DR, Blank CB (1966) Soil studies -desert microflora XII. Abundance of microflora in soil samples from the Chile Atacama Desert. space programs summary. In: *Supporting Research and Advanced Devlopments, Space Programs Summary* No. 37–38, vol IV. Jet Propulsion Lab, Pasadena, CA, pp 140–147

Carro L, Spröer C, Alonso P, Trujillo ME (2012a) Diversity of *Micromonospora* strains isolated from nitrogen fixing nodules and rhizosphere of *Pisum sativum* analyzed by multilocus sequence analysis. Syst Appl Microbiol 35:73-80 doi:10.1016/j.syapm.2011.11.003

Carro L, Pukall R, Spröer C, Kroppenstedt RM, Trujillo ME (2012b) *Micromonospora cremea* sp. nov. and *Micromonospora zamorensis* sp. nov., isolated from the rhizosphere of *Pisum sativum.* Int J Syst Evol Microbiol 62:2971-2977 doi:10.1099/ijs.0.038695-0

Carro L, Pujic P, Trujillo ME, Normand P (2013a) *Micromonospora* is a normal occupant of actinorhizal nodules. J Biosci 38:685-693

Carro L, Pukall R, Spröer C, Kroppenstedt RM, Trujillo ME (2013b) *Micromonospora halotolerans* sp. nov., isolated from the rhizosphere of a *Pisum sativum* plant. Antonie van Leeuwenhoek 103:1245-1254 doi:10.1007/s10482-013-9903-7

Carro L, Riesco R, Spröer C, Trujillo ME (2016a) *Micromonospora luteifusca* sp. nov. isolated from cultivated *Pisum sativum.* Syst Appl Microbiol 39:237-242 doi:10.1016/j.syapm.2016.04.003

Carro L, Riesco R, Spröer C, Trujillo ME (2016b) *Micromonospora ureilytica* sp. nov., *Micromonospora noduli* sp. nov. and *Micromonospora vinacea* sp. nov., isolated from Pisum sativum nodules. Int J Syst Evol Microbiol 66:3509-3514 doi:10.1099/ijsem.0.001231

Carro L et al. (2018a) Genome-based classification of micromonosporae with a focus on their biotechnological and ecological potential. Sci Rep 8(1):525 doi: 10.1038/s41598-017-17392-0

Carro L, Veyisoglu A, Riesco R, Spröer C, Klenk HP, Sahin N, Trujillo ME (2018b) *Micromonospora phytophila* sp. nov. and *Micromonospora luteiviridis* sp. nov., isolated as natural inhabitants of plant nodules. Int J Syst Evol Microbiol 68:248-253 doi:10.1099/ijsem.0.002490

Coombs JT, Franco CM (2003) Isolation and identification of actinobacteria from surface-sterilized wheat roots. Appl Environ Microbiol 69:5603-5608

Crisafuli FA, Ramos EB, Rocha MS (2015) Characterizing the interaction between DNA and GelRed fluorescent stain. Eur Biophys J 44:1-7 doi:10.1007/s00249-014-0995-4

Demain AL (2014) Importance of microbial natural products and the need to revitalize their discovery. J Ind Microbiol Biotechnol 41:185-201 doi:10.1007/s10295-013-1325-z

Ding D, Chen G, Wang B, Wang Q, Liu D, Peng M, Shi P (2013) Cultivable actinomycetes from desert ecosystem in northeast of Qinghai Plateau. Ann Microbiol 63:259-266

Edgar RC (2004) MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res 32:1792-1797 doi:10.1093/nar/gkh340

Elsayed SS, Trusch F, Deng H et al. (2015) Chaxapeptin, a lasso peptide from extremotolerant *Streptomyces leeuwenhoekii* strain C58 from the hyperarid Atacama Desert. J Org Chem 80:10252-10260 doi:10.1021/acs.joc.5b01878

Felsenstein J (1981) Evolutionary trees from DNA sequences: a maximum likelihood approach. J Mol Evol 17:368-376

Fiedler HP (2004) Screening for biodiversity. In: Bull AT (eds) Microbial Diversity and Bioprospecting. American Society of Microbiology, Washington DC, pp. 324-335

Foulerton A (1905). New species of *Streptothrix* isolated from the air. Lancet 1199-1200

Garcia LC, Martinez-Molina E, Trujillo ME (2010) *Micromonospora pisi* sp. nov., isolated from root nodules of *Pisum sativum.* Int J Syst Evol Microbiol 60:331-337 doi:10.1099/ijs.0.012708-0

Gause GF, Preobrazhenskaya TP, Sveshnikova GV, Terekhova LP, Maksimova TS (1983) A Guide for Determination of Actinomycetes. Nauka, Moscow, Russia

Genilloud O (2012) Genus I. *Micromonospora* Ørskov 1923, 156^{AL}. In: Goodfellow M, Kämpfer P, Busse HJ, Trujillo ME, Suzuki KI, Ludwig W, Whitman WB (eds) *Bergey's Manual of Systematic Bacteriology*, 2nd edn., vol 5. The *Actinobacteria*, Part B. Springer, New York, pp 1039–1057

Goodfellow M, Hill IR, Gray TRG (1967) Bacteria in a pine forest soil. In: Gray TRG, Parkinson D (eds) *The Ecology of Soil Bacteria*. University Press, Liverpool, pp 500-515.

Goodfellow M (2010) Selective isolation of actinobacteria. In: Baltz RH et al. (eds) *Manual of Industrial Microbiology and Biotechnology*. 3rd edn. American Society of Microbiology, pp 13-27

Goodfellow M, Fiedler HP (2010) A guide to successful bioprospecting: informed by actinobacterial systematics. Antonie van Leeuwenhoek 98:119-142 doi:10.1007/s10482-010-9460-2

Goodfellow M, Busarakam K, Idris H et al. (2017) *Streptomyces asenjonii* sp. nov., isolated from hyper-arid Atacama Desert soils and emended description of *Streptomyces viridosporus* Pridham et al. 1958. Antonie van Leeuwenhoek 110:1133-1148 doi:10.1007/s10482-017-0886-7

Hayakawa M, Nonomura H (1987) Humic acid vitamin agar, a new medium for the selective isolation of soil actinomycetes. J Ferment Technol 65:501-509

Hayakawa M, Sadakata T, Kajiura T, Nonomura H. (1991) New methods for the highly selective isolation of *Micromonospora* and *Microbispora* from soil. J Ferment Bioengin 72:320–326

Hoskisson PA, Hobbs G, Sharples GP (2000) Response of *Micromonospora echinospora* (NCIMB 12744) spores to heat treatment with evidence of a heat activation phenomenon. Lett Appl Microbiol 30:114-117

Idris H (2016) Actinobacterial Diversity in Atacama Deset habitats as a road map to biodiscovering. Ph. D. Thesis. Newcastle University, Newcastle upon Tyne

Idris H, Labeda DP, Nouioui I et al. (2017a) *Streptomyces aridus* sp. nov., isolated from a high altitude Atacama Desert soil and emended description of *Streptomyces noboritoensis*

441 Isono et al. 1957. Antonie van Leeuwenhoek 110:705-717 doi:10.1007/s10482-017-
442 0838-2
443 Idris H, Nouioui I, Asenjo JA, Bull AT, Goodfellow M (2017b) *Lentzea chajnantorensis* sp. nov.,
444 an actinobacterium from a very high altitude Cerro Chajnantor gravel soil in northern
445 Chile. Antonie van Leeuwenhoek 110:795-802 doi:10.1007/s10482-017-0851-5
446 Idris H, Goodfellow M, Sanderson R, Asenjo JA, Bull AT (2017c) Actinobacterial rare biospheres
447 and dark matter revealed in habitats of the chilean Atacama Desert. Sci Rep 7:8373
448 doi:10.1038/s41598-017-08937-4
449 Kimura M (1980) A simple method for estimating evolutionary rates of base substitutions
450 through comparative studies of nucleotide sequences. J Mol Evol 16:111-120
451 Krasil'nikov NA (1938) Ray Fungi and Related Organisms. *Actinomycetales*. Akad Nauk, Moscow
452 Kroppenstedt RM, Mayilraj S, Wink JM, Kallow W, Schumann P, Secondini C, Stackebrandt E
453 (2005) Eight new species of the genus *Micromonospora, Micromonospora citrea* sp.
454 nov., *Micromonospora echinaurantiaca* sp. nov., *Micromonospora echinofusca* sp. nov.
455 *Micromonospora fulviviridis* sp. nov., *Micromonospora inyonensis* sp. nov.,
456 *Micromonospora peucetia* sp. nov., *Micromonospora sagamiensis* sp. nov., and
457 *Micromonospora viridifaciens* sp. nov. Syst Appl Microbiol 28:328-339
458 doi:10.1016/j.syapm.2004.12.011
459 Kumar S, Stecher G, Tamura K (2016) MEGA7: Molecular Evolutionary Genetics Analysis
460 Version 7.0 for Bigger Datasets. Mol Biol Evol 33:1870-1874
461 doi:10.1093/molbev/msw054
462 Lane DJ (1991) 16S/23S rRNA sequencing. In: Stackebrandt E, Goodfellow M (eds) Nucleic Acid
463 Techniques in Bacterial Systematics. Wiley, New York, pp 115-175
464 Maldonado LA, Stach JE, Ward AC, Bull AT, Goodfellow M (2008) Characterisation of
465 micromonosporae from aquatic environments using molecular taxonomic methods.
466 Antonie van Leeuwenhoek 94:289-298 doi:10.1007/s10482-008-9244-0
467 Meier-Kolthoff JP, Goker M, Spröer C, Klenk HP (2013) When should a DDH experiment be
468 mandatory in microbial taxonomy?. Arch Microbiol 195:413-418 doi:10.1007/s00203-
469 013-0888-4
470 Montero-Calasanz MC, Hofner B, Göker M et al. (2014) *Geodermatophilus poikilotropi* sp. nov.:
471 a multitolerant actinomycete isolated from dolomitic marble. Biomed Res Int 914767
472 Nachtigall J, Kulik A, Helaly S et al. (2011) Atacamycins A-C, 22-membered antitumor
473 macrolactones produced by *Streptomyces* sp. C38. J Antibiot (Tokyo) 64:775-780
474 doi:10.1038/ja.2011.96
475 Nonomura H, Ohara Y (1969) Distribution of actinomycetes in soil. VI. A culture method
476 effective for both preferential isolation and enumeration of *Microbispora* and
477 *Streptosporangium* strains in soil (Part 1) J Ferment Technol 47:463–469
478 Okoro CK, Brown R, Jones AL, Andrews BA, Asenjo JA, Goodfellow M, Bull AT (2009) Diversity
479 of culturable actinomycetes in hyper-arid soils of the Atacama Desert, Chile. Antonie
480 van Leeuwenhoek 95:121-133 doi:10.1007/s10482-008-9295-2
481 Okoro CK, Bull AT, Mutreja A, Rong X, Huang Y, Goodfellow M (2010) *Lechevalieria*
482 *atacamensis* sp. nov., *Lechevalieria deserti* sp. nov. and *Lechevalieria roselyniae* sp.
483 nov., isolated from hyperarid soils. Int J Syst Evol Microbiol 60:296-300
484 doi:10.1099/ijs.0.009985-0
485 Opfell JB, Zebal GP (1967) Ecological patterns of micro-organisms in desert soils. Life Sci Space
486 Res 5:187-203
487 Ørskov J (1923) Investigations into the Morphology of the Ray Fungi. Levin and Munksgaard,
488 Copenhagen
489 Parte AC (2014) LPSN—list of prokaryotic names with standing in nomenclature. Nucleic Acids
490 Research 42:D613-D616 doi:10.1093/nar/gkt1111

Paulino-Lima IG, Fujishima K, Navarrete JU et al. (2016) Extremely high UV-C rdiation resistant microorganisms from desert environments with different manganese concentrations. J Photochem Photobiol B-Biol 163: 327-336

Pérez V, Hengst M, Kurte L et al. (2017) Bacterial survival under extreme UV radiation : a comparative proteomics study of *Rhodobacter* sp., isolated from high altitude wetlands in Chile Front Microbiol 8:1173 doi:10.3389/fmicb.2017.01173

Rateb ME, Houssen WE, Arnold M et al. (2011a) Chaxamycins A-D, bioactive ansamycins from a hyper-arid desert *Streptomyces* sp. J Nat Prod 74:1491-1499 doi:10.1021/np200320u

Rateb ME, Houssen WE, Harrison WT et al. (2011b) Diverse metabolic profiles of a *Streptomyces* strain isolated from a hyper-arid environment. J Nat Prod 74:1965-1971 doi:10.1021/np200470u

Reasoner DJ, Blannon JC, Geldreich EE (1979) Rapid seven-hour fecal coliform test. Appl Environ Microbiol 38:229-236

Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 4:406-425

Shen Y, Zhang Y, Liu C et al. (2014) *Micromonospora zeae* sp. nov., a novel endophytic actinomycete isolated from corn root (*Zea mays* L.). J Antibiot (Tokyo) 67:739-743 doi:10.1038/ja.2014.54

Shirling EB, Gottlieb D (1966) Methods for characterization of *Streptomyces* species. Int J Syst Evol Microbiol 16:313-340 doi:doi:10.1099/00207713-16-3-313

Thawai C (2015) *Micromonospora costi* sp. nov., isolated from a leaf of *Costus speciosus.* Int J Syst Evol Microbiol 65:1456-1461 doi:10.1099/ijs.0.000120

Thawai C, Kittiwongwattana C, Thanaboripat D, Laosinwattana C, Koohakan P, Parinthawong N (2016) *Micromonospora soli* sp. nov., isolated from rice rhizosphere soil. Antonie van Leeuwenhoek 109:449-456 doi:10.1007/s10482-016-0651-3

Thawai C, Tanasupawat S, Itoh T, Suwanborirux K, Suzuki K, Kudo T (2005) *Micromonospora eburnea* sp. nov., isolated from a Thai peat swamp forest. Int J Syst Evol Microbiol 55:417-422 doi:10.1099/ijs.0.63217-0

Thawai C, Tanasupawat S, Suwanborirux K, Itoh T, Kudo T (2007) *Micromonospora narathiwatensis* sp. nov., from Thai peat swamp forest soils. J Gen Appl Microbiol 53:287-293

Trujillo ME, Kroppenstedt RM, Schumann P, Carro L, Martinez-Molina E (2006) *Micromonospora coriariae* sp. nov., isolated from root nodules of *Coriaria myrtifolia.* Int J Syst Evol Microbiol 56:2381-2385 doi:10.1099/ijs.0.64449-0

Trujillo ME, Kroppenstedt RM, Fernandez-Molinero C, Schumann P, Martinez-Molina E (2007) *Micromonospora lupini* sp. nov. and *Micromonospora saelicesensis* sp. nov., isolated from root nodules of *Lupinus angustifolius.* Int J Syst Evol Microbiol 57:2799-2804 doi:10.1099/ijs.0.65192-0

Trujillo ME, Alonso-Vega P, Rodriguez R, Carro L, Cerda E, Alonso P, Martinez-Molina E (2010) The genus *Micromonospora* is widespread in legume root nodules: the example of *Lupinus angustifolius.* ISME J 4:1265-1281 doi:10.1038/ismej.2010.55

Trujillo ME, Idris H, Riesco R, Nouioui I, Igual JM, Bull AT, Goodfellow M (2017) *Pseudonocardia nigra* sp. nov., isolated from Atacama Desert rock Int J Syst Evol Microbiol 67:2980-2985 doi:10.1099/ijsem.0.002063

Versalovic J, Schneider M, De Bruijn FJ, Lupski JR (1994) Genomic fingerprinting of bacteria using repetitive sequence-based polymerase chain reaction. Meth Mol Cell Biol 5:25-40

Wichner D, Idris H, Houssen WE et al. (2017) Isolation and anti-HIV-1 integrase activity of lentzeosides A-F from extremotolerant *Lentzea* sp. H45, a strain isolated from a high-altitude Atacama Desert soil. J Antibiot 70:448-453 doi:10.1038/ja.2016.78

Williams ST, Goodfellow M, Vickers JC (1984) New microbes from old habitats? In: Carr WG, Kelly D (eds) *The Microbe 1984. II Prokaryotes and Eukaryotes*. Cambridge University Press, Cambridge, pp 219-256

Yoon S-H, Ha S-M, Kwon S, Lim J, Kim Y, Seo H, Chun J (2016) Introducing EzBioCloud: A taxonomically united database of 16S rRNA and whole genome assemblies. Int J Syst Evol Microbiol doi:doi:10.1099/ijsem.0.001755

Zhang J (2011) Improvement of an isolation medium for actinomycetes. Modern Appl Sci 5:124-127

Zhang Y, Liu H, Zhang X et al. (2014) *Micromonospora violae* sp. nov., isolated from a root of Viola philippica Car. Antonie van Leeuwenhoek 106:219-225 doi:10.1007/s10482-014-0184-6

Zhao J, Guo L, He H et al. (2014) *Micromonospora taraxaci* sp. nov., a novel endophytic actinomycete isolated from dandelion root (*Taraxacum mongolicum* Hand.-Mazz.). Antonie van Leeuwenhoek 106:667-674 doi:10.1007/s10482-014-0237-x

Zhao S, Liu C, Zheng W et al. (2017) *Micromonospora parathelypteridis* sp. nov., an endophytic actinomycete with antifungal activity isolated from the root of *Parathelypteris beddomei* (Bak.) Ching. Int J Syst Evol Microbiol 67:268-274 doi: 10.1099/ijsem.0.001614

Zhi XY, Li WJ, Stackebrandt E (2009) An update of the structure and 16S rRNA gene sequence-based definition of higher ranks of the class *Actinobacteria*, with the proposal of two new suborders and four new families and emended descriptions of the existing higher taxa. Int J Syst Evol Microbiol 59:589-608 doi:10.1099/ijs.0.65780-0

Figure legends

Figure 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.

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

Figure 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.

588

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

Location	Sampling site and code	Collection date	Altitude (masl)	Latitude (°S)	Longitude (°W)	Habitat	Nº of isolates
Cerro Chajnantor+	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	05.10.16	4174	23°03'97''	67°18'87''	Rhizosphere of *Parastrephia quadrangularis*	13

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

595 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

	STR1-41, STR1-74, STR1-85	P-H	HV	orange
Salar de Tara (rhizosphere)	STR1s-5, STR1s-6, STR1s-7, STR1s-11	S	HV	orange
	STR1-7, STR1-71, STR1-72	P-H	ZSSE	orange
	STR1s-13a, STR1s-14, STR1s-16	S	ZSSE	orange

596

597

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

BOX group	Isolates	Representative isolates	% Similarity to 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	STR1s-5	*M. chokoriensis*	99.4
XVII	STR1s-6	STR1s-6	*M. ureilytica*	99.6
XVIII	Y6-2	Y6-2	*M. pisi*	98.8

600

601