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

Lorena Carro¹, Valeria Razmilic^{1,2}, Imen Nouioui¹, Lee Richardson¹, Che Pan¹, Patrycja Golinska³, Juan A. Asenjo², Alan T. Bull⁴, Hans-Peter Klenk¹, Michael Goodfellow¹

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.

37 Introduction

38 Given their importance in biotechnology, bioprospecting and ecology, actinobacteria
39 remain a source of interest to the microbiological community (Goodfellow and Fiedler
40 2010; Demain 2014; Barka et al. 2016). Actinobacteria were initially isolated from the
41 Atacama Desert over fifty years ago (Cameron et al. 1966; Opfell and Zebal 1967)
42 though the first extensive survey of these organisms in this desert was only reported
43 recently (Okoro et al. 2009). Okoro and her colleagues isolated small numbers of
44 filamentous actinobacteria from arid, hyper-arid and extreme hyper-arid Atacama
45 Desert soils using selective isolation procedures and polyphasic taxonomic methods
46 which showed that a high proportion of the isolates belonged to the genera
47 *Amycolatopsis*, *Lechevaleria* and *Streptomyces* many of which were assigned to
48 putative new species and contained novel non-ribosomal peptide synthase genes.
49 Subsequent studies have shown that Atacama Desert habitats are not only a rich source
50 of novel streptomycetes but also of rare and poorly studied taxa, some of which produce
51 bioactive compounds and have been given validly published names (Bull et al. 2016;
52 Busarakam et al. 2014, 2016; Goodfellow et al. 2017; Idris et al. 2017a; Idris et al.
53 2017b; Trujillo et al. 2017). *Streptomyces leewenhoekii* strains (Busarakam et al. 2014),
54 for instance, synthesize novel macrolactone and polyketide antibiotics (Nachtigall et al.
55 2011; Rateb et al. 2011a; Rateb et al. 2011b) and chaxapeptin, a new lasso peptide
56 (Elsayed et al. 2015) while the type strain of *Lentzia chajnantorensis* (Idris et al. 2017b)
57 produces novel diene and monoene glycosides, several of which show anti-HIV
58 integrase activity (Wichner et al. 2017). Complementary metagenomic analyses of
59 Atacama Desert habitats have revealed a remarkable actinobacterial diversity most of
60 which has not been detected using culture-dependent methods (Bull et al. 2017; Idris
61 et al. 2017c). Improved selective isolation and cultivation methods are needed to isolate
62 components of this diversity, not least *Micromonospora* strains which are known to be a
63 rich source of new specialist metabolites and have the potential to defend plants against
64 root-infecting fungi (Carro et al. 2018a).

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

77 It is apparent from culture-independent studies that micromonosporae form a
78 small, but integral part of actinobacterial communities in Atacama Desert habitats (Bull
79 et al. 2017; Idris et al. 2017c). The failure to isolate them from such habitats may reflect
80 the use of isolation media mainly designed to be selective for streptomycetes

81 (Busarakam 2014; Idris 2016). The primary aims of the present study were to isolate
82 micromonosporae from diverse Atacama Desert soils using procedures designed to be
83 selective for members of this taxon and to determine whether any such isolates
84 represented putatively novel taxa.

85

86 **Materials and Methods**

87 *Sampling sites*

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

94 *Selective isolation*

95 Three isolation procedures were used: a) Phenol-heat protocol (P-H): a gram of each of
96 the soil samples was diluted in 0.85% sodium chloride solution containing 1.5% phenol
97 (Hayakawa et al. 1991), the preparations shaken on a rotary shaker for an hour,
98 incubated at 70°C for 40 minutes in a water bath (Nonomura and Ohara, 1969), shaken
99 again at room temperature for 2 hours, serial dilutions prepared in the saline solution,
100 and the resultant preparations shaken at room temperature prior to spreading over chitin-
101 vitamin (CHV) (Zhang 2011), Gause number 1 (G n° 1) (Gause et al. 1983), humic acid-
102 vitamin (HV) (Hayakawa and Nonomura 1987), M65 (DSMZ medium number 65),
103 R2A (Reasoner et al. 1979) and Zhang' starch soil extract agar (ZSSE) (Zhang 2011), all
104 of the media were supplemented with 50µg/ml of cycloheximide and 50µg/ml of
105 nalidixic acid; b) sprinkle protocol (S): 0.5g of soil particles of individual samples
106 which had been preheated at 120°C for 15 minutes were sprinkled directly over the
107 isolation media and c) a standard dilution plate protocol (D): dilutions of each soil
108 sample in saline were used to inoculate each of the isolation media following the
109 procedure described by Goodfellow et al. (1967). All of the inoculated plates were
110 incubated at 28°C and examined weekly for up to 28 days for the presence of
111 *Micromonospora* colonies that were detected, using a stereoscopic microscope, and then
112 transferred to the medium from which they were isolated but without the antibiotics, the
113 resultant plates were incubated at 28°C for 15 days. The 45 isolated strains were
114 maintained on either M65 or ZSSE agar and in 20% v/v glycerol at -80°C for long-term
115 preservation.

116 *Dereplication of isolates*

117 Genomic DNA for BOX-PCR was extracted from all the isolates using a Bacterial DNA
118 Extraction Kit (Sigma) following the instructions of the manufacturer and BOX-PCR
119 fingerprint profiles generated using modifications of the methods described by
120 Versalovic et al. (1994) and Trujillo et al. (2010). To this end, Bioline 2x MiFi™ mix
121 was used for PCR amplification in a final volume of 25 µl per reaction following the
122 manufacturer's recommendations; the thermal cycling parameters were: 7 minutes at
123 95 °C, 30 cycles of 1 minute at 94 °C, 1 minute at 52 °C and 3 minutes at 72 °C followed
124 by a final extension at 72 °C for 10 minutes. Five microliters of each PCR product was

125 loaded onto a 2% agarose gel containing 10 µl of GelRed™ (Crisafuli et al. 2015) per
126 100 ml and electrophoresis run at 70 V for 3 hours in freshly prepared 1x TBE-EDTA
127 buffer at pH 8.0 using a Bio-Rad Pac 300 power supply; a DNA molecular weight
128 marker 1kb HyperLadder™ (Bioline) was used as the molecular size standard. After
129 electrophoresis, gels were photographed, stored on disk as TIFF files, and manually
130 aligned into 9 multi- and 10 single-membered similarity groups.

131 *Phylogeny*

132 PCR-mediated amplification of genomic DNA of 20 representatives of the BOX-PCR
133 groups was conducted using the universal primers 27F and 1522R (Lane 1991). Bioline
134 2x MiFi™ mix was used for PCR amplification in a final volume of 50 µl per reaction
135 following the manufacturer's recommendations. The thermal cycling parameters were:
136 9 minutes at 95 °C, 30 cycles of 1 minute at 94 °C, 1 minute at 56 °C and 2 minutes at
137 72 °C followed by a final extension at 72 °C for 10 minutes. The PCR products were
138 purified using a QIAquick® PCR purification kit, according to the manufacturer's
139 instructions (Qiagen) and sequenced using the EZ-seq Barcode Service (Macrogen).
140 The resultant sequences (around 1400 nucleotides) were manually aligned using
141 MUSCLE (Edgar 2004) and then compared with corresponding sequences of
142 *Micromonospora* type strains retrieved from the EzBioCloud server (Yoon et al. 2016).
143 Phylogenetic distances were calculated with the Kimura 2-parameter model (Kimura
144 1980) and tree topologies inferred using the neighbour-joining (Saitou and Nei 1987)
145 and maximum-likelihood algorithms (Felsenstein 1981) and one thousand bootstrap
146 replications. Tree reconstructions were carried out using MEGA 7 software (Kumar et
147 al. 2016).

148 *Stress tests*

149 All of the strains were examined for their ability to grow in the presence of various
150 concentrations of sodium chloride (1, 3, 5, 7 and 9%, w/v) and at a range of temperature
151 (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
152 ZSSE agar as basal media; pH values were determined using phosphate buffers, as
153 described previously (Carro et al. 2012b). All of the plates, apart from those from the
154 temperature tests, were incubated at 28°C for 15 days. The strains were also examined
155 for their ability to grow following exposure to 100 mJoules for 30 and for 60 minutes in
156 a UV chamber (Biorad) using *Geodermatophilus poikilotrophi* DSM 44209^T as the
157 positive control (Montero-Calasanz et al. 2014)) set at a wavelength of 254 nm (UVC).
158 The capacity of the strains to grow under anaerobic conditions was tested on M65 and
159 ZSSE agar plates that were incubated for 28°C for 10 days in anaerobic atmosphere
160 generation bags (Sigma-Aldrich 68061).

161 *Antibiotic sensitivity assays*

162 The isolates were examined for their ability to inhibit the growth of wild type strains of
163 *Bacillus subtilis*, *Escherichia coli* and *Pseudomonas fluorescens* using a standard plug
164 assay (Fiedler, 2004). Lawns of each of the isolates were prepared on yeast extract-malt
165 extract and oatmeal agar (International *Streptomyces* Project [ISP] media 2 and 3;
166 Shirling and Gottlieb 1966) plates incubated at 28°C for 14 days. Agar plugs taken from
167 the incubated plates were transferred to sterile square Petri Dishes and overnight Luria
168 broth cultures [OD₆₀₀ of approximately 0.6] of each of the wild type strains in molten
169 nutrient agar added to a final concentration of OD₆₀₀ of 0.0125. The inoculated dishes

170 were incubated at 28°C and examined for zones of inhibition around the agar plugs after
171 24 and 48 hours.

172 **Results and Discussion**

173 *Isolation of micromonosporae*

174 The ability to isolate specific fractions of actinobacterial communities from the
175 Atacama Desert biome is of paramount importance especially since metagenomic
176 analyses have revealed a staggering degree of actinobacterial diversity therein: the vast
177 majority of which has gone undetected using culture-dependent procedures (Idris et al.
178 2017c), this partly reflects the use of isolation media that favour the growth of
179 streptomycetes (Williams et al. 1984). Isolation procedures designed to recover specific
180 actinobacterial genera from environmental samples are many and varied but tend to
181 reflect the biological properties of the target organisms (Goodfellow 2010), as was the
182 case in the present study. It is, for instance, known that *Micromonospora* spores are
183 activated by heat pretreatment regimes, are resistant to phenol and germinate on nutrient
184 rich media such as humic acid-vitamin agar (Hoskisson et al. 2000; Shen et al. 2014;
185 Carro et al. 2018b). It is, therefore, encouraging that over half of the *Micromonospora*
186 strains were isolated from the soil samples using the phenol-heat procedure, albeit on
187 several isolation media (Table 2).

188 Micromonosporae growing on the isolation plates were recognized, under the
189 stereoscopic microscope, by their ability to produce filamentous microcolonies that
190 were strongly attached to agar, these colonies were readily distinguished from those of
191 aerobic, endospore-forming bacilli (data not shown). It was particularly interesting that
192 most of the *Micromonospora* strains came from the ALMA 4 sample as complementary
193 culture-independent studies have shown that soil samples from this location contain
194 micromonosporal propagules (Bull et al., 2017). The strains isolated from this site
195 proved to be unusual as they formed dry, filamentous blue-green colonies covered by
196 white aerial hyphae following growth for three weeks on M65 and ZSSE media (Fig. 1).
197 The ability of these isolates to form aerial hyphae is interesting as some
198 *Micromonospora* type strains have been shown to contain putative homologous genes to
199 those associated with aerial hyphae formation and spore maturation in streptomycetes
200 (Carro et al. 2018a). Strains isolated from soil samples taken from the three remaining
201 locations showed a typical micromonosporal phenotype, one characterized by the
202 production of filamentous orange colonies that lack aerial hyphae and turn blue-black
203 upon spore formation (Genilloud 2012). Five of these strains were isolated from
204 extreme hyper-arid soil collected from the Lomas Bayas region (Idris et al. 2017c), one
205 of the driest areas in the Atacama Desert; all of these isolates were recovered by plating
206 serial dilutions of the soil onto M65 agar. In turn, the 13 strains isolated from the
207 rhizosphere of *Parastrephia quadrangularis* (*Compositae*, tribe *Asteraceae*) were
208 obtained either by sprinkling mineral particles or spreading serial dilutions of the
209 phenol-heat protocol onto HV and ZSSE agar and incubating for four weeks at 28°C.
210 *Micromonospora* strains were recovered using all three selective isolation procedures
211 (Table 2).

212 *BOX-PCR profiles*

213 The BOX-PCR profiles of the isolates encompass considerable genetic diversity with
214 fragments ranging from 0.05 to 2.0 kb (Fig. 2) thereby providing further evidence that
215 this method is effective in distinguishing between *Micromonospora* strains (Maldonado
216 et al. 2008; Trujillo et al. 2010). The isolates were assigned to seven multi- and 11
217 single-membered groups defined at the 60% similarity level (Table 3). Five of the
218 multi-membered groups and five of the singletons were composed of strains isolated
219 from the ALMA 4 soil samples, the largest of these taxa, group XI, contained seven
220 isolates. In turn, the strains isolated from *P. quadrangularis* rhizosphere soil were
221 assigned to a multi-membered group that encompassed 10 strains and to two single
222 membered groups; the five Lomas Bayas strains formed one multi- and 3 single-
223 membered taxa while the single isolates from ALMA 5 and Yungay Core Region
224 samples gave unique profiles (Fig. 2). It is interesting that the multi-membered groups
225 only contained strains isolated from a single location and that few strains were clones
226 (Fig. 2; Table 3).

227 *Phylogenetic analyses*

228 Almost complete 16S rRNA gene sequences (1372 – 1507 nucleotides [nt]) were
229 generated for the 20 isolates chosen to represent the BOX-PCR groups (Table 3). The
230 generic assignment of all of these strains was confirmed as they were recovered in the
231 *Micromonospora* 16S rRNA gene tree (Fig. 3). The 16S rRNA gene sequence
232 similarities between these isolates and the type strains of their closest phylogenetic
233 neighbours fell within the range 98.4 to 99.9 % (Table 3). Several of the isolates were
234 assigned to well-supported clusters in the *Micromonospora* tree though none of these
235 taxa included strains isolated from more than one location. Ten out of the twelve
236 ALMA 4 strains formed a well-supported phyletic line within a weakly supported clade
237 that encompassed the type strains of *Micromonospora costi* (Thawai 2015), their nearest
238 neighbour, and *Micromonospora fulviviridis* (Kroppenstedt et al. 2005); it is interesting
239 that the isolates of this clade were recovered from different samples of ALMA 4 soil
240 using two of the three isolation protocols and several selective isolation media (Table
241 2). Isolate 5R2A7, one of the remaining ALMA strains, formed a well-supported clade
242 together with the type strain of *Micromonospora coriariae* (Trujillo et al. 2006), its
243 nearest phylogenetic neighbour, and with *Micromonospora cremea* (Carro et al. 2012b)
244 whereas isolate ATA32, the remaining ALMA 4 strain, formed an unsupported clade
245 with the type strain of *Micromonospora narathiwatensis* (Thawai et al. 2007) though it
246 was most closely related to *Micromonospora eburnea* (Thawai et al. 2005).

247 It is interesting that two out of the three strains recovered from the rhizosphere
248 of *P. quadrangularis*, isolates STR1-7 and STR1s-6, were loosely associated with the
249 type strains of *Micromonospora lupini* (Trujillo et al. 2007), *Micromonospora taraxaci*
250 (Zhao et al. 2014) and *Micromonospora violae* (Zhang et al. 2014) which were isolated
251 from a root nodule of *Lupinus angustifolius* and the roots of *Taraxacum mongolicum*
252 and *Viola philippica*, respectively (Fig. 3). Moreover, the final strain, isolate STR1s-5,
253 was most closely related to the type strain of *Micromonospora ureilytica* (Carro et al.
254 2016b), which was isolated from a root nodule of *Pisum sativum*. In turn, two of the
255 four strains isolated from the extreme hyper-arid Lomas Bayas soil, isolates LB19 and
256 LB32, were phylogenetically close to the type strain of *Micromonospora saelicesensis*
257 (Trujillo et al. 2007), an isolate from a root nodule of *L. angustifolius*. It is also
258 interesting that isolate LB39 was recovered in the well supported clade that included the

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

266 To date, representatives of dereplicated groups of actinobacteria isolated from
267 Atacama Desert soils which show low pairwise 16S rRNA gene sequence similarities,
268 ($\leq 90\%$; Meier-Kolthoff et al. 2013) with the type strains of their nearest phylogenetic
269 neighbour have been invariably assigned to new species when subject to polyphasic
270 taxonomic analyses, as exemplified by *Lechevalieria*, *Pseudonocardia* and
271 *Streptomyces* species (Okoro et al. 2010; Busarakam et al. 2014; Trujillo et al. 2017).
272 This cut-off point has also proved to be a reliable indicator for the presumptive
273 recognition of novel *Micromonospora* species (Trujillo et al. 2007; Garcia et al. 2010;
274 Carro et al. 2016a) though pairwise 16S rRNA gene sequence similarities very much
275 higher than the 99.0% threshold have been found to be indicative of new
276 *Micromonospora* species, as exemplified by *M. taraxaci* and *M. violae* (Zhang et al.
277 2014; Zhao et al. 2014). Given these indicators it seems likely that further comparative
278 taxonomic analyses will show that most, if not all, of the representatives of the BOX-
279 PCR groups will be found to represent novel *Micromonospora* species. Indeed, isolates
280 ATA38, ATA39, and ATA43 from ALMA4, isolate STR1-7 from the rhizosphere of *P.*
281 *quadrangularis*, and isolate Y6_2 from Yungay are cases in point as they share 16S
282 rRNA gene sequence similarities with their closest phylogenetic neighbours at or below
283 the 99.0% threshold (Table 3).

284 *Adaptation to extreme conditions*

285 In general, the pH and temperature profiles of the isolates were typical of
286 *Micromonospora* strains (Genilloud 2012) as they grew from 20 to 37°C, at pH 6.0 to
287 8.0, but not at 4, 12 or 45°C or below pH 5.0. None of the isolates grew under anaerobic
288 conditions, but most of them were able to grow in the presence of 1%, w/v sodium
289 chloride; six out of the thirteen strains isolated from the rhizosphere of *P.*
290 *quadrangularis* grew in the presence of 3%, w/v sodium chloride. It was also interesting
291 that only 30% of the samples from ALMA soil grew on M65 agar following exposure to
292 UV light (UVC) at 100 mJoules/second for 30 minutes, this number increased to 60%
293 on ZSSE agar. Similarly, high percentages were observed for strains isolated from the
294 rhizosphere and Lomas Bayas soils on M65 (64 and 80%) and ZSSE (50 and 60%) agar,
295 respectively. These results are interesting as it has been shown that the type strain of
296 *Modestobacter caceresii*, an isolate recovered from a soil sample from the Yungay Core
297 Region, has the capacity to protect and repair damaged caused by UV radiation
298 (Busarakam et al. 2016). Interestingly, the sole *Micromonospora* strain obtained from
299 this area, isolate Y6-2, grew after an hour exposure to UV light at 100 mJoules/second,
300 only around 20% of the isolates grew under this condition. Although UVC radiation no
301 longer reaches the Earth's surface it has been used as a selective tool with reference to
302 early life on the planet and high altitude biology. Recently Paulino-Lima et al. (2016)
303 screened desert soils, including Atacama soil, for UVC-resistant bacteria and
304 interestingly over 40% of their isolates were members of the phylum *Actinobacteria*;

305 *Micromonospora* strains were not recovered by these authors. Consequently, the
306 distinctive radiation resistance of *Micromonospora* strain Y6-2 suggests that it could be
307 a novel target for detailed radiation-resistant physiological and biochemical researches
308 that should be extended to include the other radiation stressors naturally present on
309 the Earth surface. The study of these radiations will be of interest for future studies of
310 the strains and to determine if key osmoprotectant proteins are implicated in the process
311 as previously shown in *Rhodobacter* (Pérez et al 2017).

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

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

327

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334

335 **Conflict of Interest**

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

337

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

340

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566 **Figure legends**

567

568 **Figure 1.** A. Sampling sites: Lomas Bayas (LB), Salar de Tara (ST), Atacama Large
569 Millimeter Array observatory (ALMA) and Yungay (Y6); B. Salar de Tara site; C.
570 *Parastrephia. quadrangularis* plant growing at the Salar the Tara; D. Typical orange
571 colony of a *Micromonospora* isolate; E. Typical black *Micromonospora* colony
572 following spore production; F-H. Morphology observed for some Atacama
573 micromonosporae; F. Sporulating *Micromonospora* colonies; G. Mountain-shaped
574 *Micromonospora* isolates; H. Colony from ALMA4 isolate covered by white aerial
575 hyphae.

576

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

579

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

587

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	N° 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 <i>Parastrephia quadrangularis</i>	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
	4G51, 4G53, 4G55, 4G57	D	G nº 1	green
ALMA 4	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	STR1s-5, STR1s-6, STR1s-7, STR1s-11	S	HV	orange
(rhizosphere)	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	<i>M. costi</i>	99.1
II	ATA35, ATA37, ATA40	ATA40	<i>M. costi</i>	99.1
III	ATA38	ATA38	<i>M. costi</i>	98.4
IV	ATA39	ATA39	<i>M. costi</i>	98.4
V	ATA42	ATA42	<i>M. costi</i>	99.1
VI	ATA43	ATA43	<i>M. terminaliae</i>	98.8
VII	ATA32, ATA34	ATA32	<i>M. eburnea</i>	99.4
VIII	LB4, LB41	LB4	<i>M. chalcea</i>	99.6
IX	LB19	LB19	<i>M. saelicesensis</i>	99.8
X	LB32	LB32	<i>M. saelicesensis</i>	99.8
XI	LB39	LB39	<i>M. chokoriensis</i>	99.9
XII	ATA36, ATA44, 4G51, ATA 46, 4G55, 4G53, 4G57	4G51, 4G57	<i>M. costi</i>	99.1
XIII	ATA51a, ATA51b	ATA51b	<i>M. costi</i>	99.1
XIV	5R2A7	5R2A7	<i>M. coriariae</i>	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	<i>M. chokoriensis</i>	99
XVI	STR1s-5	STR1s-5	<i>M. chokoriensis</i>	99.4
XVII	STR1s-6	STR1s-6	<i>M. ureilytica</i>	99.6
XVIII	Y6-2	Y6-2	<i>M. pisi</i>	98.8

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601