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1 ***Blastococcus atacamensis* sp. nov., a novel strain adapted to life in the Yungay core**  
2 **region of the Atacama Desert**

3  
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23

24 Running title: *Blastococcus atacamensis* sp. nov.

25

26 Subject category: New taxa: Actinobacteria

27

28 The GenBank accession numbers for the 16S rRNA gene and genome sequences of strain  
29 P6<sup>T</sup> are KX926540 and POQU00000000, respectively. The genome accession number of  
30 *Blastococcus saxobsidens* DSM 44509<sup>T</sup> is POQT00000000.

31

32 Abbreviations: A<sub>2</sub>pm, diaminopimelic acid; ANI, average nucleotide identity; dDDH, digital  
33 DNA–DNA hybridization; GGDC, Genome-to-Genome Distance Calculator; NJ, neighbour-  
34 joining; ML, maximum-likelihood; MP, maximum-parsimony; MUSCLE, Multiple  
35 Sequence Comparison by Log-Expectation; T3PKS, type III polyketide synthase.

36

37 Keywords: polyphasic taxonomy, stress and biosynthetic genes, whole-genome sequences

38

### 39 **Abstract**

40 A polyphasic study was undertaken to establish the taxonomic status of a *Blastococcus* strain  
41 isolated from an extreme hyper-arid Atacama Desert soil. The isolate, strain P6<sup>T</sup>, was found  
42 to have chemotaxonomic and morphological properties consistent with its classification in  
43 the genus *Blastococcus*. It was shown to form a well-supported branch in the *Blastococcus*  
44 16S rRNA gene tree together with the type strains of *Blastococcus capsensis* and  
45 *Blastococcus saxobsidens* and was distinguished from the latter, its closest phylogenetic  
46 neighbour, by a broad range of phenotypic properties. The draft whole genome sequence of  
47 isolate P6<sup>T</sup> showed 83.6% average nucleotide identity, 83.0% average amino acid identity  
48 and a digital DNA:DNA hybridisation value of 27.8% in comparison with the genome  
49 sequence of *B. saxobsidens* DSM 44509<sup>T</sup>, values consistent with its assignment to a separate  
50 species. Based on these data it is proposed that isolate P6<sup>T</sup> (NCIMB 15090<sup>T</sup> = NRRL B-  
51 65468<sup>T</sup>) be assigned to the genus *Blastococcus* as *Blastococcus atacamensis* sp. nov.  
52 Analysis of the whole genome sequence of *B. atacamensis* P6<sup>T</sup>, with 3,778 open reading  
53 frames and a genome size of 3.9 Mb showed the presence of genes and gene clusters that  
54 encode for properties that reflect its adaptation to the extreme environmental conditions that  
55 prevail in Atacama Desert soils.

56

57 The actinobacterial genus *Blastococcus* was proposed by Ahrens and Moll [1] and the  
58 description subsequently emended by Urzì *et al.* [2], Lee [3] and Hezbri *et al.* [4],  
59 respectively. The genus *Blastococcus* together with the genera *Cumulibacter* [5],  
60 *Geodermatophilus* [6], *Klenkia* [7] and *Modestobacter* [8] belong to the family  
61 *Geodermatophilaceae* [9, 10] of the order *Geodermatophilales* [11]. Members of all of these  
62 taxa share genomic features, as exemplified by multiple copies of the *trwC* gene (conjugative

63 relaxase) [12], have modest growth requirements [13], show unusual resistance to oxidative  
64 stress [14] and tend to be associated with arid biomes, such as desert and high altitude soils  
65 and with the surfaces of ancient monuments and natural stones [2, 15, 16].

66 Blastococci form a well-supported clade in the *Geodermatophilaceae* 16S rRNA gene tree  
67 [4, 17] and can be distinguished from members of other genera classified in this family using  
68 a combination of phenotypic features [13]. They are Gram-stain positive, coccoid-shaped  
69 bacteria that may be motile or non-motile and which may propagate by budding and multiple  
70 fission; they have *meso*-A<sub>2</sub>pm in the peptidoglycan, mainly unsaturated and iso-branched  
71 fatty acids; and complex phospholipid profiles which may include diphosphatidylglycerol,  
72 phosphatidylcholine, phosphatidylethanolamine and phosphatidylinositol [4, 18]. At the time  
73 of writing the genus encompasses 5 validly named species, namely *Blastococcus aggregatus*  
74 [1, 4, 19], the type species, *Blastococcus capsensis* [4], *Blastococcus endophyticus* [4, 17],  
75 *Blastococcus jejunensis* [3, 4], and *Blastococcus saxobsidens* [4, 19], and one validly named  
76 strain, *Blastococcus colisei* [20]. These bacteria were isolated from the Baltic Sea, an  
77 archaeological Roman pool in Tunisia, the leaves of the medicinal Chinese plant  
78 *Camptotheca acuminata*, beach sand off the coast of South Korea, a limestone sample from  
79 a church in Malta and an archaeological amphitheatre, respectively, and can be distinguished  
80 using a range of phenotypic properties [4]. In addition, “*Candidatus Blastococcus*  
81 *massiliensis*” was identified, from a stool sample of a patient with anorexia nervosa [21].

82 In a continuation of our studies on actinobacterial diversity in Atacama Desert habitats,  
83 several strains were recovered from an extreme hyper-arid soil that had colonial and  
84 morphological properties typical of blastococci. One of these isolates, strain P6<sup>T</sup>, was the  
85 subject of a polyphasic taxonomic study which showed that it represents a new *Blastococcus*  
86 species, for which the name *Blastococcus atacamensis* sp. nov. is proposed.

87

88 *Blastococcus* strains were isolated from an extreme hyper-arid soil sample in November 2011  
89 from the Yungay core region of the Atacama Desert on the eastern flank of the Cerro Aguas  
90 Blancas (24°06'18.6"S/70°01'55.6W) at 1,033 metres above sea level. One gram of the soil  
91 sample was suspended in 4.0 ml of ¼ strength Ringer's solution (Oxoid, product No.  
92 BO0332D), this suspension was shaken on a tumble shaker prior to heating at 55°C for six  
93 minutes. Aliquots (100µl) of the 10<sup>-1</sup> and 10<sup>-2</sup> dilutions were spread, in triplicate, over GYM

94 *Streptomyces* (DSMZ medium No. 65) and *Geodermatophilus obscurus* media [22]  
95 supplemented with nalidixic acid ( $10\mu\text{g}\cdot\text{ml}^{-1}$ ), cycloheximide and nystatin (each at  $25\mu\text{g}\cdot\text{ml}^{-1}$ ).  
96 The isolation plates were dried for 15 minutes at room temperature before incubation, as  
97 recommended by Vickers and Williams [23]. After incubation at  $28^{\circ}\text{C}$  for 2 weeks, the  
98 presumptive *Blastococcus* isolates were counted and expressed as the number of colony  
99 forming units (cfu) per gram dry weight soil.

100 Small numbers of strains growing on the isolation plates were assigned to the genus  
101 *Blastococcus* as they formed characteristic small, circular, reddish pink colonies with entire  
102 margins; the highest count,  $3.7\cdot 10^3$  cfu/g dry weight soil, was recorded from the *G. obscurus*  
103 medium plates and the corresponding number on the GYM *Streptomyces* medium plates was  
104  $2.7\cdot 10^3$  cfu/g dry weight soil. A representative *Blastococcus* strain, isolate P6<sup>T</sup>, was taken  
105 from one of the GYM *Streptomyces* plates and along with the type strains of *Blastococcus*  
106 species was maintained on GYM slopes at room temperature and as suspensions of cells in  
107 20%, v/v glycerol at  $-20^{\circ}\text{C}$  and  $-80^{\circ}\text{C}$ .

108 The isolate was examined for motility and morphological properties using procedures  
109 described by Trujillo *et al.* [24]. Cultural features were recorded on modified Bennett's  
110 (DSMZ medium No. 894), GPHF (DSMZ medium No. 553), GYM *Streptomyces* (DSMZ  
111 medium No. 65), Luedemann's [6], potato dextrose (DSMZ medium No. 129), Reasoner's  
112 2A (DSMZ medium No. 830) and from tryptone-yeast extract, yeast extract-malt extract,  
113 oatmeal, inorganic salts-starch, glycerol-asparagine, peptone-yeast extract-iron and tyrosine  
114 agar (International *Streptomyces* Project [ISP] media 1–7; [25]) plates following incubation  
115 at  $28^{\circ}\text{C}$  for 3 weeks. The isolate was found to be a Gram-stain positive, non-motile  
116 actinobacterium that formed rods and coccoid shaped cells with evidence of budding (Fig.  
117 1). Strain P6<sup>T</sup> was observed to grow well on GYM *Streptomyces*, GPHF, modified Bennett's,  
118 potato dextrose, Luedemann's, Reasoner's 2A and yeast extract-malt extract agar, as  
119 exemplified in Figure S1, but poorly on ISP media 1, 3 to 7, generally producing red-orange  
120 or yellowish pink pigments; diffusible pigments were not observed on any of these media.

121 Biomass for most of the chemotaxonomic analyses carried out on isolate P6<sup>T</sup> was harvested  
122 from 1,000 ml yeast extract-malt extract broth ISP medium 2 that had been shaken in 500 ml  
123 baffled Erlenmeyer flasks, each flask containing 200 ml of medium, at 180 revolutions per  
124 minute (rpm) at  $28^{\circ}\text{C}$  for 2 weeks; the biomass was washed twice in distilled water and

125 freeze-dried. Biomass for the fatty acid analysis was prepared on PYGV agar (DSMZ  
126 medium No. 621), modified by the inclusion of 2 g of peptone instead of casein, 2 g of yeast  
127 extract and 10 ml of a 20% w/v glucose, after incubation at 20°C for 16 days and washed  
128 twice in sterile distilled water.

129 Isolate P6<sup>T</sup> was examined for chemotaxonomic markers known to be of value in the  
130 systematics of microorganisms classified in the genus *Blastococcus* [4, 18]. Standard  
131 chromatographic procedures were used to determine the isomers of A<sub>2</sub>pm [26], isoprenoid  
132 quinones [27], whole-cell sugars [28] and polar lipids [29], as modified by Kroppenstedt and  
133 Goodfellow [30]. Isoprenoid quinones extracted from *Micromonospora luteifusca* GUI2<sup>T</sup>  
134 [31] were used as standards. In turn, fatty acids extracted from the isolate were methylated,  
135 analysed using the protocol of the Sherlock Microbial Identification (MIDI) system, version  
136 5 [32] and the resultant peaks identified using the ACTIN 6 database.

137 In general, the chemotaxonomic properties of isolate P6<sup>T</sup> are consistent with its classification  
138 in the genus *Blastococcus* [4, 18, 20]. The organism contains *meso*-A<sub>2</sub>pm as the diagnostic  
139 diamino acid (Fig. S2); MK-9(H<sub>2</sub>) and MK-9(H<sub>4</sub>) as predominant isoprenologues in a  
140 proportion of 3:2 (Fig. S3); iso-C<sub>16:0</sub> (38.9%), iso-C<sub>16:1</sub> H (17.7%), iso-C<sub>15:0</sub> (14.2%) and 9-  
141 methyl-C<sub>16:0</sub> (5.7%) as major fatty acids; a polar lipid profile that includes  
142 diphosphatidylglycerol, phosphatidylcholine, phosphatidylethanolamine,  
143 phosphatidylglycerol and phosphatidylinositol (Fig. S4) and galactose and glucose as major  
144 sugars with a lesser proportion of ribose and traces of arabinose and xylose (Fig. S5).

145 Genomic DNA for 16S rRNA gene sequencing was extracted from 25 ml ISP 2 broth  
146 incubated at 28°C, shaken at 180 rpm for 10 days; 5 ml of the fully grown culture were used  
147 for genomic DNA extraction, following the protocol used by Kieser *et al.* [33] though in this  
148 case incubation with protease K was conducted at 60°C until the solution became clear  
149 (~1.5h); the quality of the isolated genomic DNA was checked in a 1%, w/v agarose gel run  
150 at 70V for 1.5h. PCR-mediated amplification of the 16S rRNA gene was performed in a final  
151 volume of 25 µl using the standard primers 27F and 1525r [34], 100 ng of genomic DNA and  
152 MyFi™ Mix (Bioline, UK), following the manufacturer's instructions; the PCR conditions  
153 were 5min at 95°C followed by 30 cycles of 30sec at 95°C, 30sec at 55°C and 23sec at 72°C.  
154 Two µl of the resulting PCR mixture was passed through a 1%, w/v agarose gel from which  
155 a single band of the expected size (about 1,500 bp) was visualised. The rest of the PCR

156 mixture was cleaned with exonuclease I and shrimp alkaline phosphatase (NEB, UK;  
157 #E2622S) and sent to Geneius (Cramlington, UK) for sequencing, using a BigDye®  
158 terminator v3.1 cycle sequencing kit (Thermo Fisher Scientific Inc.) on an ABI sequencer  
159 model 3730xl; the sequence was assembled using Pregap4 and Gap4 from Staden Package  
160 version 2.0.0b9 [35].

161

162 The fragment of the 16S rRNA gene sequence of isolate P6<sup>T</sup> (1,340 bp) was compared with  
163 corresponding 16S rRNA gene sequences of the type strains of the *Blastococcus* species, the  
164 sequence of “*Candidatus B. massiliensis*” AP3 and those of *Cumulibacter manganoferans*  
165 DSM 103787<sup>T</sup>, *G. obscurus* DSM 43160<sup>T</sup>, ‘*Klenkia marina*’ DSM 45722<sup>T</sup> and  
166 *Modestobacter multiseptatus* DSM 44406<sup>T</sup> all of which were retrieved from the EzBioCloud  
167 server (<http://www.ezbiocloud.net/>; [36]). Alignment of the nucleotide sequences was  
168 achieved with MUSCLE [37]. Phylogenetic trees were inferred with the MEGA7 suite of  
169 programs version 7.0 [38] using the NJ [39], ML [40] and MP algorithms [41] with 1,000  
170 bootstrap repetitions [42]; evolutionary distances were calculated with the Kimura 2-  
171 parameters model [43]. Sequence similarity values were calculated based on the alignment  
172 generated with MUSCLE [37], using PHYDIT software version 3.0. In addition, pairwise  
173 sequence similarities and phylogenetic reconstruction were performed using the method  
174 recommended by Meier-Kolthoff *et al.* [44] and the GGDC web server [45] available at  
175 <http://ggdc.dsmz.de/phylogeny-service.php#> and the Genome-to-Genome Distance  
176 Calculator (GGDC; [44]) to validate the results obtained using the MEGA7 software. The  
177 resultant trees were rooted with *G. obscurus* DSM 43160<sup>T</sup>, *K. marina* YIM M1315 and *M.*  
178 *multiseptatus* DSM 44406<sup>T</sup> using FigTree version 1.4.2  
179 (<http://tree.bio.ed.ac.uk/software/figtree/>). All of the Figures were edited in Inkscape version  
180 0.9 (<https://inkscape.org/en/download/>).

181 Strain P6<sup>T</sup> was found to form a well-supported subclade in the *Blastococcus* 16S rRNA gene  
182 tree together with the type strains of *B. capsensis* and *B. saxobsidens* (Fig. 2). It is most  
183 closely related to type strain of the *B. saxobsidens* sharing a 16S rRNA gene similarity of  
184 99.5%, a value that corresponds to 7 nucleotide (nt) differences at 1,339 locations. The  
185 corresponding 16S rRNA gene sequence similarity value between P6<sup>T</sup> and *B. capsensis* BMG  
186 804<sup>T</sup> was 99.1%, which equates to 10 nt differences at 1,241 sites. The 16S rRNA gene

187 sequence similarities between strain P6<sup>T</sup> and the other *Blastococcus* type strains fell within  
188 the range 97.7 and 98.1%, values corresponding to between 26 and 31 nt differences. The  
189 same pairwise similarity values were recorded between the isolate and the type strains of *B.*  
190 *capsensis* and *B. saxobsidens* using the GGDC server.

191 Isolate P6<sup>T</sup> and *B. saxobsidens* DSM 44509<sup>T</sup> were examined for a broad range of phenotypic  
192 properties. Enzyme profiles were determined using API ZYM strips (BioMérieux) by  
193 following the manufacturer's instructions, while GEN III microplates (Biolog Inc., Hayward,  
194 CA, USA) were used to test for the ability of the strains to oxidise carbon and nitrogen  
195 sources and to determine resistance to inhibitory compounds using inoculating fluid (IF-C,  
196 Biolog Inc.) and a cell density of 86% transmittance in an OmniLog instrument (Biolog Inc.)  
197 set at 28°C. Data from the triplicated cultures recorded in Phenotypic Mode from the GENIII  
198 microplates were analysed using opm package 1.0.6 [46] for R [47], using R studio [48].  
199 Many of the remaining tests were carried out using ISP 2 agar [25] as the basal medium. The  
200 strains were examined for their ability to grow at a range of pH values (pH 5–10 at single  
201 unit intervals; pH was adjusted by adding drops of either 1N NaOH or 1N HCl as described  
202 by Montero-Calasanz *et al.* [49]) and temperatures (4, 10, 20, 28, 37, 40, 45 and 50°C) and  
203 in the presence of various concentrations of sodium chloride (1.0, 1.5, 3.0, 5.0, 7.0, 15.0 and  
204 20%, w/v). Apart from the temperature tests these features were recorded after incubation at  
205 28°C. Results of all of these tests were recorded after incubation for 3 weeks. The ISP 2  
206 medium was also used to test the capacity of the strains to degrade casein (1%), cellulose  
207 (1%), elastin (0.3%), guanine (0.5%), hypoxanthine (0.4%), L-tyrosine (0.4%), uric acid  
208 (0.5%) and xanthine (0.4%), their ability to degrade tributyrin was determined using  
209 tributyrin agar (Sigma-Aldrich). Results of all of these tests were recorded after incubation  
210 at 28°C for 14 days. Aesculin (0.1%) and arbutin (0.1%) hydrolysis was established using the  
211 media and methods described by Williams *et al.* [50], the hydrolysis of urea (0.2%, w/v) after  
212 Christensen [51] and nitrate reduction following Schaal *et al.* [52]. Catalase production was  
213 detected by the formation of bubbles after mixing a fresh drop of 3% hydrogen peroxide to  
214 fresh growth of the cultures on glass slides. Oxidase activity was determined in a 1%, w/v  
215 solution of *N-N-N'-N'*-tetramethyl-1,4-phenyldiamine (Sigma-Aldrich) and the development  
216 of a blue purple colour was recorded as a positive result [53]. The degradation and tolerance



217 tests were carried out in triplicate using a cell suspension equivalent to 5.0 on the McFarland  
218 scale [54].

219 The triplicated tests on isolate P6<sup>T</sup> and *B. saxobsidens* DSM 44509<sup>T</sup> gave identical results for  
220 all of the phenotypic tests, apart from the ability of isolate P6<sup>T</sup> to degrade arbutin.

221 The phenotypic properties of isolate P6<sup>T</sup> and *B. saxobsidens* DSM 44509<sup>T</sup> strains were  
222 compared with those of the other type strains of *Blastococcus* species, which had mainly been  
223 examined using the same procedures, as exemplified by the API, GENIII microplate,  
224 tolerance and catalase tests [4, 20]. It can be seen from Table 1 that the isolate can be  
225 distinguished from all of the *Blastococcus* type strains, including *B. capsensis* DSM 46835<sup>T</sup>  
226 and *B. saxobsidens* DSM 44509<sup>T</sup>, its nearest phylogenetic neighbours, using a combination  
227 of phenotypic features. It can be distinguished from each of these organisms by its ability to  
228 grow at 10 and 45°C, to reduce nitrate to nitrite and use dextrin,  $\alpha$ -keto-butyric acid and D-  
229 malic acid, and by its inability to use glucuronamide and D-saccharic acid as sole carbon  
230 sources. It can also be separated from the *B. saxobsidens* type strain by its capacity to oxidise  
231 pectin and D-salicin. It is also evident from Table 1 that several chemotaxonomic features  
232 support the separation of isolate P6<sup>T</sup> from the type strains of *Blastococcus* species. Isolate  
233 P6<sup>T</sup> and *B. saxobsidens* DSM 44509<sup>T</sup> were also found to share many phenotypic features:  
234 they produce acid and alkaline phosphatases,  $\alpha$ -chymotrypsin, cysteine arylamidase, esterase  
235 (C4), esterase lipase (C8),  $\alpha$ -glucosidase, naphthol-AS-B1-phosphohydrolase and valine  
236 arylamidase; degraded aesculin, tributyrin and starch; but do not produce lipase (C14),  $\alpha$ -  
237 mannosidase, or  $\alpha$ -fucosidase or hydrolyse allantoin and urea, or degrade casein, cellulose,  
238 elastin, guanine, hypoxanthine, L-tyrosine, uric acid, or xanthine. In addition, they can utilise  
239  $\alpha$ - and  $\beta$ -hydroxy-butyric acid, D-cellobiose, D-fucose,  $\beta$ -gentiobiose, D-gluconic acid,  $\alpha$ -D-  
240 lactose, D-salicin, sucrose and D-turanose but not L-alanine, D-arabitol, D-aspartic acid,  
241 glycyl-proline, gelatin, mucic acid or *p*-hydroxy-phenylacetic acid.

242 Biomass for sequencing the whole-genome of isolate P6<sup>T</sup> was prepared in a 1.5 ml of brain-  
243 heart infusion broth at 28°C in a shaking incubator (180 rpm) for 2 days. Genomic DNA of  
244 strains was extracted using the QIAamp DNA extraction kit (Qiagen, USA) according to the  
245 manufacturer's instructions. The purity and concentration of the extracted genomic DNA  
246 were measured using the Nanodrop spectrophotometer (NanoDrop Technologies, UK).  
247 Genome sequencing was performed on an Illumina MiSeq instrument (Illumina); the reads

248 were assembled by using SPAdes 3.9.0 [55] and contigs smaller than 1,000 bp in size were  
249 discarded. The draft assemblies have been submitted to the GenBank (accession numbers:  
250 POQU000000000 and POQT000000000) and is publicly available.

251 The genomes were annotated using the RAST annotation pipeline [56] and a sequenced based  
252 comparison performed in the SEED Viewer [56, 57]. A digital DNA:DNA hybridisation  
253 (dDDH) value was calculated between the genomes of strain P6<sup>T</sup> and *B. saxobsidens* DSM  
254 44509<sup>T</sup> using the GGDC server [45]. BLAST-based ANI and AAI between the strains were  
255 calculated using the online resource from the K. Konstantinidis group (<http://enve-omics.ce.gatech.edu/>; [58]).

257 The draft genomes of strain P6<sup>T</sup> and *B. saxobsidens* DSM 44509<sup>T</sup>, contained 3,778 and 4,348  
258 open reading frames, respectively, and were 3.9 Mb and 4.5 Mb in size with average *in silico*  
259 DNA G+C contents of 73.1 and 74.3 mol%. The dDDH value between the genome of the  
260 two strains was 27.8% (C. I. 25.4-30.3%), which is well below the 70% threshold used to  
261 confirm the species status of novel strains [59]. The corresponding ANI and AAI indices  
262 were 84.6± 4.5 and 83.0± 13.0, values below the threshold used for prokaryotic species  
263 delineation [58, 60, 61].

264 The draft genomes of isolates P6<sup>T</sup> and *B. saxobsidens* DSM 44509<sup>T</sup> were examined using the  
265 antiSMASH server [62] to detect putative biosynthetic gene clusters. The genome of isolate  
266 P6<sup>T</sup> was found to encode for a T3PKS and corresponding residues that make up the catalytic  
267 triad found in RppA, a T3PKS involved in the biosynthesis of pentaketide 1,3,6,8-  
268 tetrahydroxynaphthalene in *Streptomyces griseus* [63, 64]. The T3PKS of isolate P6<sup>T</sup> showed  
269 94% sequence identity with a corresponding sequence detected in the genome of the *B.*  
270 *saxobsidens* strain and 83% identity with a putative T3PKS encoded in the genome of *G.*  
271 *obscurus* DSM 43160<sup>T</sup> (Gobs\_4821; UniProt [65], accession number: D2S5V1). The gene  
272 that encodes for the T3PKS of isolate P6<sup>T</sup> was surrounded by other biosynthetic genes, such  
273 as one encoding for a methyltransferase and others encoding regulatory and transport  
274 proteins, thereby suggesting the presence of a biosynthetic gene cluster though the  
275 functionality and product generated by this putative biocluster has still to be established. The  
276 genomes of isolate P6<sup>T</sup> and the *B. saxobsidens* strain were also found to harbour genes  
277 encoding for polyprenyl synthetase and phytoene synthase, enzymes involved in the  
278 biosynthesis of terpenoid compounds [66, 67]. The genome of the *B. saxobsidens* strain

279 contains two genes that encode for proteins that contain the IucA/IucC domain (Pfam [68]  
280 accession: pfam04183) which is involved in the biosynthesis of siderophore compounds [69].  
281 The genome of the type strain of *Modestobacter caceserii* which, like P6<sup>T</sup>, was isolated from  
282 an extreme hyper-arid soil sample collected from the Yungay core region of the Atacama  
283 Desert, contained a siderophore gene cluster predicted to encode for deferoxamine; the  
284 genome of this organism also contained gene clusters encoding for type II and III polyketides  
285 and terpenes [70]. These preliminary datasets suggest that the genomes of  
286 *Geodermatophilaceae* strains have the capacity to produce specialised metabolites such as  
287 polyketides and siderophores. However, antiSMASH does not necessarily detect all of the  
288 gene clusters in genomes, as exemplified by the failure to identify the hygromycin A gene  
289 cluster in *Streptomyces leewenhoekii* C34<sup>T</sup> [71], moreover an improved genome assembly is  
290 required for a more precise interpretation of predicted biosynthesis gene clusters in the  
291 genome of isolate P6<sup>T</sup>.

292

293 A comparison of the genomes of isolate P6<sup>T</sup> and *B. saxobsidens* DSM 44509<sup>T</sup> showed that  
294 the genome of the former contains 474 genes that are absent from the genome of the latter,  
295 including those involved in stress responses (2 copies of *terA* and 3 copies of *terD* genes; P6-  
296 peg 850-P6-peg 855) [72]. In contrast, the genome of the *B. saxobsidens* strain harbours  
297 2,848 genes that are absent from isolate P6<sup>T</sup> (data not shown); these genes include multiple  
298 copies of the *tetA*, *tetB*, and *tetC* genes that are involved in tricarboxylate/citrate transport.  
299 However, most of the unique genes (54-66%) found in the genomes of these strains encode  
300 for hypothetical proteins.

301 The genomes of isolate P6<sup>T</sup> and the *B. saxobsidens* DSM 44509<sup>T</sup> contained 119 and 147  
302 genes, respectively that are associated with stress responses (Table S1). Each of the strains  
303 contained two genes involved in carbon starvation, one encoding for carbon starvation  
304 protein A and the other for a carbon storage regulatory protein indicating that they are adapted  
305 to life in low carbon environments [73-75]. Similarly, four genes belonging to the CspA  
306 family associated with responses to cold-shock [76], 13 genes of the *dnaK* gene cluster that  
307 respond to heat shock [77] and seven genes associated with the biosynthesis, uptake and  
308 utilisation of trehalose, which are considered to help in responses to heat and desiccation  
309 stress [78], are conserved in each of the strains. The genomes of the strains also contain genes

310 belonging to the *uvrABC* DNA repair system that assists in UV resistance [79], as well as  
311 Rec proteins (RecA, RecX and those involved in the RecBCD and RecFOR pathways) that  
312 are responsible for stabilising genomes [80]. The P6<sup>T</sup> genome contains a *coxGMLS* gene  
313 cluster and a *coxD* gene, whereas the *B. saxobsidens* strain has five copies of the *coxM* gene  
314 and two copies of the *coxS* gene, though the gene encoding for the *coxG* protein is absent;  
315 *cox* genes code for the utilisation of carbon monoxide, thereby indicating that these  
316 organisms may have a chemolithoautotrophic lifestyle [81].

317 The proteins involved in responses to oxidative stress are, with minor exceptions, conserved  
318 in the genomes of isolate P6<sup>T</sup> and *B. saxobsidens* DSM 44509<sup>T</sup> (Table S1). The P6<sup>T</sup> genome  
319 contains eight genes involved in responses to osmotic stress, notably choline dehydrogenase,  
320 and ABC transporter proteins for betaine, glycine and L-proline uptake and a high affinity  
321 choline uptake protein (*betT*) [82, 83]. In turn, only the *B. saxobsidens* strain has a *sox* gene  
322 cluster encoding the subunit of sarcosine oxidase, along with additional copies of *betT* and  
323 transport proteins. Sarcosine oxidase is associated with responses to osmotic stress [84, 85].  
324

325 It is clear from the chemotaxonomic, genomic, morphological and phylogenetic data that  
326 isolate P6<sup>T</sup> is a *bona fide* member of the genus *Blastococcus*. It can be distinguished from the  
327 type strains of *Blastococcus* species using a broad range of phenotypic features and from *B.*  
328 *saxobsidens* BC448<sup>T</sup>, its close phylogenetic relative, by low ANI and AAI indices and by a  
329 low *in silico* DNA:DNA pairing value. It can be concluded that the isolate should be  
330 recognised as a new *Blastococcus* species, for which we propose the name *Blastococcus*  
331 *atacamensis* sp. nov.

332  
333 This is the first description of a novel *Blastococcus* species from the Atacama Desert though  
334 there are grounds for believing that others will follow [86], especially since culture-  
335 independent studies show that blastococci are part of the core microbiome of hyper- and  
336 extreme hyper-arid soils of the desert landscape [87]. It is also interesting that the genomes  
337 of *B. atacamensis* P6<sup>T</sup> and *M. caceserii* KNN 45-2b [70], another isolate from the Yungay  
338 core region, contain genes or gene clusters associated with an ability to cope with low levels  
339 of carbon [73-75], osmotic stress [82], high UV radiation [79] and heat tolerance and  
340 desiccation (biosynthesis and uptake of trehalose; [78]). The genomes of these strains also

341 contain multiple *cox* genes suggesting that *Geodermatophilaceae* strains from the Atacama  
342 may be able to use carbon monoxide as a carbon and energy source, an observation in line  
343 with the suggestion that facultatively chemoautotrophic bacteria may sustain microbial  
344 communities in the nutrient impoverished high altitude Atacama Desert soils [88, 89].  
345 Biological adaptations such as these may account for the presence of blastococci in habitats  
346 characterised by scarcity of available water, low nutrient availability and extremes of  
347 temperature and UV radiation levels [2, 14].

348

349 **Description of *Blastococcus atacamensis* sp. nov.**

350 *Blastococcus atacamensis* (a.ta.cam.en'sis. N.L. masc. adj. *atacamensis*; belonging to the  
351 Atacama Desert, the source of the isolate).

352

353 Gram-stain positive, oxidase-negative actinobacterium which forms non-motile, rod-and  
354 coccoid-elements with evidence of budding. Round orange colonies with entire margins are  
355 formed on yeast extract-malt extract agar. Grows from 10–45°C, optimally ~35°C, from pH  
356 6–12, optimally ~pH 7.0 and in the presence of 3%, w/v sodium chloride. Degrades starch  
357 and tributyrin but not guanine. Arbutin is hydrolysed. Utilises L-glutamic acid (amino acid),  
358 D-glucose, glycerol,  $\alpha$ -methyl-D-glucoside, *N*-acetyl-D-glucosamine (sugars), acetic acid,  
359 acetoacetic acid,  $\gamma$ -amino-*n*-butyric acid,  $\alpha$ - and  $\beta$ -hydroxybutyric acid,  $\alpha$ -ketobutyric acid,  
360 D-gluconic acid,  $\alpha$ -ketoglutaric acid, D-malic acid and propionic acid (organic acids), but not  
361 D-mannose, D-melibiose, *N*-acetyl-neuraminic acid or L-rhamnose (sugars) or butyric acid,  
362 mucic acid, D-saccharic acid,  $\alpha$ -hydroxyphenylacetic acid or bromosuccinic acid (organic  
363 acids); is resistant to aztreonam (antibiotic), lithium chloride and potassium tellurite (heavy  
364 metals) and Tween 40 (surfactant) but is sensitive to fusidic acid, lincomycin, minocycline,  
365 rifamycin SV, troleandomycin and vancomycin (antibiotics), guanidine HCl (chaotropic  
366 agent), tetrazolium blue and tetrazolium violet (redox indicators), sodium bromide, sodium  
367 formate and sodium lactate (salts) and niaproof (surfactant) (GENIII microplates). Additional  
368 phenotypic features are cited either in the text or in Table 1. The predominant fatty acids are  
369 iso-C<sub>16:0</sub>, iso-C<sub>16:1</sub> H, iso-C<sub>15:0</sub> and 9-methyl-C<sub>16:0</sub>; the major sugars are galactose, glucose  
370 and ribose; the polar lipid profile contains diphosphatidylglycerol, phosphatidylcholine,  
371 phosphatidylethanolamine, phosphatidylglycerol and phosphatidylinositol, an unidentified

372 lipid and an unidentified phosphoglycolipid. Additional chemotaxonomic properties are  
373 typical of the genus. The *in silico* DNA G+C content of the type strain is 73.1 mol%. The  
374 type strain, P6<sup>T</sup> (=NCIMB 15090<sup>T</sup> = NRRL B-65468<sup>T</sup>) was isolated from an extreme hyper-  
375 arid soil sample from the Yungay core region of the Atacama Desert.

376

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### 389 **Conflicts of interest**

390 The authors declare that they do not have any conflict of interest.

### 391 **Ethical statement**

392 The authors have not carried out any studies involving human participants or animals.

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636

639 **Table 1:** Phenotypic properties that differentiate isolate P6<sup>T</sup> from the *Blastococcus* type strains.

| <b>Characteristics</b>            | <b>1</b>                      | <b>2</b>                          | <b>3</b>       | <b>4</b>             | <b>5</b>                            | <b>6</b>                 | <b>7</b>           |
|-----------------------------------|-------------------------------|-----------------------------------|----------------|----------------------|-------------------------------------|--------------------------|--------------------|
| Cell shape                        | Cocci                         | Cocci, rods, vibrios <sup>d</sup> | Cocci          | Cocci, rods, vibrios | Cocci <sup>e</sup>                  | Cocci, rods <sup>c</sup> | Cocci <sup>d</sup> |
| Bud formation                     | +                             | + <sup>d</sup>                    | –              | +                    | – <sup>e</sup>                      | + <sup>c</sup>           | – <sup>d</sup>     |
| Germ tube                         | +                             | + <sup>d</sup>                    | –              | +                    | – <sup>e</sup>                      | – <sup>c</sup>           | – <sup>d</sup>     |
| Motility                          | –                             | + <sup>d</sup>                    | –              | –                    | – <sup>e</sup>                      | + <sup>c</sup>           | + <sup>d</sup>     |
| Pigmentation                      | Light-pink to mild red-orange | Pink                              | Bright orange  | Coral                | White to pink to black <sup>e</sup> | Apricot                  | Pink to orange     |
| Temperature growth range (°C)     | 10–45                         | 10–40 <sup>d</sup>                | 20–30          | 10–40                | 10–45 <sup>e</sup>                  | 10–37 <sup>c</sup>       | 20–37 <sup>d</sup> |
| <b>Biochemical tests:</b>         |                               |                                   |                |                      |                                     |                          |                    |
| Catalase                          | +                             | +                                 | –              | –                    | + <sup>e</sup>                      | + <sup>c</sup>           | + <sup>*</sup>     |
| Nitrate to nitrite reduction      | +                             | – <sup>e</sup>                    | – <sup>b</sup> | –                    | – <sup>e</sup>                      | + <sup>e</sup>           | – <sup>e</sup>     |
| <b>API ZYM tests:</b>             |                               |                                   |                |                      |                                     |                          |                    |
| Acid phosphatase                  | +                             | – <sup>e</sup>                    | – <sup>b</sup> | –                    | + <sup>e</sup>                      | – <sup>e</sup>           | + <sup>‡</sup>     |
| Alkaline phosphatase              | +                             | – <sup>e</sup>                    | + <sup>b</sup> | +                    | + <sup>e</sup>                      | – <sup>e</sup>           | + <sup>e</sup>     |
| Esterase lipase (C 8)             | +                             | + <sup>e</sup>                    | + <sup>b</sup> | –                    | + <sup>e</sup>                      | – <sup>e</sup>           | + <sup>e</sup>     |
| α-Glucosidase                     | +                             | – <sup>e</sup>                    | + <sup>b</sup> | +                    | + <sup>e</sup>                      | – <sup>e</sup>           | + <sup>‡</sup>     |
| Naphthol-AS-BI-phosphohydrolase   | +                             | – <sup>e</sup>                    | – <sup>b</sup> | –                    | + <sup>e</sup>                      | – <sup>e</sup>           | + <sup>e</sup>     |
| Valine arylamidase                | +                             | – <sup>e</sup>                    | – <sup>b</sup> | +                    | + <sup>e</sup>                      | + <sup>e</sup>           | + <sup>‡</sup>     |
| <b>GENIII Biolog microplates:</b> |                               |                                   |                |                      |                                     |                          |                    |
| <b>Oxidation of Amino acids:</b>  |                               |                                   |                |                      |                                     |                          |                    |
| L-Alanine                         | –                             | +                                 | –              | +                    | v                                   | +                        | –                  |

|                                  |   |                       |                         |   |                        |                                 |                                    |
|----------------------------------|---|-----------------------|-------------------------|---|------------------------|---------------------------------|------------------------------------|
| Glycyl-L-proline(dipeptide)      | –                                       | +                     | v                       | –   | –                      | –                               | –                                  |
| <b>Monosaccharides:</b>          |   |                       |                         |   |                        |                                 |                                    |
| <i>N</i> -Acetyl-neuraminic acid | –                                       | +                     | –                       | –   | –                      | –                               | v                                  |
| Glucuronamide                    | –                                       | v                     | +                       | –   | +                      | v                               | +                                  |
| <b>Disaccharide:</b>             |   |                       |                         |   |                        |                                 |                                    |
| $\beta$ -Gentiobiose             | +                                       | +                     | –                       | +   | +                      | +                               | +                                  |
| <b>Sugar alcohol:</b>            |   |                       |                         |   |                        |                                 |                                    |
| D-Salicin                        | +                                       | –                     | –                       | v   | v                      | –                               | +                                  |
| <b>Polymers:</b>                 |   |                       |                         |   |                        |                                 |                                    |
| Dextrin                          | +                                       | +                     | –                       | +   | +                      | –                               | –                                  |
| Gelatin                          | –                                       | +                     | –                       | –   | v                      | +                               | –                                  |
| Pectin                           | +                                       | –                     | +                       | v   | –                      | –                               | –                                  |
| Tween 40                         | +                                       | +                     | –                       | +   | +                      | +                               | v                                  |
| <b>Organic acids:</b>            |   |                       |                         |   |                        |                                 |                                    |
| D-Gluconic acid                  | +                                       | –                     | +                       | +   | +                      | v                               | +                                  |
| $\beta$ -hydroxy-Butyric acid    | +                                       | –                     | +                       | +   | v                      | +                               | +                                  |
| $\alpha$ -keto-Butyric acid      | +                                       | v                     | –                       | +   | –                      | +                               | –                                  |
| D-Malic acid                     | +                                       | +                     | +                       | v   | +                      | +                               | –                                  |
| Methyl pyruvate                  | +                                       | –                     | –                       | v   | –                      | –                               | v                                  |
| Mucic acid                       | –                                       | –                     | –                       | v   | –                      | +                               | –                                  |
| D-Saccharic acid                 | –                                       | –                     | –                       | +   | +                      | v                               | +                                  |
| <b>Phospholipids</b>             | DPG, L, PC<br>PE, PG,<br>PGL, PI        | DPG, PI,<br>GPL, PC   | DPG, PE,<br>PC, PI, 2PL | DPG, PC,<br>PI, GPL,<br>PE, OH-PE,<br>6PL | DPG, PI,<br>PE, PC     | DPG, PC,<br>PME, PE,<br>PI, GPL | DPG, PE, PC,<br>PI, GLP, 3PL       |
| <b>Diagnostic sugars</b>         | Glu, Gal,<br>Rib; traces<br>of Ara, Xyl | Rib, Ara,<br>Man, Glu | Glu, Rha,<br>Rib        | Glu, Gal,<br>Rib                          | Glu, Gal;<br>traces of | Rha, Rib,<br>Xyl, Glu;          | Glu, Gal;<br>traces of Rib,<br>Man |

|                                       |   |   |   |   |  |  |  |
|---------------------------------------|---|---|---|---|--|--|--|
| <b>Menaquinones (MK)</b>              | MK-9(H <sub>4</sub> ),<br>MK-9(H <sub>2</sub> ) | MK-8(H <sub>4</sub> ),<br>MK-9(H <sub>4</sub> ) | MK-9(H <sub>4</sub> ),<br>MK-9, MK-<br>9(H <sub>2</sub> ) | MK-9(H <sub>4</sub> ),<br>MK-9, MK-<br>8(H <sub>4</sub> ) | Rha, Rib,<br>Man, Ara<br>MK-9(H <sub>4</sub> ),<br>MK-9, MK-<br>8(H <sub>4</sub> ) | traces of<br>Man<br>MK-9(H <sub>4</sub> ),<br>MK-9 | MK-9(H <sub>4</sub> ),<br>MK-8(H <sub>4</sub> ),<br>MK-9, MK-<br>9(H <sub>6</sub> ), MK-<br>9(H <sub>2</sub> ) |
| <b>DNA G + C content (mole<br/>%)</b> | 73.1  | 73.9 <sup>d</sup>                               | 73.7  | 73.2  | 71.6 <sup>e</sup>  | 72.3 <sup>c</sup>                                  | 73.5   |

640

641

642 Strains: **1**, Isolate P6<sup>T</sup>; **2**, *B. aggregatus* DSM 4725<sup>T</sup>; **3**, *B. capsensis* DSM 46835<sup>T</sup>; **4**, *B. colisei* DSM 46837<sup>T</sup>; **5**, *B. endophyticus* DSM  
643 45413<sup>T</sup>; **6**, *B. jejuensis* DSM 19597<sup>T</sup>; **7**, *B. saxobsidens* DSM 44509<sup>T</sup>. Data for **1** and **7** are from this study; those for **2** to **6** are from  
644 Hezbri et al. [20] unless indicated. + positive; – negative; v variable. Data taken from <sup>a</sup> Ahrens and Moll [1]; <sup>b</sup> Hezbri et al. [4]; <sup>c</sup> Lee [3];  
645 <sup>d</sup> Urzì et al. [19] and <sup>e</sup> Zhu et al. [17]; \* recorded as negative by Hezbri et al. [4]; † recorded as negative by Zhu et al. [17]. Abbreviations:  
646 Ara, arabinose; Glu, glucose; Gal, galactose; Man, mannose; Rha, rhamnose; Rib, ribose; Xyl, xylose; DPG, diphosphatidylglycerol;  
647 GPI, glycoposphatidylinositol; PE, phosphatidylethanolamine; PME, phosphatidyl-*N*-methylethanolamine; PC, phosphatidylcholine;  
648 PI, phosphatidylinositol; unidentified: GPL, glycopospholipid, L, lipid, PL, phospholipid; MK, menaquinone.

649 **Legends for Figures**

650

651 **Figure 1.** Phase contrast image of isolate P6<sup>T</sup> following growth on ISP 2 at 28°C for 7 days  
652 showing the presence of coccoid and rod-shaped elements and evidence of budding. Scale  
653 bar: 5µm.

654 **Figure 2.** Neighbour-joining tree based on partial 16S rRNA gene sequences (1,239  
655 nucleotides) showing the relationships between isolate P6<sup>T</sup> and the type strains of  
656 *Blastococcus* species and the candidatus strain. Asterisks indicate branches of the tree that  
657 were also found using the maximum-likelihood (ML) and maximum-parsimony (MP) tree-  
658 making algorithms. Numbers at the nodes indicate levels of bootstrap support (%) above 50%  
659 based on a neighbour-joining analysis of 1,000 resampled datasets. Genbank accession  
660 numbers are indicated in parentheses. The scale bar indicates the number of substitutions per  
661 nucleotide position.

662

663 **Supplementary Figures**

664 Fig. S1. Light microscopy images of colonies of isolate P6<sup>T</sup> following growth on ISP 2 at  
665 28°C for 3 weeks.

666 Fig. S2. Thin-layer chromatographs showing (a) the presence of diaminopimelic acid (A<sub>2</sub>pm)  
667 isomers and (b) the presence of *meso*-A<sub>2</sub>pm in whole-cell hydrolysates of isolate P6<sup>T</sup>.

668 Fig. S3. Menaquinone profile of isolate P6<sup>T</sup>. Isoprenoid quinones extracted from  
669 *Micromonospora luteifusca* GUI2<sup>T</sup> [1] were used as standards.

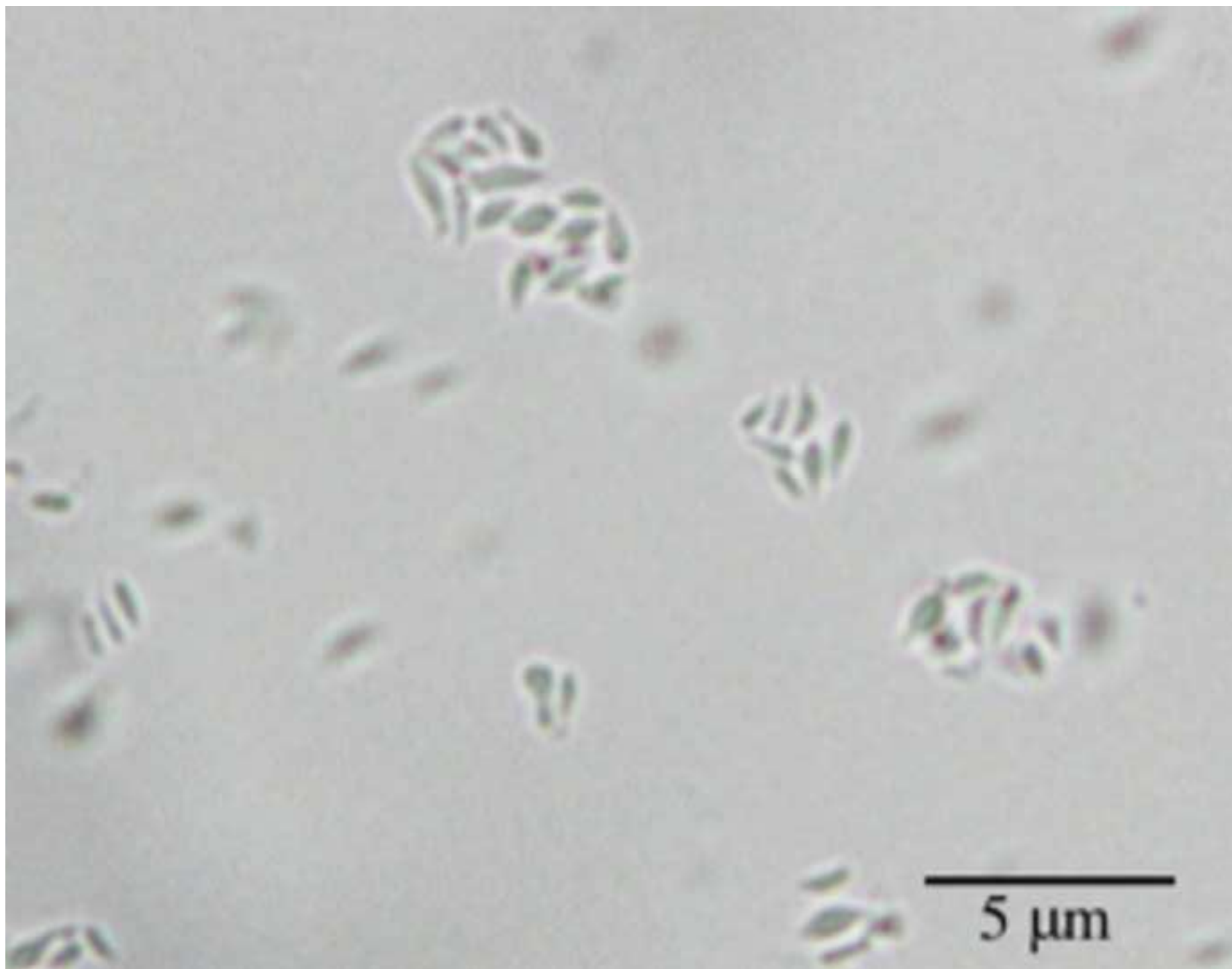
670 Fig. S4. Bi-dimensional thin-layer chromatography of polar lipids of isolate P6<sup>T</sup>.

671 Fig. S5. Thin-layer chromatographs showing the presence of (a) standard sugars and (b)  
672 sugars in whole-cell hydrolysates of isolate P6<sup>T</sup>.

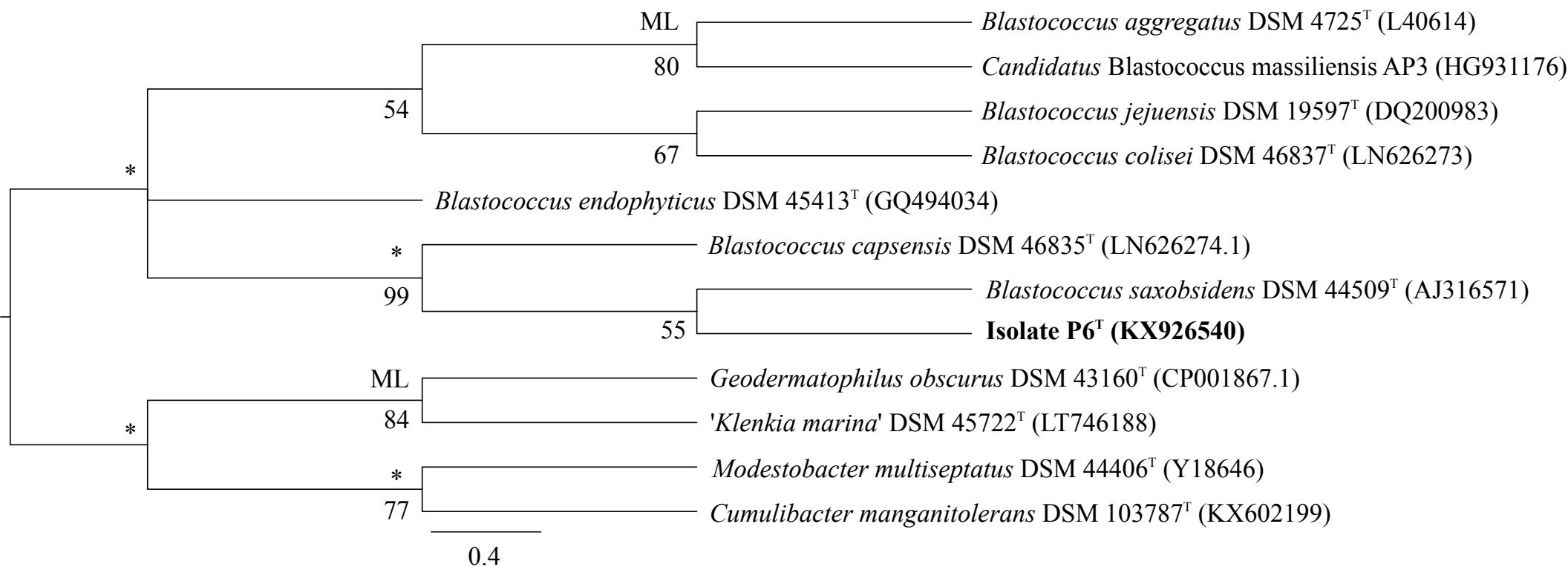
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**International Journal of Systematic and Evolutionary Microbiology****Supplementary data*****Blastococcus atacamensis* sp. nov., a novel strain adapted to life in the Yungay core region of the Atacama Desert**

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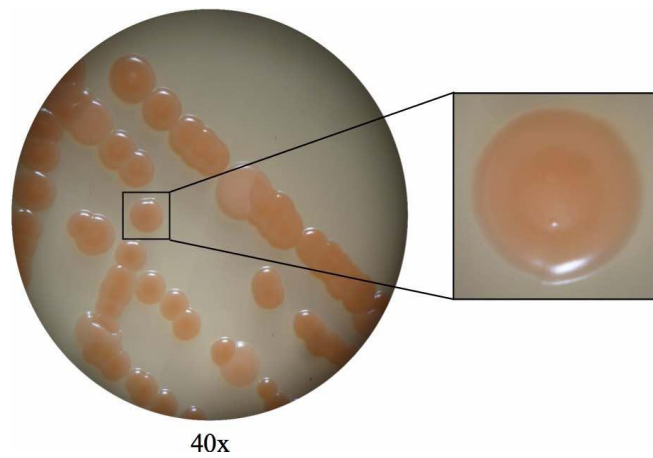
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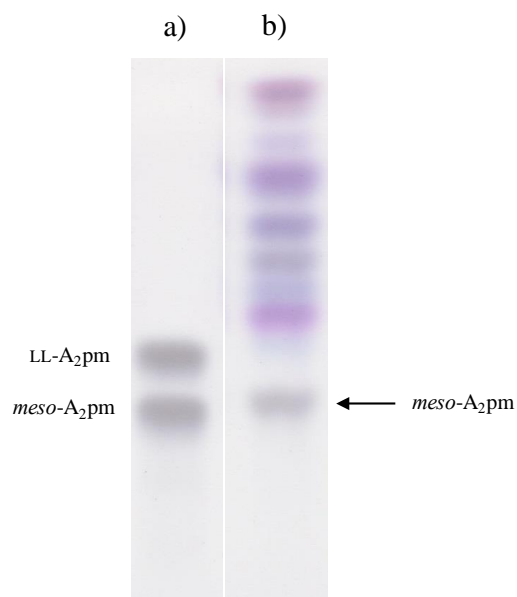
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<sup>6</sup> School of Biosciences, University of Kent, Canterbury CT2 7NJ, Kent, United Kingdom

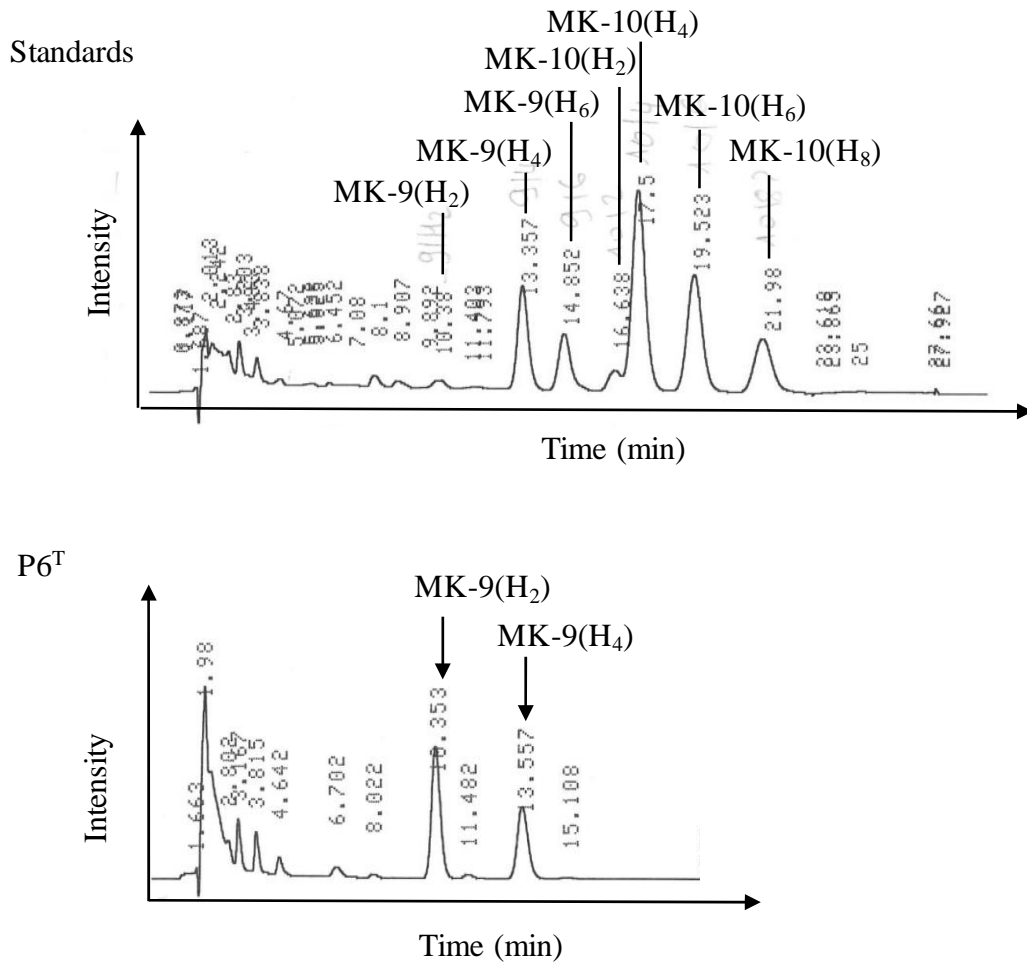
\*To whom correspondence should be addressed: phone +44-(0)191 2087706; email: [m.goodfellow@ncl.ac.uk](mailto:m.goodfellow@ncl.ac.uk).



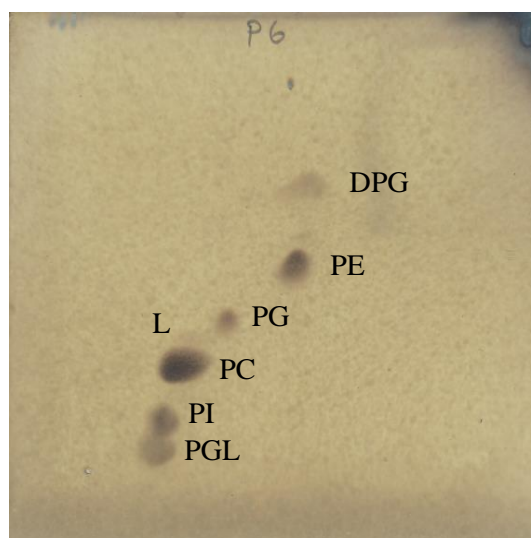
**Supplementary Figure S1:** Light microscopy images of colonies of isolate P6<sup>T</sup> following growth on ISP 2 at 28°C for 3 weeks.



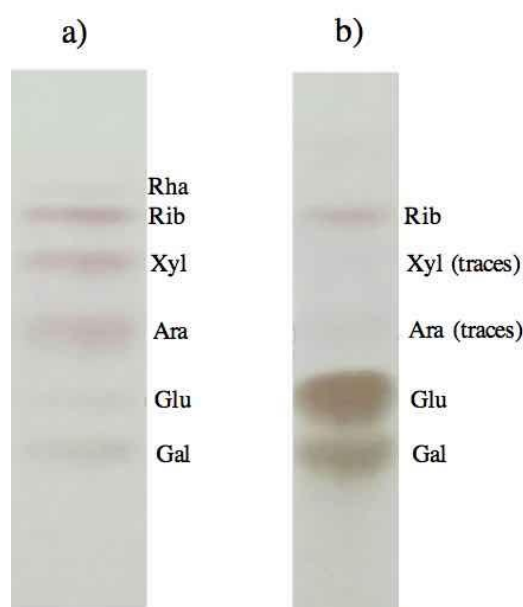
**Supplementary Fig. S2:** Thin-layer chromatographs showing (a) the presence of diaminopimelic acid (A<sub>2</sub>pm) isomers and (b) the presence of *meso*-A<sub>2</sub>pm in whole-cell hydrolysates of isolate P6<sup>T</sup>. a) Standards A<sub>2</sub>pm; b) isolate P6<sup>T</sup>.



**Supplementary Fig. S3:** Menaquinone profile of isolate P6<sup>T</sup>. Isoprenoid quinones extracted from *Micromonospora luteifusca* GUI2<sup>T</sup> [1] were used as standards.



**Supplementary Fig. S4:** Bi-dimensional thin-layer chromatography of polar lipids of isolate P6<sup>T</sup> using molybdato-phosphoric acid reagent (5 %). DPG: diphosphatidylglycerol; L: unidentified lipid; PC: phosphatidylcholine; PE: phosphatidylethanolamine; PG: phosphatidylglycerol; PGL: Unknown phosphoglycolipid; PI: phosphatidylinositol. Solvent 1: chloroform:methanol:distilled water (65:25:4); solvent 2: chloroform:glacial acetic acid:methanol:distilled water (80:12:15:4).



**Supplementary Fig. S5.** Thin-layer chromatographs showing the presence of (a) standard sugars and (b) sugars in whole-cell hydrolysates of isolate P6<sup>T</sup>. a) Standard sugars; b) sugars present in isolate P6<sup>T</sup>. Rha: rhamnose; Rib: ribose; Xyl: xylose; Ara: arabinose; Glu: glucose; Gal: galactose.

**Supplementary Table 1. A list of stress related genes present in strains P6<sup>T</sup> and DSM 44509<sup>T</sup>**

| Presence                                   | Category       | Role   | Organism A             | Organism B                        |
|--|----------------|--|------------------------|-----------------------------------|
| P6 <sup>T</sup> and DSM 44509 <sup>T</sup> | Cold shock     | Cold shock protein CspA  | P6_peg1370, P6_peg2786 | DSM44509_peg149, DSM44509_peg3572 |
| P6 <sup>T</sup> and DSM 44509 <sup>T</sup> | Cold shock     | Cold shock protein CspC  | P6_peg575              | DSM44509_peg176                   |
| P6 <sup>T</sup> and DSM 44509 <sup>T</sup> | Cold shock     | Cold shock protein CspG  | P6_peg2467             | DSM44509_peg2911                  |
| P6 <sup>T</sup>                            | Detoxification | Various polyols ABC transporter, periplasmic substrate-binding protein                                 | P6_peg1164, P6_peg1169 |                                   |
| P6 <sup>T</sup> and DSM 44509 <sup>T</sup> | Heat shock     | Chaperone protein DnaJ   | P6_peg274, P6_peg1954  | DSM44509_peg382, DSM44509_peg2184 |
| P6 <sup>T</sup> and DSM 44509 <sup>T</sup> | Heat shock     | Chaperone protein DnaK   | P6_peg226              | DSM44509_peg384                   |
| P6 <sup>T</sup> and DSM 44509 <sup>T</sup> | Heat shock     | Heat shock protein GrpE  | P6_peg227              | DSM44509_peg383                   |
| P6 <sup>T</sup> and DSM 44509 <sup>T</sup> | Heat shock     | Heat-inducible transcription repressor HrcA  | P6_peg1955             | DSM44509_peg2183                  |
| P6 <sup>T</sup> and DSM 44509 <sup>T</sup> | Heat shock     | HspR, transcriptional repressor of DnaK operon   | P6_peg275              | DSM44509_peg381                   |
| P6 <sup>T</sup> and DSM 44509 <sup>T</sup> | Heat shock     | Hypothetical radical SAM family enzyme in heat shock gene cluster, similarity with CPO of BS HemN-type | P6_peg1959             | DSM44509_peg2178                  |
| P6 <sup>T</sup> and DSM 44509 <sup>T</sup> | Heat shock     | Nucleoside 5-triphosphatase RdgB (dHATP, dITP, XTP-specific) (EC 3.6.1.15)                             | P6_peg6                | DSM44509_peg2317                  |
| P6 <sup>T</sup> and DSM 44509 <sup>T</sup> | Heat shock     | Ribonuclease PH (EC 2.7.7.56)  | P6_peg5                | DSM44509_peg2316                  |
| P6 <sup>T</sup> and DSM 44509 <sup>T</sup> | Heat shock     | Ribosomal RNA small subunit methyltransferase E (EC 2.1.1.-)   | P6_peg1953             | DSM44509_peg2185                  |
| P6 <sup>T</sup> and DSM 44509 <sup>T</sup> | Heat shock     | Translation elongation factor LepA   | P6_peg1981             | DSM44509_peg2165                  |
| P6 <sup>T</sup> and DSM 44509 <sup>T</sup> | Heat shock     | rRNA small subunit methyltransferase I   | P6_peg2916             | DSM44509_peg235                   |
| P6 <sup>T</sup> and DSM 44509 <sup>T</sup> | Heat shock     | tmRNA-binding protein SmpB   | P6_peg3717             | DSM44509_peg3854                  |

|  |                  |   |                       |   |
|--|------------------|---|-----------------------|---|
| P6 <sup>T</sup> and DSM 44509 <sup>T</sup> | Osmotic stress   | Choline dehydrogenase (EC 1.1.99.1)   | P6_peg827, P6_peg3514 | DSM44509_peg321, DSM44509_peg1542                                     |
| P6 <sup>T</sup> and DSM 44509 <sup>T</sup> | Osmotic stress   | Glycine betaine ABC transport system permease protein   | P6_peg1488            | DSM44509_peg112   |
| P6 <sup>T</sup> and DSM 44509 <sup>T</sup> | Osmotic stress   | High-affinity choline uptake protein BetT   | P6_peg2419            | DSM44509_peg286, DSM44509_peg1388, DSM44509_peg1393, DSM44509_peg1579 |
| P6 <sup>T</sup> and DSM 44509 <sup>T</sup> | Osmotic stress   | L-proline glycine betaine ABC transport system permease protein ProV (TC 3.A.1.12.1)                    | P6_peg944, P6_peg1490 | DSM44509_peg110, DSM44509_peg1084                                     |
| P6 <sup>T</sup> and DSM 44509 <sup>T</sup> | Osmotic stress   | L-proline glycine betaine ABC transport system permease protein ProW (TC 3.A.1.12.1)                    | P6_peg1489            | DSM44509_peg111, DSM44509_peg1082, DSM44509_peg1083                   |
| P6 <sup>T</sup> and DSM 44509 <sup>T</sup> | Osmotic stress   | L-proline glycine betaine binding ABC transporter protein ProX (TC 3.A.1.12.1)                          | P6_peg1487            | DSM44509_peg113, DSM44509_peg279                                      |
| DSM 44509 <sup>T</sup>                     | Osmotic stress   | Sarcosine oxidase alpha subunit (EC 1.5.3.1)  |                       | DSM44509_peg1385  |
| DSM 44509 <sup>T</sup>                     | Osmotic stress   | Sarcosine oxidase beta subunit (EC 1.5.3.1)   |                       | DSM44509_peg1383  |
| DSM 44509 <sup>T</sup>                     | Osmotic stress   | Sarcosine oxidase delta subunit (EC 1.5.3.1)  |                       | DSM44509_peg1384  |
| DSM 44509 <sup>T</sup>                     | Osmotic stress   | Sarcosine oxidase gamma subunit (EC 1.5.3.1)  |                       | DSM44509_peg1386  |
| DSM 44509 <sup>T</sup>                     | Osmotic stress   | Aquaporin Z   |                       | DSM44509_peg31  |
| P6 <sup>T</sup> and DSM 44509 <sup>T</sup> | Oxidative stress | Putative Holliday junction resolvase YggF   | P6_peg2241            | DSM44509_peg1712  |
| P6 <sup>T</sup> and DSM 44509 <sup>T</sup> | Oxidative stress | Ribosomal RNA small subunit methyltransferase E (EC 2.1.1.-)  | P6_peg1953            | DSM44509_peg2185  |
| P6 <sup>T</sup> and DSM 44509 <sup>T</sup> | Oxidative stress | Flavohemoprotein (Hemoglobin-like protein) (Flavohemoglobin) (Nitric oxide dioxygenase) (EC 1.14.12.17) | P6_peg3406            | DSM44509_peg3484, DSM44509_peg3485                                    |
| P6 <sup>T</sup> and DSM 44509 <sup>T</sup> | Oxidative stress | Hydroxyacylglutathione hydrolase (EC 3.1.2.6)   | P6_peg65, P6_peg2230  | DSM44509_peg1701, DSM44509_peg2761                                    |
| P6 <sup>T</sup> and DSM 44509 <sup>T</sup> | Oxidative stress | Lactoylglutathione lyase (EC 4.4.1.5)   | P6_peg2435            | DSM44509_peg1561  |
| P6 <sup>T</sup> and DSM 44509 <sup>T</sup> | Oxidative stress | Acetyl-CoA:Cys-GlcN-Ins acetyltransferase, mycothiol synthase MshD                                      | P6_peg3623            | DSM44509_peg3541  |
| P6 <sup>T</sup> and DSM 44509 <sup>T</sup> | Oxidative stress | Formaldehyde dehydrogenase MscR, NAD/mycothiol-dependent (EC 1.2.1.66)                                  | P6_peg2339            | DSM44509_peg334, DSM44509_peg1381                                     |
| P6 <sup>T</sup> and DSM 44509 <sup>T</sup> | Oxidative stress | Glycosyltransferase MshA involved in mycothiol biosynthesis (EC 2.4.1.-)                                | P6_peg3580            | DSM44509_peg3513  |
| P6 <sup>T</sup> and DSM 44509 <sup>T</sup> | Oxidative stress | L-cysteine:1D-myo-inositol 2-amino-2-deoxy-alpha-D-glucopyranoside ligase MshC                          | P6_peg3347            | DSM44509_peg1070  |
| P6 <sup>T</sup> and DSM 44509 <sup>T</sup> | Oxidative stress | Mycothiol S-conjugate amidase Mca   | P6_peg321             | DSM44509_peg2370  |

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| P6 <sup>T</sup> and DSM 44509 <sup>T</sup> | Oxidative stress | N-acetyl-1-D-myo-inosityl-2-amino-2-deoxy-alpha-D-glucopyranoside deacetylase MshB | P6_peg1013             | DSM44509_peg1794                                    |
| P6 <sup>T</sup> and DSM 44509 <sup>T</sup> | Oxidative stress | Putative hydrolase in cluster with formaldehyde/S-nitrosomycothioli reductase MscR | P6_peg2338             | DSM44509_peg333                                     |
| P6 <sup>T</sup> and DSM 44509 <sup>T</sup> | Oxidative stress | S-nitrosomycothioli reductase MscR   | P6_peg2339             | DSM44509_peg334, DSM44509_peg1381                   |
| P6 <sup>T</sup> and DSM 44509 <sup>T</sup> | Oxidative stress | Uncharacterized protein Rv0487/MT0505 clustered with mycothiol biosynthesis gene   | P6_peg3579             | DSM44509_peg3512                                    |
| P6 <sup>T</sup> and DSM 44509 <sup>T</sup> | Oxidative stress | Alkyl hydroperoxide reductase subunit C-like protein                               | P6_peg3238             | DSM44509_peg984                                     |
| P6 <sup>T</sup> and DSM 44509 <sup>T</sup> | Oxidative stress | Catalase (EC 1.11.1.6)   | P6_peg613              | DSM44509_peg1819, DSM44509_peg3715                  |
| P6 <sup>T</sup> and DSM 44509 <sup>T</sup> | Oxidative stress | Organic hydroperoxide resistance protein   | P6_peg279              | DSM44509_peg379                                     |
| P6 <sup>T</sup> and DSM 44509 <sup>T</sup> | Oxidative stress | Organic hydroperoxide resistance transcriptional regulator                         | P6_peg280              | DSM44509_peg378                                     |
| P6 <sup>T</sup> and DSM 44509 <sup>T</sup> | Oxidative stress | Phytochrome, two-component sensor histidine kinase (EC 2.7.3.-)                    | P6_peg28, P6_peg2209   | DSM44509_peg2040, DSM44509_peg4012                  |
| P6 <sup>T</sup> and DSM 44509 <sup>T</sup> | Oxidative stress | Redox-sensitive transcriptional regulator (AT-rich DNA-binding protein)            | P6_peg3266, P6_peg3267 | DSM44509_peg3930, DSM44509_peg3931                  |
| P6 <sup>T</sup> and DSM 44509 <sup>T</sup> | Oxidative stress | Transcriptional regulator, FUR family  | P6_peg612, P6_peg3592  | DSM44509_peg3526, DSM44509_peg3714                  |
| P6 <sup>T</sup> and DSM 44509 <sup>T</sup> | Oxidative stress | Zinc uptake regulation protein ZUR   | P6_peg1798             | DSM44509_peg407, DSM44509_peg1351, DSM44509_peg4073 |
| P6 <sup>T</sup> and DSM 44509 <sup>T</sup> | Oxidative stress | bacteriophytochrome heme oxygenase BphO  | P6_peg29               | DSM44509_peg4013                                    |
| P6 <sup>T</sup> and DSM 44509 <sup>T</sup> | Oxidative stress | Catalase (EC 1.11.1.6)   | P6_peg613              | DSM44509_peg1819, DSM44509_peg3715                  |
| P6 <sup>T</sup> and DSM 44509 <sup>T</sup> | Oxidative stress | NAD-dependent glyceraldehyde-3-phosphate dehydrogenase (EC 1.2.1.12)               | P6_peg874              | DSM44509_peg3078                                    |
| P6 <sup>T</sup> and DSM 44509 <sup>T</sup> | Oxidative stress | NAD-dependent protein deacetylase of SIR2 family                                   | P6_peg1289, P6_peg2601 | DSM44509_peg219, DSM44509_peg3132                   |
| P6 <sup>T</sup> and DSM 44509 <sup>T</sup> | Oxidative stress | Nicotinamidase (EC 3.5.1.19)   | P6_peg366              | DSM44509_peg1160, DSM44509_peg2303                  |
| P6 <sup>T</sup> and DSM 44509 <sup>T</sup> | Oxidative stress | Nicotinate phosphoribosyltransferase (EC 2.4.2.11)                                 | P6_peg365              | DSM44509_peg2304                                    |
| P6 <sup>T</sup>                            | Oxidative stress | CoA-disulfide reductase (EC 1.8.1.14)  | P6_peg1645             |   |
| DSM 44509 <sup>T</sup>                     | Oxidative stress | Peroxidase (EC 1.11.1.7)   |                        | DSM44509_peg3715                                    |
| DSM 44509 <sup>T</sup>                     | Oxidative stress | transcriptional regulator, Crp/Fnr family  |                        | DSM44509_peg2379                                    |



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| P6 <sup>T</sup> and DSM 44509 <sup>T</sup> | no subcategory | Flavoheomoprotein (Hemoglobin-like protein) (Flavoheomoglobin) (Nitric oxide dioxygenase) (EC 1.14.12.17) | P6_peg3406   | DSM44509_peg3484, DSM44509_peg3485   |
| P6 <sup>T</sup> and DSM 44509 <sup>T</sup> | no subcategory | Hemoglobin-like protein HbO   | P6_peg1865   | DSM44509_peg685  |
| P6 <sup>T</sup> and DSM 44509 <sup>T</sup> | no subcategory | diguanylate cyclase/phosphodiesterase (GGDEF & EAL domains) with PAS/PAC sensor(s)                        | P6_peg301, P6_peg436, P6_peg521, P6_peg665, P6_peg699, P6_peg726, P6_peg1016, P6_peg1019, P6_peg1054, P6_peg1080, P6_peg1176, P6_peg1199, P6_peg1204, P6_peg1205, P6_peg1216, P6_peg1449, P6_peg1524, P6_peg1682, P6_peg1833, P6_peg1919, P6_peg1934, P6_peg1935, P6_peg2120, P6_peg2153, P6_peg3156, P6_peg3327, P6_peg3329, P6_peg3342, P6_peg3368, P6_peg3376, P6_peg3647, P6_peg3742 | DSM44509_peg440, DSM44509_peg460, DSM44509_peg522, DSM44509_peg523, DSM44509_peg538, DSM44509_peg739, DSM44509_peg915, DSM44509_peg916, DSM44509_peg924, DSM44509_peg947, DSM44509_peg1062, DSM44509_peg1369, DSM44509_peg1372, DSM44509_peg1510, DSM44509_peg1787, DSM44509_peg1791, DSM44509_peg1814, DSM44509_peg1826, DSM44509_peg1922, DSM44509_peg2018, DSM44509_peg2150, DSM44509_peg2427, DSM44509_peg2461, DSM44509_peg2476, DSM44509_peg2477, DSM44509_peg2814, DSM44509_peg2873, DSM44509_peg3354, DSM44509_peg3770, DSM44509_peg3968, DSM44509_peg4177, DSM44509_peg4271 |
| P6 <sup>T</sup> and DSM 44509 <sup>T</sup> | no subcategory | Carbon starvation protein A   | P6_peg1951   | DSM44509_peg975  |
| P6 <sup>T</sup> and DSM 44509 <sup>T</sup> | no subcategory | Carbon storage regulator  | P6_peg3731   | DSM44509_peg4059   |
| P6 <sup>T</sup> and DSM 44509 <sup>T</sup> | no subcategory | ABC-type Fe <sup>3+</sup> -siderophore transport system, permease 2 component                             | P6_peg780, P6_peg1125  | DSM44509_peg709, DSM44509_peg3042  |
| P6 <sup>T</sup> and DSM 44509 <sup>T</sup> | no subcategory | Flavoheomoprotein (Hemoglobin-like protein) (Flavoheomoglobin) (Nitric oxide dioxygenase) (EC 1.14.12.17) | P6_peg3406   | DSM44509_peg3484, DSM44509_peg3485   |
| P6 <sup>T</sup> and DSM 44509 <sup>T</sup> | no subcategory | GTP-binding protein HflX  | P6_peg208  | DSM44509_peg1601   |
| P6 <sup>T</sup> and DSM 44509 <sup>T</sup> | no subcategory | Anti-sigma B factor antagonist RsbV   | P6_peg1497   | DSM44509_peg715, DSM44509_peg716, DSM44509_peg1537   |
| P6 <sup>T</sup> and DSM 44509 <sup>T</sup> | no subcategory | RNA polymerase sigma factor SigB  | P6_peg600, P6_peg2515  | DSM44509_peg2957, DSM44509_peg3720   |
| P6 <sup>T</sup> and DSM 44509 <sup>T</sup> | no subcategory | Serine phosphatase RsbU, regulator of sigma subunit   | P6_peg602, P6_peg1036, P6_peg1041, P6_peg1190, P6_peg1192, P6_peg1237, P6_peg1322, P6_peg2184, P6_peg2763, P6_peg2953, P6_peg2961, P6_peg3019  | DSM44509_peg298, DSM44509_peg490, DSM44509_peg714, DSM44509_peg1766, DSM44509_peg2084, DSM44509_peg2260, DSM44509_peg2460, DSM44509_peg2490, DSM44509_peg2491, DSM44509_peg2564, DSM44509_peg2572, DSM44509_peg2959, DSM44509_peg3627, DSM44509_peg3647, DSM44509_peg4104, DSM44509_peg4267, DSM44509_peg4269  |
| P6 <sup>T</sup> and DSM 44509 <sup>T</sup> | no subcategory | Serine-protein kinase RsbW (EC 2.7.11.1)  | P6_peg601  | DSM44509_peg2958   |
| DSM 44509 <sup>T</sup>                     | no subcategory | Putative SigmaB associated two-component system sensor protein  |  | DSM44509_peg2261   |