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

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The GenBank accession numbers for the 16S rRNA gene and genome sequences of strain P6^T are KX926540 and POQU00000000, respectively. The genome accession number of *Blastococcus saxobsidens* DSM 44509^T is POQT00000000.

Abbreviations: A_2pm, diaminopimelic acid; ANI, average nucleotide identity; dDDH, digital DNA–DNA hybridization; GGDC, Genome-to-Genome Distance Calculator; NJ, neighbour-joining; ML, maximum-likelihood; MP, maximum-parsimony; MUSCLE, Multiple Sequence Comparison by Log-Expectation; T3PKS, type III polyketide synthase.

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

Abstract

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

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

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

Blastococci form a well-supported clade in the *Geodermatophilaceae* 16S rRNA gene tree [4, 17] and can be distinguished from members of other genera classified in this family using a combination of phenotypic features [13]. They are Gram-stain positive, coccoid-shaped bacteria that may be motile or non-motile and which may propagate by budding and multiple fission; they have *meso*-A_2pm in the peptidoglycan, mainly unsaturated and iso-branched fatty acids; and complex phospholipid profiles which may include diphosphatidylglycerol, phosphatidylcholine, phosphatidylethanolamine and phosphatidylinositol [4, 18]. At the time of writing the genus encompasses 5 validly named species, namely *Blastococcus aggregatus* [1, 4, 19], the type species, *Blastococcus capsensis* [4], *Blastococcus endophyticus* [4, 17], *Blastococcus jejunensis* [3, 4], and *Blastococcus saxobsidens* [4, 19], and one validly named strain, *Blastococcus colisei* [20]. These bacteria were isolated from the Baltic Sea, an archaeological Roman pool in Tunisia, the leaves of the medicinal Chinese plant *Camptotheca acuminata*, beach sand off the coast of South Korea, a limestone sample from a church in Malta and an archaeological amphitheatre, respectively, and can be distinguished using a range of phenotypic properties [4]. In addition, "*Candidatus* Blastococcus massiliensis*"* was identified, from a stool sample of a patient with anorexia nervosa [21].

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

Blastococcus strains were isolated from an extreme hyper-arid soil sample in November 2011 from the Yungay core region of the Atacama Desert on the eastern flank of the Cerro Aguas Blancas (24º06'18.6"S/70º01º55.6W) at 1,033 metres above sea level. One gram of the soil sample was suspended in 4.0 ml of ¼ strength Ringer's solution (Oxoid, product No. BO0332D), this suspension was shaken on a tumble shaker prior to heating at 55ºC for six minutes. Aliquots (100µl) of the 10^{-1} and 10^{-2} dilutions were spread, in triplicate, over GYM

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

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

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

Biomass for most of the chemotaxonomic analyses carried out on isolate P6^T was harvested from 1,000 ml yeast extract-malt extract broth ISP medium 2 that had been shaken in 500 ml baffled Erlenmeyer flasks, each flask containing 200 ml of medium, at 180 revolutions per minute (rpm) at 28ºC for 2 weeks; the biomass was washed twice in distilled water and

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

Isolate $P6^T$ was examined for chemotaxonomic markers known to be of value in the systematics of microorganisms classified in the genus *Blastococcus* [4, 18]. Standard chromatographic procedures were used to determine the isomers of A_2pm [26], isoprenoid quinones [27], whole-cell sugars [28] and polar lipids [29], as modified by Kroppenstedt and Goodfellow [30]. Isoprenoid quinones extracted from *Micromonospora luteifusca* $GUI2^T$ [31] were used as standards. In turn, fatty acids extracted from the isolate were methylated, analysed using the protocol of the Sherlock Microbial Identification (MIDI) system, version 5 [32] and the resultant peaks identified using the ACTIN 6 database.

In general, the chemotaxonomic properties of isolate $P6^T$ are consistent with its classification in the genus *Blastococcus* [4, 18, 20]. The organism contains *meso*-A_2pm as the diagnostic diamino acid (Fig. S2); MK-9(H_2) and MK-9(H_4) as predominant isoprenologues in a proportion of 3:2 (Fig. S3); iso-$C_{16:0}$ (38.9%), iso-$C_{16:1}$ H (17.7%), iso-$C_{15:0}$ (14.2%) and 9-methyl-$C_{16:0}$ (5.7%) as major fatty acids; a polar lipid profile that includes diphosphatidylglycerol, phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol and phosphatidylinositol (Fig. S4) and galactose and glucose as major sugars with a lesser proportion of ribose and traces of arabinose and xylose (Fig. S5).

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

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

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

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

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

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

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

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

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

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

were assembled by using SPAdes 3.9.0 [55] and contigs smaller than 1,000 bp in size were discarded. The draft assemblies have been submitted to the GenBank (accession numbers: POQU00000000 and POQT00000000) and is publicly available.

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

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

The draft genomes of isolates P6^T and *B. saxobsidens* DSM 44509^T were examined using the antiSMASH server [62] to detect putative biosynthetic gene clusters. The genome of isolate P6^T was found to encode for a T3PKS and corresponding residues that make up the catalytic triad found in RppA, a T3PKS involved in the biosynthesis of pentaketide 1,3,6,8-tetrahydroxnaphthalene in *Streptomyces griseus* [63, 64]. The T3PKS of isolate P6^T showed 94% sequence identity with a corresponding sequence detected in the genome of the *B. saxobsidens* strain and 83% identity with a putative T3PKS encoded in the genome of *G. obscurus* DSM 43160^T (Gobs_4821; UniProt [65], accession number: D2S5V1). The gene that encodes for the T3PKS of isolate P6^T was surrounded by other biosynthetic genes, such as one encoding for a methyltransferase and others encoding regulatory and transport proteins, thereby suggesting the presence of a biosynthetic gene cluster though the functionality and product generated by this putative biocluster has still to be established. The genomes of isolate P6^T and the *B. saxobsidens* strain were also found to harbour genes encoding for polyprenyl synthetase and phytoene synthase, enzymes involved in the biosynthesis of terpenoid compounds [66, 67]. The genome of the *B. saxobsidens* strain

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

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

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

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

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

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

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

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

Description of *Blastococcus atacamensis* sp. nov.

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

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

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

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Conflicts of interest

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

Ethical statement

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^{T} from the *Blastococcus* type strains.

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

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

Menaquinones (MK)	MK-9(H_4), MK-9(H_2)	MK-8(H_4), MK-9(H_4)	MK-9(H_4), MK-9, MK-9(H_2)	MK-9(H_4), MK-9, MK-8(H_4)	Rha, Rib, Man, Ara MK-9(H_4), MK-9, MK-8(H_4)	traces of Man MK-9(H_4), MK-9	MK-9(H_4), MK-8(H_4), MK-9, MK-9(H_6), MK-9(H_2)
DNA G + C content (mole %)	73.1	73.9 [d]	73.7	73.2	71.6 [e]	72.3 [c]	73.5

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

Legends for Figures

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

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

Supplementary Figures

Fig. S1. Light microscopy images of colonies of isolate P6^T following growth on ISP 2 at 28°C for 3 weeks.

Fig. S2. Thin-layer chromatographs showing (a) the presence of diaminopimelic acid (A_2pm) isomers and (b) the presence of *meso*-A_2pm in whole-cell hydrolysates of isolate P6^T.

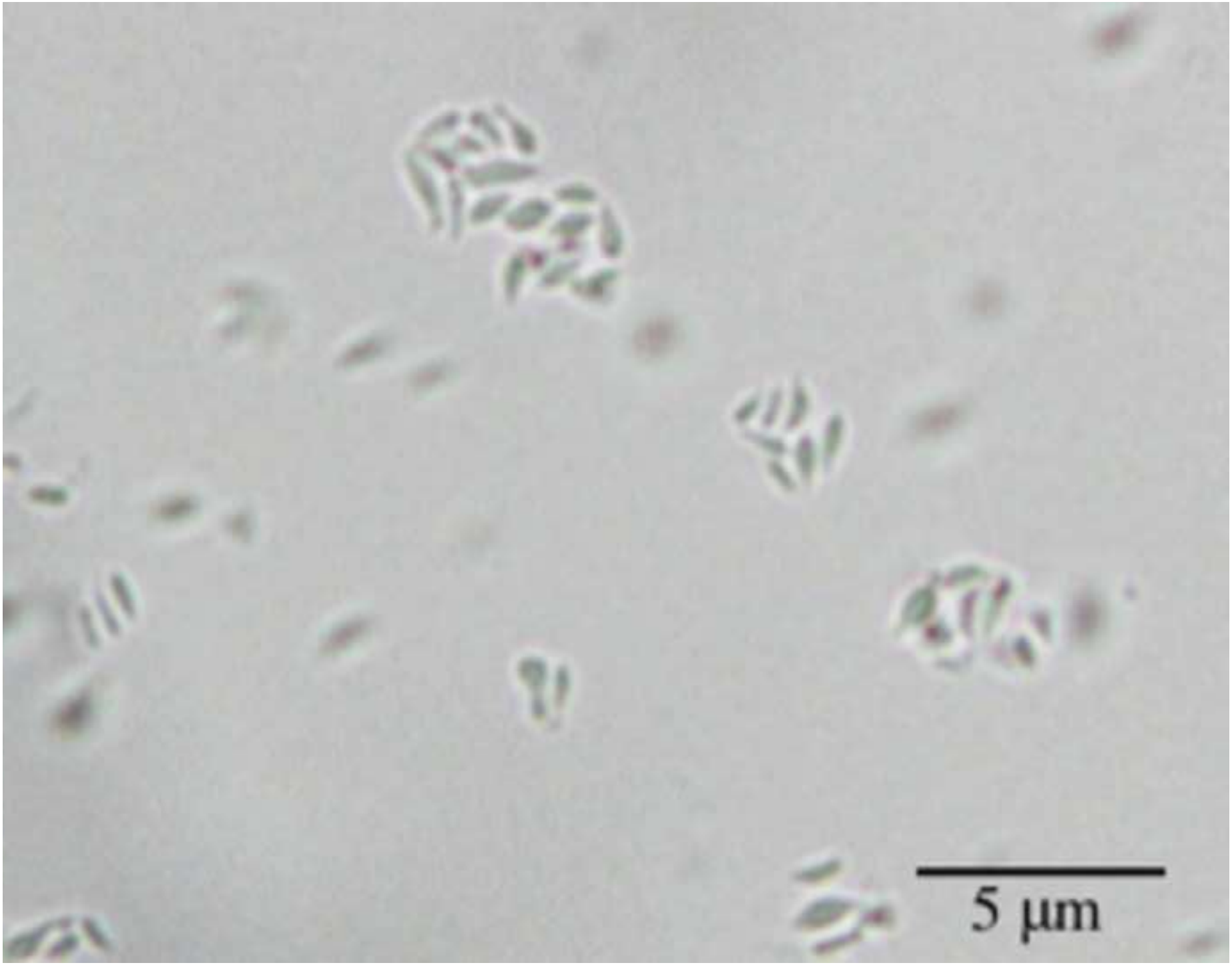
Fig. S3. Menaquinone profile of isolate P6^T. Isoprenoid quinones extracted from *Micromonospora luteifusca* GUI2^T [1] were used as standards.

Fig. S4. Bi-dimensional thin-layer chromatography of polar lipids of isolate P6^T.

Fig. S5. Thin-layer chromatographs showing the presence of (a) standard sugars and (b) sugars in whole-cell hydrolysates of isolate P6^T.

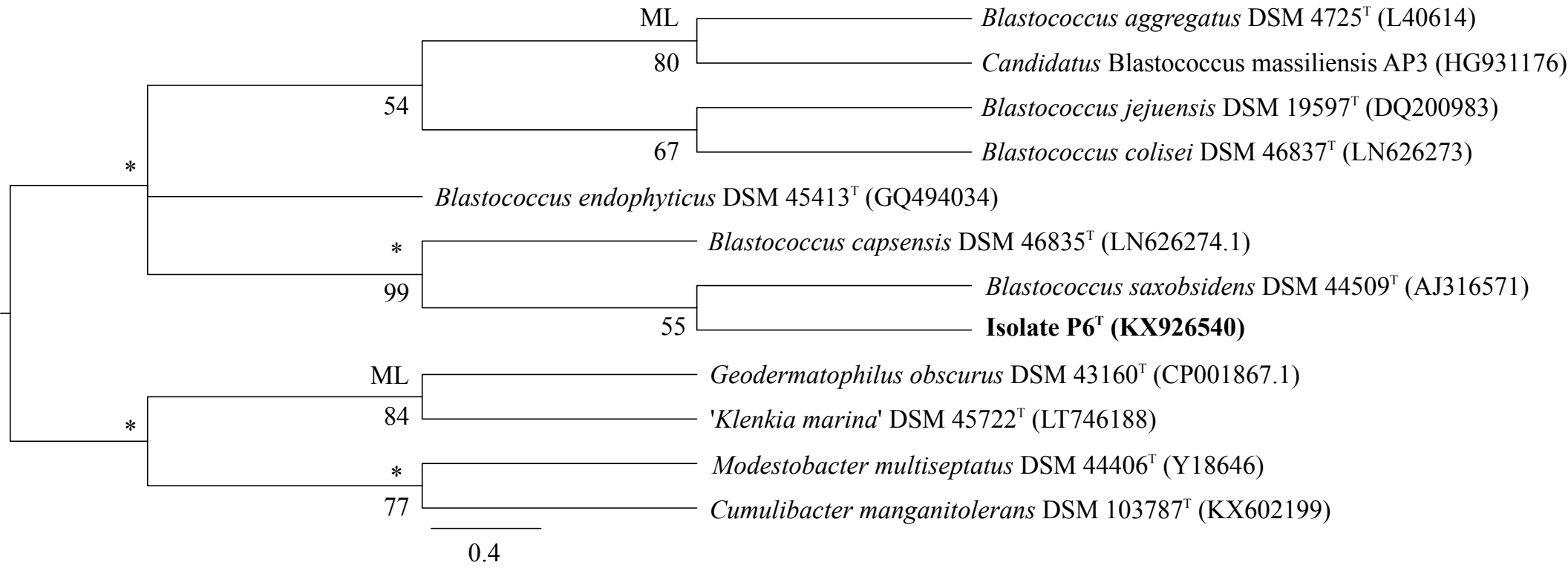
Figure

Click here to download Figure Figure 1.tiff



Figure

Click here to download Figure Figure 2.eps



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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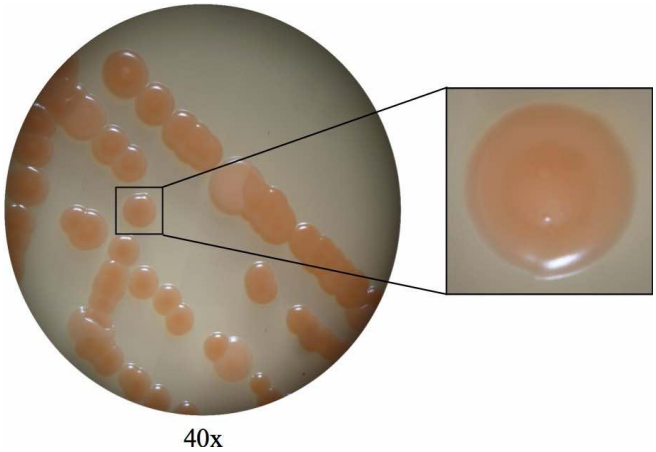
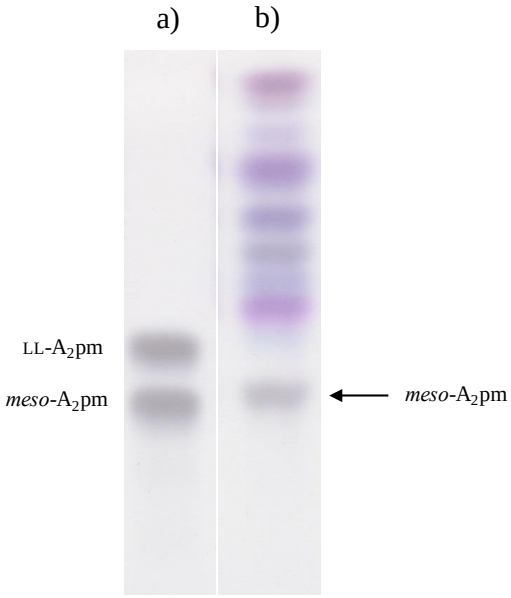
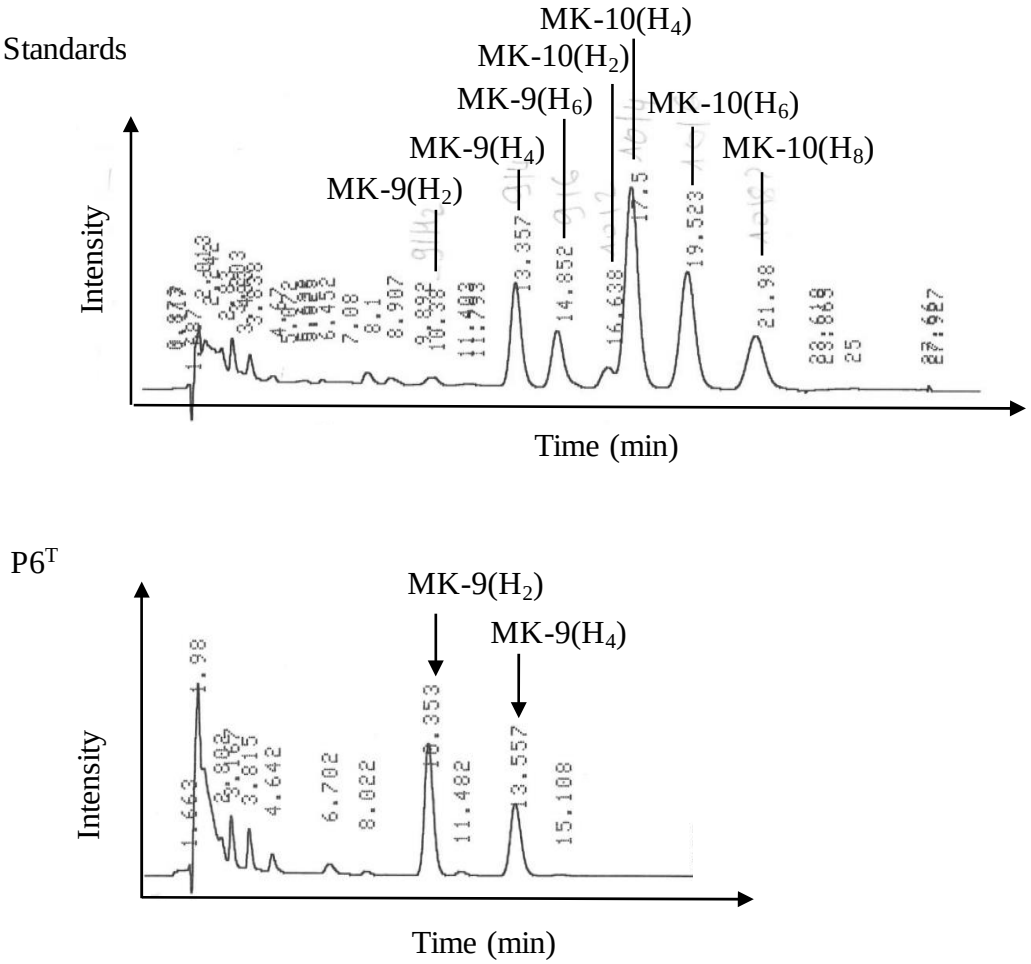


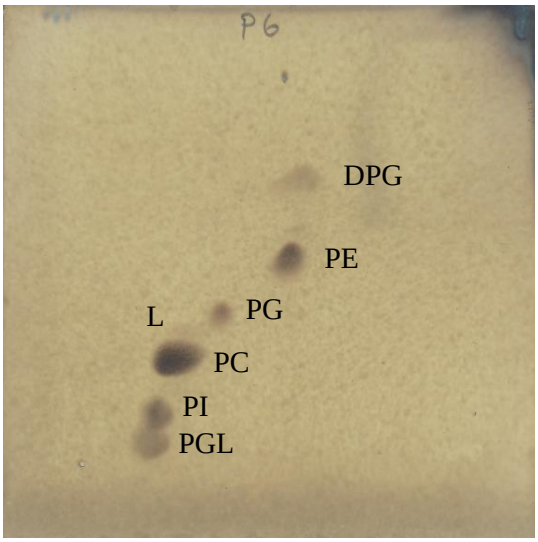
Supplementary Figure S1: Light microscopy images of colonies of isolate P6^T following growth on ISP 2 at 28°C for 3 weeks.



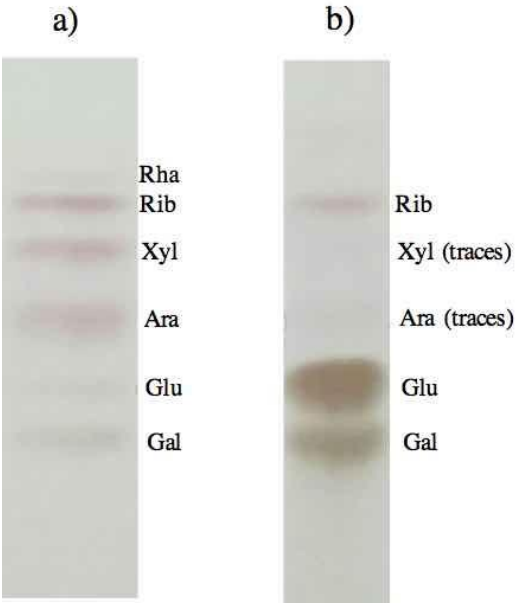
Supplementary Fig. S2: Thin-layer chromatographs showing (a) the presence of diaminopimelic acid (A_2pm) isomers and (b) the presence of *meso*-A_2pm in whole-cell hydrolysates of isolate P6^T. a) Standards A_2pm; b) isolate P6^T.



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



Supplementary Fig. S4: Bi-dimensional thin-layer chromatography of polar lipids of isolate P6^T using molybdatophosphoric 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^T. a) Standard sugars; b) sugars present in isolate P6^T. Rha: rhammnose; Rib: ribose; Xyl: xylose; Ara: arabinose; Glu: glucose; Gal: galactose.

Supplementary Table 1. A list of stress related genes present in strains P6^T and DSM 44509^T

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

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

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

P6^{T} and DSM 44509^{T}	no subcategory	Flavohemoprotein (Hemoglobin-like protein) (Flavohemoglobin) (Nitric oxide dioxygenase) (EC 1.14.12.17)	P6_peg3406	DSM44509_peg3484, DSM44509_peg3485
P6^{T} and DSM 44509^{T}	no subcategory	Hemoglobin-like protein HbO	P6_peg1865	DSM44509_peg685
P6^{T} and DSM 44509^{T}	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^{T} and DSM 44509^{T}	no subcategory	Carbon starvation protein A	P6_peg1951	DSM44509_peg975
P6^{T} and DSM 44509^{T}	no subcategory	Carbon storage regulator	P6_peg3731	DSM44509_peg4059
P6^{T} and DSM 44509^{T}	no subcategory	ABC-type Fe3+-siderophore transport system, permease 2 component	P6_peg780, P6_peg1125	DSM44509_peg709, DSM44509_peg3042
P6^{T} and DSM 44509^{T}	no subcategory	Flavohemoprotein (Hemoglobin-like protein) (Flavohemoglobin) (Nitric oxide dioxygenase) (EC 1.14.12.17)	P6_peg3406	DSM44509_peg3484, DSM44509_peg3485
P6^{T} and DSM 44509^{T}	no subcategory	GTP-binding protein HflX	P6_peg208	DSM44509_peg1601
P6^{T} and DSM 44509^{T}	no subcategory	Anti-sigma B factor antagonist RsbV	P6_peg1497	DSM44509_peg715, DSM44509_peg716, DSM44509_peg1537
P6^{T} and DSM 44509^{T}	no subcategory	RNA polymerase sigma factor SigB	P6_peg600, P6_peg2515	DSM44509_peg2957, DSM44509_peg3720
P6^{T} and DSM 44509^{T}	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^{T} and DSM 44509^{T}	no subcategory	Serine-protein kinase RsbW (EC 2.7.11.1)	P6_peg601	DSM44509_peg2958
DSM 44509^{T}	no subcategory	Putative SigmaB asociated two-component system sensor protein		DSM44509_peg2261