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Modestobacter caceresii sp. nov., novel actinobacteria with an insight into their adaptive mechanisms for survival in extreme hyper-arid Atacama Desert soils☆

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ABSTRACT

A polyphasic study was designed to determine the taxonomic provenance of three *Modestobacter* strains isolated from an extreme hyper-arid Atacama Desert soil. The strains, isolates KNN 45-1a, KNN 45-2b^T and KNN 45-3b, were shown to have chemotaxonomic and morphological properties in line with their classification in the genus *Modestobacter*. The isolates had identical 16S rRNA gene sequences and formed a branch in the *Modestobacter* gene tree that was most closely related to the type strain of *Modestobacter marinus* (99.6% similarity). All three isolates were distinguished readily from *Modestobacter* type strains by a broad range of phenotypic properties, by qualitative and quantitative differences in fatty acid profiles and by BOX fingerprint patterns. The whole genome sequence of isolate KNN 45-2b^T showed 89.3% average nucleotide identity, 90.1% (SD: 10.97%) average amino acid identity and a digital DNA–DNA hybridization value of 42.4 ± 3.1 against the genome sequence of *M. marinus* DSM 45201^T, values consistent with its assignment to a separate species. On the basis of all of these data, it is proposed that the isolates be assigned to the genus *Modestobacter* as *Modestobacter caceresii* sp. nov. with isolate KNN 45-2b^T (CECT 9023^T = DSM 101691^T) as the type strain. Analysis of the whole-genome sequence of *M. caceresii* KNN 45-2b^T, with 4683 open reading frames and a genome size of ∽4.96 Mb, revealed the presence of genes and gene-clusters that encode for properties relevant to its adaptability to harsh environmental conditions prevalent in extreme hyper arid Atacama Desert soils.

Introduction

The genus *Modestobacter* [37] belongs to the family *Geodermatophilaceae* [41,42] of the order *Geodermatophilales* [59] which is a member of the class *Actinobacteria* [63]. Members of the genus are currently recognised by a combination of chemotaxonomic, morphological and physiological properties [43,69]. They are aerobic, Gram-positive, non-spore-forming, heterotrophic actinobacteria which form rod- and coccoid-shaped elements which tend to remain aggregated and have a tendency to form multiseptate filaments and an ability to grow on oligotrophic media; the wall peptidoglycan contains *meso*-diaminopimelic acid, the major fatty acid is *iso*-$C_{16:0}$, the predominant respiratory quinone is tetrahydrogenated menaquinone with nine isoprene units (MK-9 [H_4]) and the major polar lipids include diphosphatidylglycerol, phosphatidylethanolamine and phosphatidylinositol.

The genus *Modestobacter* currently encompasses six species, *Modestobacter multiseptatus* [37], the type species, *Modestobacter lapidis* [69], *Modestobacter marinus* [75], *Modestobacter muralis* [69], *Modestobacter roseus* [48] and *Modestobacter versicolor* [50] which form a distinct clade in the *Geodermatophilaceae* 16S rRNA gene

☆ The GenBank EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of *Modestobacter caceserii* isolates KNN 45-1a, KNN 45-2b^T and KNN 45-3b are LN898186, LN898173 and LN898185, respectively. The partial *gyrB* gene sequences of isolates KNN45-1a, KNN 45-3b and the type strains of *M. lapidis*, *M. marinus*, *M. multiseptatus*, *M. muralis*, *M. roseus* and *M. versicolor* are LN898184, LN898183, LN898182, LN898181, LN898179, LN898180, LN898178 and LN898177, respectively. The whole-genome sequence number of *M. caceserii* KNN 45-2b^T is JPMX00000000.

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tree. The small number of *Modestobacter* strains assigned to these species were isolated from markedly different ecosystems [69] though there is evidence that members of the genus are associated with extreme biomes, including regoliths and desert soils and with the surfaces of rocks and ancient monuments [19,44,45,70]. The presence of *Modestobacter* strains in such hostile environments may be partly due to their ability to form black pigments [12], melanin-like pigments which may prove to be a source of sun screens.

To date, *Modestobacter* strains have not been isolated from Atacama Desert soil. Members of the genus have been considered to access trace carbon sources on stone surfaces that are characterized by low organic carbon availability [12] while *M. marinus* strain BC501 has been reported to be highly resistant to gamma and high energy UV radiation [19].

Normand et al. [44] found that the 5.6 Mb genome of this strain contained several genes in multiple copies, such as *coxSML* (carbon monoxide dehydrogenase), *katA* (manganese-containing catalase) and *trwC* (conjugative relaxase) and *uvrACD* (UV resistance). The analysis of the proteome of isolate BC501 has provided additional insight into how *Modestobacter* strains cope with stressful environmental conditions [61].

The present study was designed to establish the taxonomic status of three *Modestobacter* strains isolated from an extreme hyper-arid Atacama Desert soil. The isolates, strains KNN 45-1a, KNN 45-2b^T and KNN 45-3b, were compared with the type strains of the six validly published *Modestobacter* species using a range of genotypic and phenotypic properties shown to be of value in the circumscription of *Modestobacter* species [69]. The strains were found to form a novel species of *Modestobacter*; the name proposed for this species is *Modestobacter caceresii* with isolate KNN 45-2b^T as the type strain. Analysis of the whole-genome sequence generated for this strain provided an insight into how the organism has adapted to harsh environmental conditions prevalent in extreme hyper-arid Atacama Desert soils.

Materials and methods

Isolation of strains

Modestobacter strains were recovered from an extreme hyper-arid soil sample collected from the Yungay core region of the Atacama Desert (24° 06′ 18.6″ S/70° 01° 55.6″ W) using Gause's No. 1 agar [76] and humic acid-vitamin agar [21]; these media were supplemented with actidione (25 μg ml^{-1}) and in the case of the humic acid agar with nalidixic acid (10 μg ml^{-1}). Aliquots (100 μl) of a 10^{-1} suspension of the soil prepared in ¼ strength Ringer's solution (Oxoid) were spread over the plates of each of the isolation media which had been dried for 15 min at room temperature prior to inoculation, as recommended by Vickers and Williams [72]. After incubation at 28 °C for 3 weeks, the presumptive *Modestobacter* isolates were counted and expressed as the number of colony forming units (cfu) per gram dry weight soil.

Test strains, maintenance and cultural conditions

Three representative strains were taken from the isolation plates, isolate KNN 45-1a was from one of the Gause's No. 1 agar plates and isolates KNN 45-2b^T and KNN 45-3b from humic acid agar plates. The isolates together with *M. lapidis* MON 3.1^T, *M. marinus* DSM 45201^T, *M. multiseptatus* DSM 44406^T and *M. muralis* MDVD1^T, *M. roseus* DSM 45764^T and *M. versicolor* DSM 16678^T were maintained on modified Bennett's agar slopes [23] at room temperature and as suspensions of cells in 20% v/v glycerol at −20 °C and −80 °C. Biomass for the fatty acid and molecular systematic analyses carried out on the isolates was harvested from yeast extract-malt extract agar plates (International *Streptomyces* Project [ISP] medium 2; [62] that had been incubated at 28 °C for 5 days; the biomass preparations were washed twice in distilled water and stored at −20 °C. Biomass for the additional chemotaxonomic studies carried out on isolate KNN 45-2b^T was prepared in shake flasks (200 revolutions per minute) of ISP 2 broth after incubation for 14 days at 28 °C; cells were harvested by centrifugation, washed twice in distilled water and freeze-dried.

Phylogenetic analyses

Genomic DNA was extracted from isolates KNN 45-1a, KNN 45-2b^T and KNN 45-3b and PCR-mediated amplification of 16S rRNA and gyrase B (*gyrB*) genes and direct sequencing of the purified PCR products realised following procedures described by Carro et al. [10]. The resultant 16S rRNA gene sequences (1390–1405 bp) were aligned using CLUSTAL X [66] against corresponding sequences of the *Modestobacter* type strains retrieved from the GenBank database using the EzTaxon-e server [26]. Phylogenetic trees were inferred using the maximum-likelihood [14], maximum-parsimony [16] and neighbour-joining [55] algorithms with 1000 bootstrap replicates [15] after removing the gaps and missing data from the nucleotide sequence alignment using the MEGA 6 software package [65]. The neighbour-joining and maximum-parsimony trees were obtained using the Max-mini branch-and-bound algorithm [47]. The phylogenetic position of the three isolates was established using representative sequences from members of the family *Geodermatophilaceae*.

Partial *gyrB* gene sequences of all of the *Modestobacter* type strains generated in this study were used to determine the potential value of this gene as a phylogenetic marker. All sequences (1043–1361 bp) were aligned and the corresponding phylogenetic trees were constructed as explained above for the 16S rRNA gene. In this analysis, *Geodermatophilus obscurus* DSM 43160^T was used as an outgroup.

BOX typing

BOX-PCR fingerprinting profiles from genomic DNA extracted from the isolates and *Modestobacter* type strains were generated using the BOXAIR primer [71] and previously described experimental conditions [68]. Cluster analysis based on the Pearson moment correlation coefficient was carried out with the software Gel-Compar II (Applied Maths).

Chemotaxonomy

The isolates were examined for the presence of the isomers of diaminopimelic acid (A_2pm) following the procedure described by Hasegawa et al. [20]. In turn, fatty acids extracted from the isolates were methylated, analysed using the protocol of the Sherlock Microbial Identification (MIDI) system, version 5 [57]; the resultant peaks were named using the SACTIN 6 database and the results compared with those of the *Modestobacter* type strains which had been examined under the same experimental conditions [69]. Using standard chromatographic procedures isolate KNN 45-2b^T was examined for the presence of diagnostic menaquinones and polar lipids [38] and whole-organism sugars [64].

Cultural and morphological properties

The isolates were examined for motility and micromorphological properties using procedures described by Trujillo et al. [69]. Cultural properties of the isolates were recorded on

tryptone-yeast extract, yeast extract malt extract oatmeal, inorganic salts-starch, glycerol-asparagine, peptone-yeast extract-iron and tyrosine agar plates (ISP media 1–7; [62]) following incubation at 28 °C for 14 days.

Phenotypic properties

The isolates were screened for a combination of biochemical, degradation and physiological properties shown to be of value in an earlier study of *Modestobacter* strains [69]. All of the tests were carried out in duplicate using a standard inoculum equivalent to 5.0 on the McFarland scale [39]. In addition, the ability of the isolates to grow in the presence of carbon dioxide as a sole carbon source was examined using a Thermo Forma Series II Water Jacket CO_2 incubator and carbon utilisation agar plates (ISP medium 9; [62]) was determined following incubation at 28 °C for 14 days.

Whole-genome sequencing of isolate KNN 45-2b^T and genomic analyses

Isolate KNN 45-2b^T was grown in tryptone soy broth supplemented with 10% sucrose-yeast extract-malt extract (1%, v/v), 5 mM $MgCl_2$ and 0.5% glycine at 30 °C for 48 h. Cells were spun down, and resuspended in 10 mM NaCl, 20 mM Tris–HCl (pH 8.0) and 1 mM EDTA then incubated with lysozyme at 37 °C for 1–30 min until they were lysed. SDS (0.5% final concentration) and proteinase K (40 μg) were added and the cell extract incubated at 50 °C for 6 h when a standard phenol–chloroform extraction was carried out on the lysate. The pH of the extract was adjusted to 5.5 with 0.3 M sodium acetate and DNA spooled with a glass rod following the addition of 2 volumes of 96% ethanol. After washing and drying the DNA was dissolved in TE buffer, DNA quality was verified by *sal1* digestion and agarose gel electrophoresis.

The genome of strain KNN 45-2b^T was sequenced on an Illumina platform (Service SX, Leiden, The Netherlands). The quality of the 100-nt pair end reads was verified using FastQC [1] and depending on the quality, reads were trimmed to various lengths at both ends. The trimmed reads were assembled using Velvet [77]. The genome was annotated using the RAST server [3] with default options. Predictions of gene clusters for natural products were performed using antiSMASH [35]. Protein sequences of genes belonging to *cox* and *uvr* gene clusters in *M. marinus* strain BC501 were BLAST searched in the genome of strain KNN 45-2b^T on the SEED server using default settings [4].

A BLAST based average nucleotide identity (ANIb) of the genome of strain KNN 45-2b^T was calculated against the genome sequence of *M. marinus* DSM 45201^T (Sangal and Goodfellow, unpublished data) using Jspecies [52]. A two-way average amino acid identity (AAI) was calculated using the protein sequences of these strains by an online resource from the K. Konstantinidis group (http://enve-omics.ce.gatech.edu/aai/). The digital DNA–DNA hybridization (dDDH) values between these genomes were calculated using the genome-to-genome distance calculator, GGDC 2.0 [2,36].

Results

Isolation, enumeration, cultural and morphological properties and phylogeny

Small numbers of strains growing on the isolation plates were assigned to the genus *Modestobacter* as they formed characteristically round, slightly mucoid colonies that were initially orange to beige in colour but later turned black. The highest count, 6.0×10^{-1} cfu/g dry weight soil, was recorded on the humic acid-vitamin agar plates. All of the isolates were shown to be

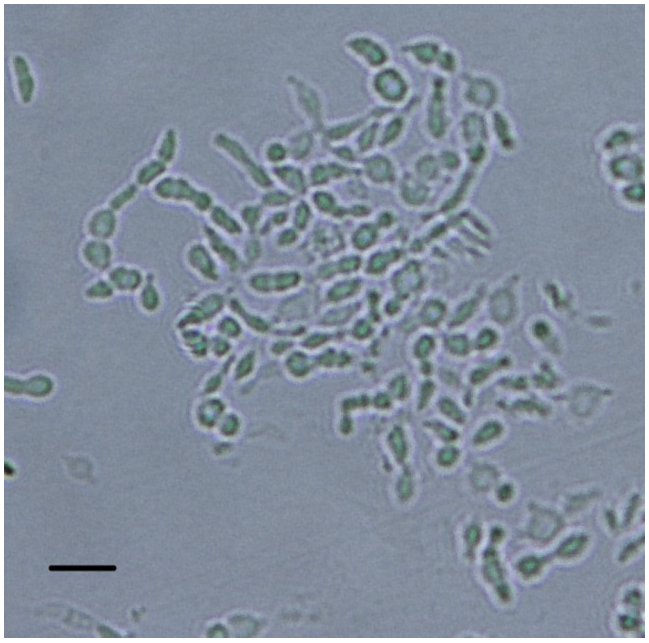

Fig. 1. Phase contrast image of strain KKNN 45-2b^T grown on ISP 2 agar at 28 °C for 3 days. Bar, 5 μm.

Gram-stain-positive, non-motile and formed short-rod and coccoid shaped cells that had a tendency to remain aggregated (Fig. 1). Colonies were olive to yellowish in colour, but turned black on prolonged incubation. The isolates and the *Modestobacter* type strains were found to grow well on most of the ISP media producing pigments that ranged from yellowish white to black (Table 1). The colonies were flat, round and mucoid with entire margins.

The isolates were shown to have identical 16S rRNA gene sequences, which, when compared with corresponding sequences of the *Modestobacter* type strains showed that they formed a distinct lineage within the evolutionary radiation of the genus *Modestobacter*, one that was supported by the neighbour-joining, maximum-likelihood and maximum-parsimony algorithms and by an 86% bootstrap value (Fig. 2). The strains formed a well delineated branch in the *Modestobacter* 16S rRNA gene tree together with the type strains of *M. marinus* and *M. roseus*, a relationship supported by a 96% bootstrap value and by all three tree-making algorithms. They were shown to be most closely related to the type strain of *M. marinus* sharing a 99.6% 16S rRNA gene similarity with the latter, a

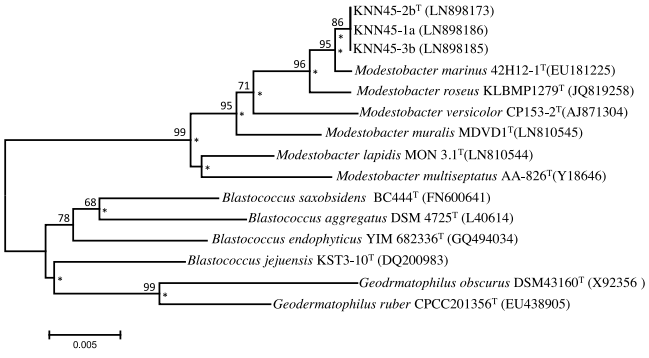

Fig. 2. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences of isolates KNN 45-1a, KNN 45-2b^T and KNN 45-3b and representative type strains of the family *Geodermatophilaceae*. *Asterisks* indicate that the corresponding branches were recovered in the maximum-likelihood and maximum-parsimony trees. Only bootstrap values above 50% are shown. Bar, 0.005 substitutions per nucleotide position.

Table 1
Growth and cultural characteristics of isolates and *Modestobacter* type strains on ISP media after incubation for 14 days at 28 °C.

Media	Growth	Colony colour	Diffusible pigment
Glycerol-asparagine agar (ISP5)	++	Olive-Black[a]	None
Inorganic salts-starch agar (ISP 4)	+	Yellowish-white	None
Oatmeal agar (ISP 3)	+++	Olive-black	None
Peptone-yeast extract -iron agar (ISP 6)	+++	Black/orange[b]	None/light yellow[b]
Tryptone-yeast extract agar (ISP 1)	+++	Yellowish-white	None
Tyrosone agar (ISP 7)	+++	Yellowish-white	None/light yellow[b]
Yeast extract-malt extract agar (ISP 2)	++++	White yellow-black	None/light yellow[b]

Key: ++++, abundant growth; +++, very good growth; ++, good growth; +, poor growth.
[a] The *M. lapidis* and *M. muralis* colonies were orange-black and brown-black, respectively.
[b] Results for isolates KNN 45-1a, KNN 45-2b^T and KNN 45-3b and for the type strain of *M. versicolor*.

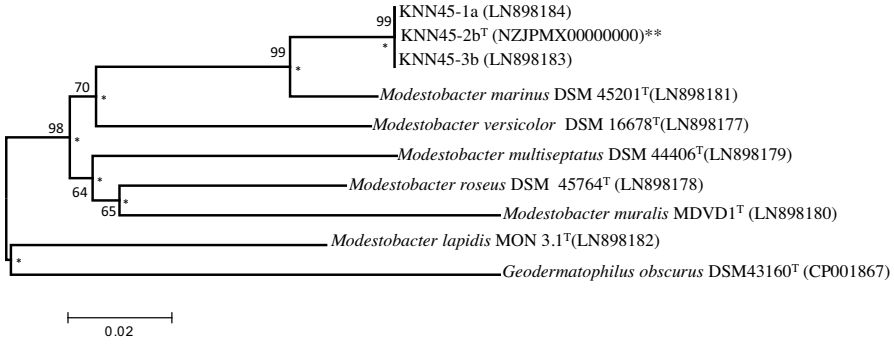

Fig. 3. Neighbour-joining phylogenetic tree based on partial *gyrB* gene sequences of isolates KNN 45-1a, KNN 45-2b^T and KNN 45-3b and all of the *Modestobacter* type strains. *G. obscurus* was used as an outgroup. Asterisks indicate the corresponding branches recovered in the maximum-likelihood and maximum-parsimony trees. Only bootstrap values above 50% are shown. Scale bar represents 0.02 substitutions per nucleotide position. ***gyrB* gene sequence was extracted from the whole genome sequence deposited in public databases under accession number JPMX01000080 (Region: 2895-4931).

value that corresponds to 5 nucleotide (nt) differences at between 1387 and 1403 locations; the corresponding figures between the isolates and the *M. roseus* type strain were 99.4% 16S rRNA gene sequence similarity and 9 nt differences at between 1385 and 1409 sites.

The three isolates were recovered as a well-defined cluster in the *gyrB* gene tree with *M. marinus* as the most closely related species, a result supported by a bootstrap value of 99% (Fig. 3). Sequence similarities between the isolates were identical while a value of 96.4% was obtained between them and the type strain of *M. marinus*. The same tree topology was obtained when the different algorithms used for the 16S rRNA gene sequence analyses were applied. Overall, the *Modestobacter* species had sequence similarities between 87.8% and 91.1% and were well separated; the *gyrB* gene phylogeny showed better resolution than the corresponding 16S rRNA phylogeny.

BOX-PCR profiles of the isolates and selected reference *Modestobacter* type strains clearly showed the diversity of their genetic profiles (Fig. S1). The isolates have very similar banding patterns which sharply distinguish them from the reference strains, notably from the type strain of *M. marinus*.

Chemotaxonomy

The three isolates were found to contain *meso*-A_2m as the diamino acid, *iso*-$C_{16:0}$ as the predominant fatty acid, but lacked mycolic acids. The fatty acid profiles of the isolates were seen to show qualitative and quantitative differences when compared with corresponding profiles of the *Modestobacter* type strains, as exemplified by the presence of predominant amounts of $C_{17:0}$ and *iso*-$C_{15:0}$ in the type strains of *M. marinus* and *M. multiseptatus*, respectively (Table 2). Isolate KNN 45-2b^T was found to contain tetrahydrogenated menaquinone as the sole isoprenologue, whole-cell hydrolysates rich in arabinose, glucose, ribose and rhamnose, and diphosphatidylglycerol, phosphatidylethanolamine (taxonomically significant component), phosphatidylglycerol, phosphatidylinositol, phosphatidylinositol mannoside, and three unidentified lipids (Fig. S2).

Phenotypic tests

Duplicated strains of KNN 45-1a, KNN 45-2b^T and KNN 45-3b were found to give identical results for all of the phenotypic tests. The isolates and the *Modestobacter* type strains were shown to grow at 20 °C and 28 °C, at pH 7.0 and pH 8.0, produce acid phosphatase, esterase (C4), leucine arylamidase, naphthol-AS-B1 phosphohydrolase and valine arylamidase (API tests), reduce nitrate to nitrite, hydrolyse urea, use acetoacetic acid and dextrin as sole carbon sources and grow in the presence of fusidic acid, minocycline and potassium tellurite (Biolog GENIII microplates). Scant growth was detected in the presence of 5% CO_2 as the sole carbon source. In contrast, none of the strains were found to grow at 40 °C, at pH 4, 10 or 11, to produce α-fucosidase, α- or β-galactosidases (API ZYM tests) or to assimilate α-amino-butyric acid, formic acid, α-methyl-D-glucoside, glycyl-proline or methyl pyruvate as sole carbon sources (Biolog GEN III microplates).

The remaining phenotypic tests were found to distinguish the three isolates from the *Modestobacter* type strains (Table 3) which had been examined using the same media, methods and cultivation conditions [69]. In particular, the isolates were separated from the type strain of *M. marinus*, as exemplified by their ability to produce α-chymotrypsin and esterase lipase (C8) (API ZYM tests), to assimilate acetic acid, gentiobiose, D-raffinose, D-serine, D-trehalose and D-turanose and grow in the presence of tetrazolium violet (Biolog GEN III microplates).

Table 2
Fatty acid composition (%) of the isolates and the type strains of *Modestobacter* species. Strains: 1, isolates KNN 45-1a, KNN 45-2b^T and KNN 45-3b; 2, *M. lapidis* MON 3.1^T; 3, *M. marinus* DSM 45201^T; 4, *M. multiseptatus* DSM 44406^T; 5, *M. muralis* MDVD1^T; 6, *M. roseus* DSM 45764^T; 7, *M. versicolor* DSM 16678^T.

Fatty acid	1	2	3	4	5	6	7
$C_{12:0}$	0.2–0.7	–	–	–	–	–	–
iso-$C_{13:0}$	–	0.1	–	–	–	–	–
$C_{13:0}$	–	–	0.4	–	–	–	–
iso-$C_{14:0}$	1.6–1.9	2.1	1.3	1.1	1.4	2.4	1.0
$C_{14:0}$	0.3–0.9	2.1	1.0	0.4	0.9	0.3	0.3
iso-$C_{15:1}$G	0.7–1.5	5.3	0.7	1.7	–	–	2.8
iso-$C_{15:0}$	2.9–7.4	17.4	9.7	21.5	8.2	11.0	19.9
anteiso-$C_{15:0}$	0.6–1.0	2.5	4.2	3.7	1.5	4.5	2.8
$C_{15:1\ B}$	0.3–0.4	1.2	0.7	–	–	–	–
$C_{15:0}$	0.6–1.7	2.3	5.6	1.3	3.9	1.3	0.7
iso-$C_{16:1}$ H	1.3–9.4	3.6	0.7	0.5	–	5.3	–
iso-$C_{16:0}$	22.6–39.1	21.8	10.3	19.7	16.1	21.9	22.2
$C_{16:1\omega9c}$	7.3–11.1	10.0	3.2	1.0	3.6	–	1.3
$C_{16:0}$	5.7–8.8	7.6	11.2	3.8	8.3	7.2	3.2
9-methyl $C_{16:0}$	0.7–0.9	0.5	0.3	–	–	1.2	2.0
anteiso-$C_{17:1C}$	0.2–0.4	0.5	0.2	0.2	–	–	0.4
iso-$C_{17:0}$	0.9–3.1	1.0	2.8	8.8	3.2	3.1	8.7
anteiso-$C_{17:0}$	0.7–1.4	1.6	3.7	5.9	3.2	2.1	4.9
$C_{17:1\omega9c}$	8.5–13.8	6.9	13.7	4.8	19.4	15.0	5.5
Cyclo $C_{17:0}$	0.7–2.5	2.3	0.2	–	–	1.0	–
$C_{17:0}$	1.2–5.3	1.5	20.0	8.6	17.2	9.6	4.8
10-methyl $C_{17:0}$	0.8–2.0	0.2	–	0.4	0.5	1.0	0.9
$C_{18:3\omega6c,12,14c}$	1.2–4.1	–	1.3	0.9	1.0	–	1.0
iso-$C_{18:0}$	0.3–0.5	–	–	0.5	–	–	0.4
$C_{18:1\omega9c}$	4.4–10.2	3.9	2.1	5.5	5.4	2.1	9.8
$C_{18:0}$	0.6–3.0	0.8	3.7	6.6	2.8	0.6	3.9
iso-$C_{17:0}$ OH	1.6–2.1	2.7	2.6	2.8	3.2	–	0.9
$C_{16:1\ \omega7c}/C_{17:1\ \omega6c}$	–	–	–	–	–	8.4	–
$C_{18:1\ \omega7c}/C_{18:1\ \omega9c}/C_{18:1\ \omega12t}$	0–0.5	0.1	–	–	–	0.4	–
iso-$C_{17:0\ \omega9c}$/10-methyl $C_{16:0}$	0–0.5	1.0	0.4	0.8	1.0	1.5	1.3

Genomic analyses resolving the taxonomic status of KNN45-2b^T

The whole genome sequence of strain KNN45-2b^T was compared to that of its nearest phylogenetic neighbour, namely *M. marinus* DSM 45201^T (Sangal and Goodfellow, unpublished data). ANIb values of 89.3% and 90. 4% were observed when the genome sequence of KNN45-2b^T and *M. marinus* DSM 45201^T were used as reference against one another, respectively. Similarly, an AAI value of 90.1% (SD: 11%) was observed between these strains. The dDDH value was 42.4 ± 3.1 between isolate KNN45-2b^T and *M. marinus* DSM 45201^T, a value well below the recommended cut-off value of 70% for the assignment of strains to the same species [74]. These results provide further evidence that these strains belong to two different species.

Detection of genes associated with stress responses

The genome of isolate KNN 45-2b^T was assembled into 140 contigs to give a total genome size of ∽4.96 Mb with an average GC content of 73.6 mol%. The genome sequence has been deposited at DDBJ/EMBL/GenBank under accession number JPMX00000000. The whole genome was annotated to include 4683 protein coding sequences and 50 RNA (47 tRNA) genes by the RAST pipeline.

The SEED analyses [4] of the KNN 45-2b^T genome identified 110 genes that are associated with stress responses, including *hrcA* and *grpE* genes and the *dnaK-J* gene cluster involved in the heat shock response [29] and four genes encoding the CspA family of proteins that respond to cold shock (Supplementary Table 1; [12]). Multiple copies of *bet* genes (2 copies of *betA*, one copy of *betB* and two copies of *betT*) and two *proU* and one *sox* gene cluster involved in the uptake of choline and betaine and betaine biosynthesis are also present [7,25,33,46,73]. These metabolic activities contribute to the response against osmotic stress [6,40]. Two genes involved in carbon starvation were identified (fig|6666666.51110.peg.3264 and fig|6666666.51110.peg.1467) that encode a carbon storage regulator CsrA and carbon starvation protein A, respectively (Supplementary Table 1), CsrA is a global regulator involved in repression of multiple genes/pathways, including glycogen biosynthesis [53,54]. Carbon starvation protein A may help the strain to survive in low carbon habitats by activating peptide uptake [31,49,58]. Although the SEED analyses did not identify any genes associated with the response to desiccation stress, a number of genes involved in the biosynthesis and uptake of trehalose were scattered across the genome; trehalose has been linked with tolerance to heat and desiccation in bacteria [51].

The BLAST search of the genes involved in UV resistance in *M. marinus* strain BC501 revealed the presence of two copies of *uvrA*, one *uvrB* and three copies of *uvrD* genes in strain KNN 45-2b^T (Supplementary Table 2), the *uvrC* gene of strain BC501 showed partial similarity with a gene in strain KNN 45-2b^T. The KNN 45-2b^T genome also contained a *recO* gene and three copies of *recQ* DNA helicase that are known to be involved in stabilizing the genome [22,34]. The BLAST searches also revealed the presence of two *coxGLSM* gene clusters, an additional cluster of *coxLSM*, as well as *coxD and coxE* genes (Supplementary Table 2). The *coxGLSM* cluster encodes different subunits of carbon monoxide (CO) dehydrogenase that contain domains for molybdopterin, Fe-S and FAD-binding. Carbon monoxide dehydrogenases enable chemolithoautotrophic lifestyles in bacteria through utilization of CO as a carbon and energy source [30].

Biosynthetic gene clusters for secondary metabolites

Antibiotics and Secondary Metabolite Analysis Shell (antiSMASH version 3.0.4; [35]) identified a siderophore gene cluster that is predicted to encode desferrioxamine B. Four of the five biosynthetic genes of this gene cluster show significant homology with the desferrioxamine B gene cluster in *Streptomyces coelicolor* strain A3(2) [5,67]. In addition, five other putative gene clusters were identified in the KNN 45-2b^T genome, including

Table 3
Phenotypic properties that distinguish isolates KNN 45-1a, KNN 45-2b^T and KNN 45-3b from the type strains of *Modestobacter* species.

	Isolates: KNN 45-1a, KNN 45-2^T and 45-3b	*M. lapidis* MON 3.1^T	*M. marinus* DSM 45201^T	*M. multiseptatus* DSM 44406^T	*M. muralis* MDVD1^T	*M. roseus* DSM 45764^T	*M. versicolor* DSM 16678^T
API-ZYM tests							
Alkaline phosphatase	+	–	+	–	+	+	+
α-Chymotrypsin	+	+	–	+	–	+	+
Cysteine arylamidase	+	+	+	+	+	+	–
Esterase lipase (C8)	+	–	–	–	–	+	+
α-Glucosidase	–	+	–	+	–	–	–
β-Glucosidase	–	+	–	–	–	–	–
Trypsin	–	–	–	–	–	+	–
BIOLOG GEN III microplate tests							
a. Assimilation of:							
Acetic acid, D-turanose	+	+	–	+	–	+	–
N-Acetyl-β-D-glucosamine, N-acetyl-β-D-mannose, D-fructose, D-lactose, L-mannose	+	–	–	+	–	+	–
N-acetyl-muramic acid, L-lactic acid	+	–	–	+	–	+	+
L-Alanine	+	–	–	+	–	+	+
D-Arabitol, D-fucose, L-rhamnose, D-saccharic acid	–	–	–	+	–	–	+
L-Arginine, α-*keto*-butyric acid, L-histidine, *myo*-inositol	–	–	–	+	–	–	+
D-Aspartic acid, D-fructose-6PO_4	+	+	–	+	–	+	+
L-Aspartic acid	+	+	–	+	–	–	+
α-Hydroxy-butyric acid, glucuronamide	–	–	–	–	+	–	+
β-Hydroxy-DL-butyric acid, D-saccharic acid, b-Hydroxy-DL-butyric acid	–	–	–	+	–	+	–
D-Cellobiose	–	+	–	+	–	+	–
Citric acid, D-fucose, D-sorbitol	–	+	–	–	–	–	–
D-Galactose, D-mannose	–	–	+	+	+	–	–
D-Galacturonic acid, α-D-glucose, D-pectin	+	+	–	–	+	+	+
L-Galacturonic acid, glucuronamide	–	+	–	–	–	–	+
Gelatin	–	+	–	–	+	–	–
Gentiobiose	+	+	–	–	–	–	+
D-Gluconic acid, sodium bromide	–	–	+	+	+	–	+
α-D-Glucose, D-pectin	–	–	+	–	+	+	+
α-*keto*-Glutaric acid	–	+	+	+	–	–	–
3-Methyl-glucose	–	–	–	–	+	–	–
D-Glucuronic acid	–	–	+	–	–	–	+
L-Glutamic acid	–	–	–	+	+	–	+
Glycerol	–	–	–	+	–	+	+
Inosine	+	+	+	+	–	–	+
D-Lactic acid methyl ester	+	–	–	–	+	+	+
D-Malic acid	–	+	+	–	+	–	+
L-Malic acid, quinic acid	–	+	–	+	+	–	+
D-Maltose	–	+	–	+	–	–	+
Mucic acid	–	–	–	+	+	+	–
D-Glucose-PO_4, D-melibiose	+	+	–	–	–	+	+
p-Hydroxy-phenylacetic acid, bromo-succinic acid, D-sorbitol	–	+	–	–	–	–	–
Propionic acid, L-pyroglutamic acid	–	+	–	–	+	+	–
D-Raffinose, D-trehalose	+	–	+	+	–	+	–
D-Salicin	–	–	–	+	–	–	+
D-Serine	+	+	–	–	–	+	+
Stachyose	–	–	–	–	–	+	–
Sodium butyrate	+	–	+	–	+	–	+
Sodium lactate	–	+	+	+	+	–	+
D-Sucrose	+	+	–	+	–	+	+
D-Trehalose	+	+	–	+	–	+	–
b. Growth in presence of inhibitory compounds							
Aztreonam	+	–	+	+	+	+	+
Guanidine HCl	+	–	–	–	+	–	+
Lincomycin	+	+	+	–	+	–	+
Lithium chloride	+	+	+	+	+	+	–
Nalidixic acid	+	+	+	+	+	–	+
Niaproof 4	–	–	–	+	+	+	+
Rifamycin SV	–	–	+	+	+	+	+
Tetrazolium blue	–	+	–	+	–	–	+
Tetrazolium violet	+	+	–	+	–	+	+
Troleandomycin	–	+	–	+	+	–	+
Vancomycin	+	+	–	+	+	–	+
Growth at pH 5.0	+	–	+	+	+	–	+
Growth in presence of 8% w/v NaCl	–	–	+	+	+	–	+
Resistance to antibiotics (μg ml^{-1}):							
Ampicillin (4), Cephaloridine (2),	–	–	+	–	–	–	+
Ciprofloxacin (2)	–	–	+	–	–	+	+
Lincomycin (3)	–	–	–	–	+	–	+

Key: +, positive; –, negative.

one type II polyketide, one type III polyketide and two terpene biosynthetic clusters. One gene cluster was not assigned to any functional category. Although this strain was isolated from an extreme environment, biosynthetic gene clusters for the synthesis of antibiotics or other special biomolecules were not identified. However, antiSMASH may have limited capabilities to detect all of the biosynthetic gene clusters in the genome, as observed for *Streptomyces leeuwenhoekii* C34^T where the hygromycin A gene cluster was not identified by antiSMASH [8,17].

Discussion

The three representative strains taken from the isolation plates were found to share morphological and phenotypic properties and fatty acid profiles consistent with their classification in the genus *Modestobacter* [43,69], a point underlined by the menaquinone, whole cell sugar and lipid composition of isolate KNN 45-2b^T. In addition, the isolates formed a branch in the *Modestobacter* 16S rRNA and *gyrB* gene trees (Figs. 2 and 3) that were most closely related to the type strain of *M. marinus* but were distinguished readily from the latter based on fatty acid (Table 2) and BOX-PCR (Fig. S1) profiles and by a broad range of phenotypic properties (Table 3).

Whole genome sequence analyses such as ANI [27,28] and AAI between orthologous genes [60] are powerful and reliable tools for species delineation [56]. Genome-to-genome sequence comparison has also been widely used to delineate prokaryotic species and dDDH values found to highly correlate with genetic distances based on variations in 16S rRNA genes [2,56]. It was, therefore, encouraging that the ANIb, AAI and dDDH values between the genomes of isolate KNN45-2b^T and *M. marinus* DSM 45201^T indicated that these strains clearly belong to distinct species within the genus *Modestobacter*.

It is evident from this broad-ranging polyphasic taxonomic study that isolates KNN 45-1a, KNN 45-2b^T and 45-3b form a centre of taxonomic variation within the genus *Modestobacter* that merits recognition as a new species. It is proposed that these isolates be recognised as *Modestobacter caseresii* sp. nov.

Description of *M. caceresii* (ca. ce. res'. i.i. sp. nov. *caceresii*, named in honour of Luis Cáceres in recognition of his studies on relative humidity patterns and water availability in arid Atacama Desert soils.

Aerobic, Gram-stain-positive, non-motile actinobacteria which form short rods and coccoid-like elements and grow especially well on ISP 2 agar as black mucoid colonies. Grows from 20 to 37 °C, optimally ∽28 °C, and from pH 5–9, optimally ∽pH 7.5 and in the presence of 8% (w/v) NaCl. Additional phenotypic properties are cited in the text and in Tables 1 and 3. The predominant fatty acid is *iso*-$C_{16:0}$. Other chemotaxonomic markers match those described for members of the genus *Modestobacter*. The G + C content of the type strain is 72.5 ± 1.0 mol%. Strain KNN 45-2b^T (CECT 9023^T = DSM 101691^T) was isolated from an extreme hyper-arid soil from the Yungay core region of the Atacama Desert in Chile. The GenBank accession number for the 16S rRNA gene sequence of isolate KNN 45-2b^T is LN898173 and that of its whole-genome sequence JPMX 00000000.

This first report of *Modestobacter* strains from Atacama Desert soil provides further evidence that members of this poorly studied genus are present in habitats characterised by low water and nutrient availability, high solar radiation and sharp variations in temperature [9,12,13,45]. Given this context, it is particularly interesting that genes and gene clusters identified in the genome of isolate KNN 45-2b^T encode for attributes relevant to its ability to counter harsh conditions found in extreme hyper-arid Atacama Desert soils, as witnessed by genes involved in responses to osmotic stress (*bet A-B* genes and the *sox* gene cluster [6,40]), heat shock (*hrcA, grpE* and *dna K-J* gene, [29]), cold shock (cspA family genes; [13]) and heat tolerance and desiccation (biosynthesis and uptake of trehalose [51]). The organism is also equipped to survive low nutrient conditions and the presence in the genome of multiple *sox* genes is consistent with a chemolithotrophic metabolism as the isolate has the capacity to use CO as a sole carbon and energy source [11,30]. It also has the potential to metabolise environmental proteins and peptides under starvation conditions as it has a gene that encodes for carbon starvation protein A, this gene has been reported to activate peptide uptake during energy starvation thereby allowing bacteria to use alternative energy sources [49,58].

Microorganisms in Atacama Desert soils need strategies to survive high levels of UV radiation which can damage DNA by a number of photochemical reactions; nucleotide excision repair plays a key role in repairing damaged DNA [18,24]. Isolate KNN 45-2^T has the ability to protect and repair damage caused by UV radiation as it has genes that encode for Uvr ABCD proteins, excision proteins that have been reported in a number of bacteria [18]. Mutations in *uvr ABC* genes have been shown to be associated with UV sensitivity in *Rhodobacter sphaeroides* [32].

Conflict of interest

The authors declare no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.syapm.2016.03.007.

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