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

Kanungnid Busarakam[1], Alan T. Bull[2], Martha E. Trujillo[3], Raul Riesco[3], Vartul Sangal[4], Gilles P. van Wezel[5] and Michael Goodfellow[1,*]

1. School of Biology, Newcastle University, Newcastle upon Tyne NE1 7RU, UK

2. School of Biosciences, University of Kent, Canterbury CT2 7NJ, UK

3. Departamento de Microbiología y Genética, Universidad de Salamanca, Campus Miguel de Unamuno, 37007, Salamanca, Spain

4. Faculty of Health and Life Sciences, Northumbria University, Newcastle upon Tyne NE1 8ST, UK

5. Molecular Biotechnology, Institute of Pathology, Leiden University, P.O. Box 9505 230 RA Leiden, The Netherlands

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*To whom correspondence should be addressed: Telephone: +44(0)191208 7706: email: m.goodfellow@ncl.ac.uk

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

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 ∽ 4.96Mb, 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.

Keywords: Modestobacter caceserii, polyphasic taxonomy, whole genome sequence, hyper arid Atacama Desert soil, average nucleotide identity, average amino acid identity.

Introduction

The genus Modestobacter [37] belongs to the family Geodermatophilaceae [41,42] of the order Geodermatophilales [60] 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 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

77 multiple copies, such as cox SML (carbon monoxide dehydrogenase), kat A (manganese
78 containing catalase) and trwC (conjugative relaxase) and uvr ACD (UV resistance). The
79 analysis of the proteome of isolate BC501 has provided additional insight into how
80 Modestobacter strains cope with stressful environmental conditions [61].
81 The present study was designed to establish the taxonomic status of three
82 Modestobacter strains isolated from an extreme hyper-arid Atacama Desert soil. The
83 isolates, strains KNN 45-1a, KNN 45-2b^T and KNN 45-3b, were compared with the type
84 strains of the six validly published Modestobacter species using a range of genotypic and
85 phenotypic properties shown to be of value in the circumscription of Modestobacter species
86 [69]. The strains were found to form a novel species of Modestobacter; the name proposed
87 for this species is Modestobacter caceresii with isolate KNN 45-2b^T as the type strain.
88 Analysis of the whole-genome sequence generated for this strain provided an insight into
89 how the organism has adapted to harsh environmental conditions prevalent in extreme hyper arid
90 Atacama Desert soils.
91
92 **Materials and methods**
93 Isolation of strains
94 Modestobacter strains were recovered from an extreme hyper-arid soil sample
95 collected from the Yungay core region of the Atacama Desert (24^o 06' 18.6" S / 70^o 01^o 55.6"
96 W) using Gause's No 1 agar [76] and humic acid-vitamin agar [21]; these media were
97 supplemented with actidione (25 · g ml^{-1}) and in the case of the humic acid agar with
98 nalidixic acid (10 · g ml^{-1}). Aliquots (100 µl) of a 10^{-1} suspension of the soil prepared in ¼
99 strength Ringer's solution (Oxoid) were spread over the plates of each of the isolation media
100 which had been dried for 15 minutes at room temperature prior to inoculation, as
101 recommended by Vickers and Williams [72]. After incubation at 28^{o}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^{o}C and -80^{o}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^{o}C for 5 days; the biomass preparations were washed twice in distilled water and stored at -20^{o}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^{o}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 to 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 1,000 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, G. 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 micro-morphological 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^{o}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^{o}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), 5mM $MgCl_2$ and 0.5% glycine at 30^{o}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^{o}C for 1-30 minutes until they were lysed. SDS (0.5% final concentration) and proteinase K (40 · g) were added and the cell extract incubated at 50^{o}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 0.3M 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, http://www.bioinformatics.bbsrc.ac.ukprojects/fastgc/) 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 x 10^{-1} cfu/g dry weight soil, was recorded on the humic acid-vitamin agar plates. All of the isolates were

shown to be 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 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 – 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^{o}C and 28^{o}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

40oC, at pH 4, 10 or 11, to produce · -fucosidase, · - or · · galactosidases (API ZYM tests) or to assimilate · -amino-butyric acid, formic acid, · -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 · -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).

Genomic analyses resolving the taxonomic status of KNN45-2bT

The whole genome sequence of strain KNN45-2bT was compared to that of its nearest phylogenetic neighbour, namely M. marinus DSM 45201T (Sangal and Goodfellow, unpublished data). ANIb values of 89.3% and 90. 4% were observed when the genome sequence of KNN45-2bT and M. marinus DSM 45201T 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 KNN45-2bT and M. marinus DSM 45201T, a value well below the recommended cut-off value of 70% recommended 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 45-2bT 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 4,683 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 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 Modestobacter 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 20-37^{o}C, optimally $\backsim$ 28^{o}C, and from pH 5-9, optimally $\backsim$ 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 2. 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

45-2bT 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 [12,13,45]. Given this context, it is particularly interesting that genes and gene clusters identified in the genome of isolate KNN 45-2bT 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 45-2T 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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626

627 **Table 1.**

628 Fatty acid composition (%) of the isolates and the type strains of Modestobacter species.

629 Strains: 1, isolates KNN 45-1a, KNN 45-2b^T and KNN 45-3b; 2, M. lapidis MON 3.1^T; 3, M.

630 marinus DSM 45201^T; 4, M. multiseptatus DSM 44406^T; 5, M. muralis MDVD1^T; 6, M.

631 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

635 **Table 2**

636 Growth and cultural characteristics of isolates and Modestobacter-type strains on ISP media

637 after incubation for 14 days at 28^{o}C.

Media	Growth	Colony colour	Diffusible pigment
Glycerol-asparagine agar (ISP5)	++	Olive-Black*	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**	None/light yellow**
Tryptone-yeast extract agar (ISP 1)	+++	Yellowish-white	None
Tyrosone agar (ISP 7)	+++	Yellowish-white	None/light yellow**
Yeast extract-malt extract agar (ISP 2)	++++	White yellow-black	None/light yellow**

638

639 Key: ++++, abundant growth; +++, very good growth; ++, good growth; +, poor growth

640 *The M. lapidis and M. muralis colonies were orange-black and brown-black, respectively;
641 **, results for isolates KNN 45-1a, KNN 45-2b^{T} and KNN 45-3b and for the type strain of M.
642 versicolor.

643

644

645

646 Table 3.

647 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):							
Ampicillin (4),	-	-	+	-	-	-	+
cephaloridine (2),							
Ciprofloxacin (2)	-	-	+	-	-	+	+
Lincomycin (3)	-	-	-	-	+	-	+

649 Key: +, positive; -, negative.

Legends

Fig. 1. 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 also recovered in the maximum-likelihood and maximum-parsimony trees. Only bootstrap values above 50% are shown. Bar, 0.005 substitutions per nucleotide position.

Fig 2. 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 Modestobacter type strains. G. obscures was used as outgroup. Asterisks indicate that the corresponding branches were also recoved in the maxmum-likelihood and maximum-parsimony trees. Only bootstrap values above 50% are shown. Scale bar represents 0.02 substitutions per nucleotide position.

Fig. 3. BOX fingerprint profiles of selected Modestobacter type strains and isolates KNN 45-1a, KNN 45-2b^T and KNN 45-3b. UPGMA dendrogram based on Pearson's correlation coefficient.

Figure 1. 16S rRNA gene phylogeny

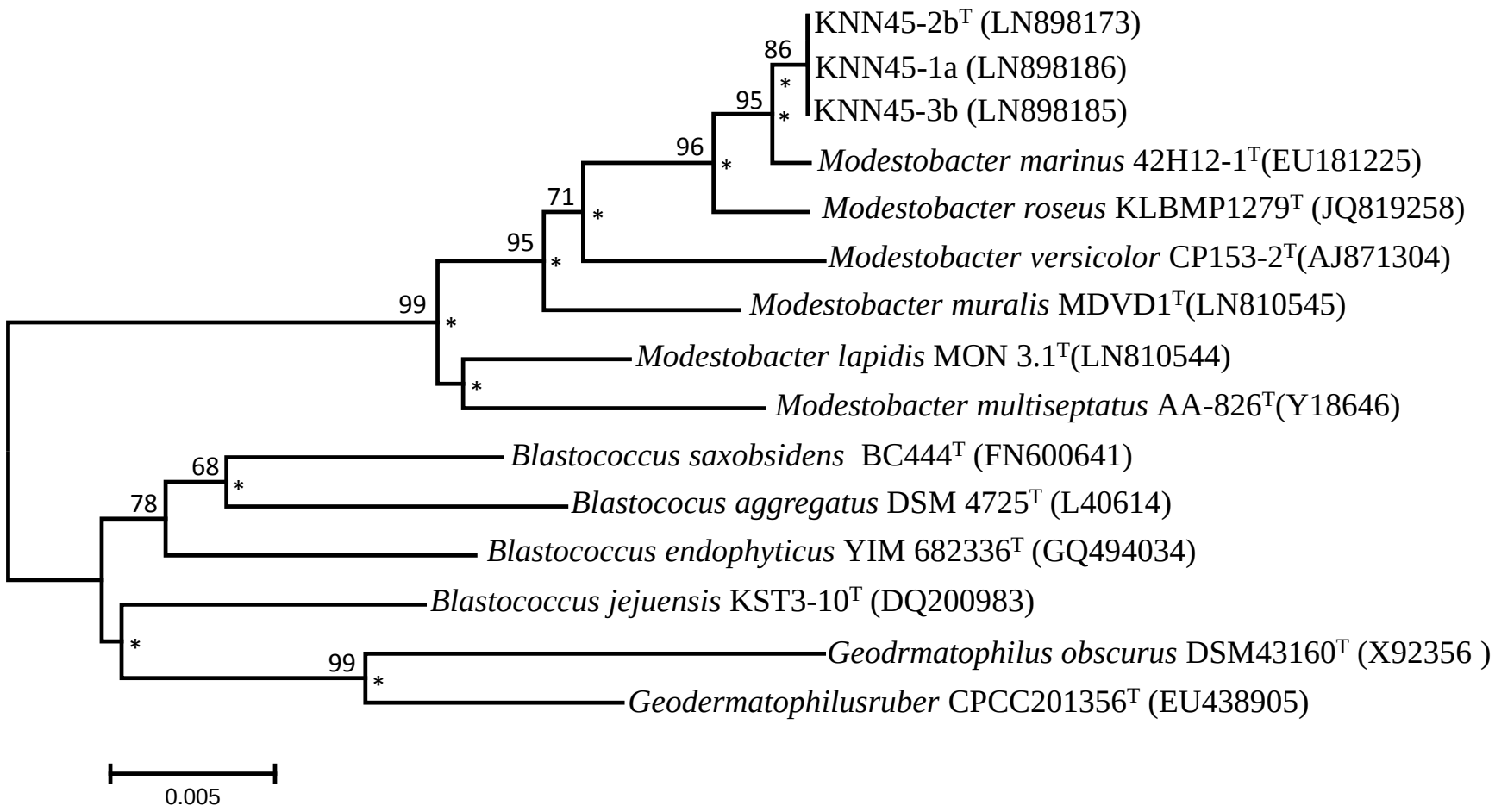
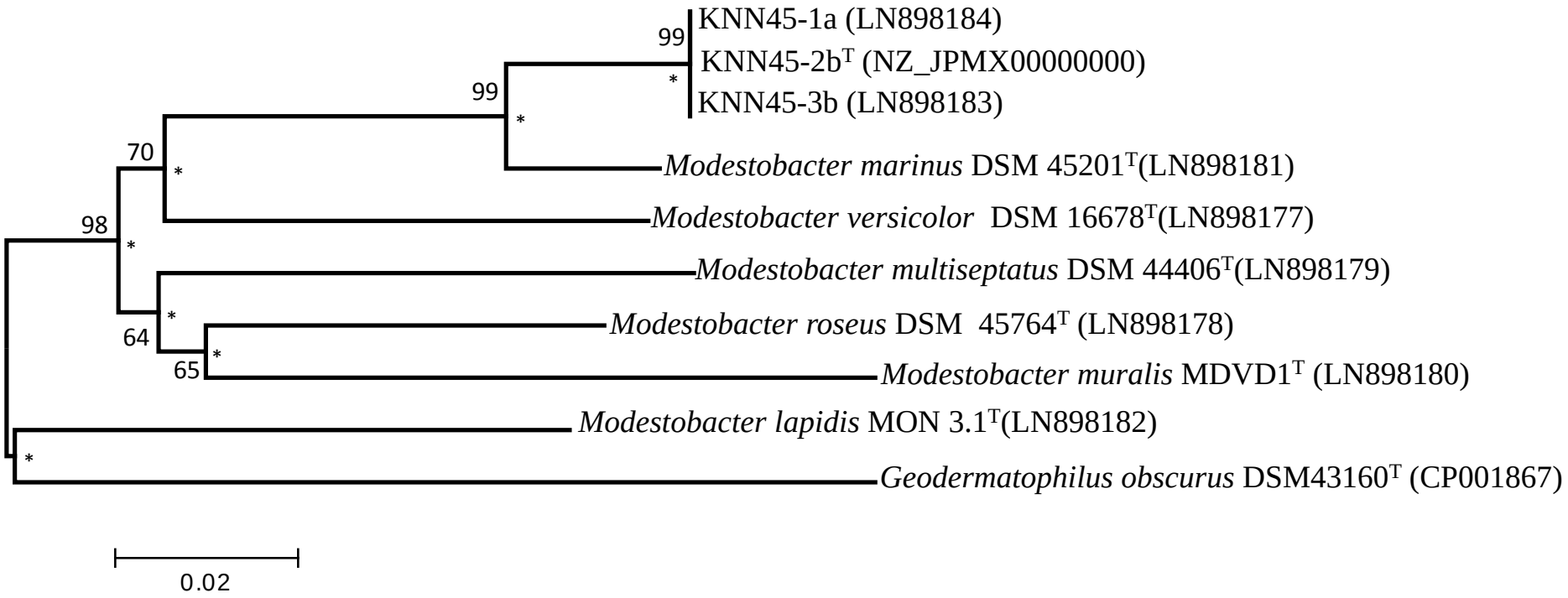


Figure 2


KNN45-1a (LN898184)
KNN45-2b^{T} (NZ_JPMX00000000)
KNN45-3b (LN898183)
Modestobacter marinus DSM 45201^{T}(LN898181)
Modestobacter versicolor DSM 16678^{T}(LN898177)
Modestobacter multiseptatus DSM 44406^{T}(LN898179)
Modestobacter roseus DSM 45764^{T} (LN898178)
Modestobacter muralis MDVD1^{T} (LN898180)
Modestobacter lapidis MON 3.1^{T}(LN898182)
Geodermatophilus obscurus DSM43160^{T} (CP001867)
99
99
70
98
64
65
0.02

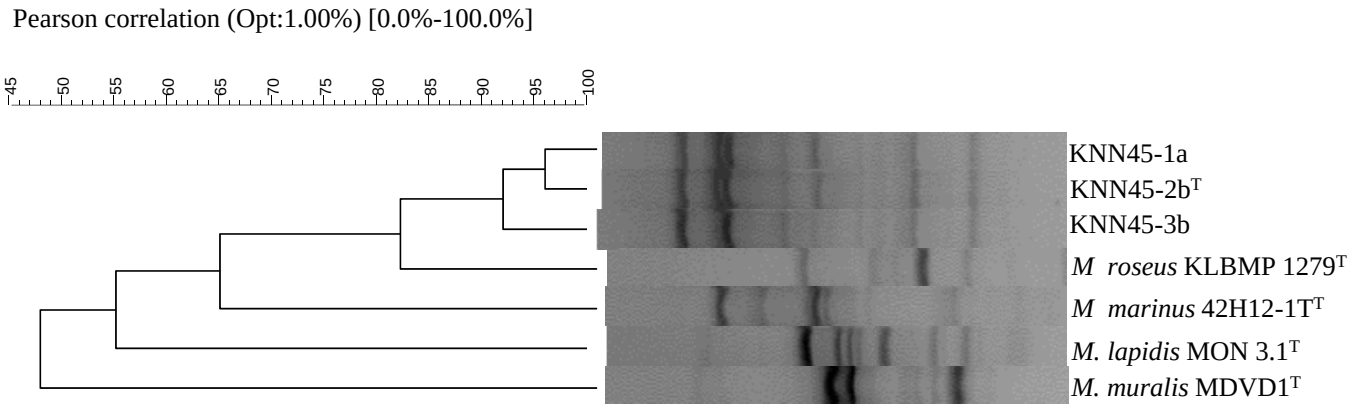
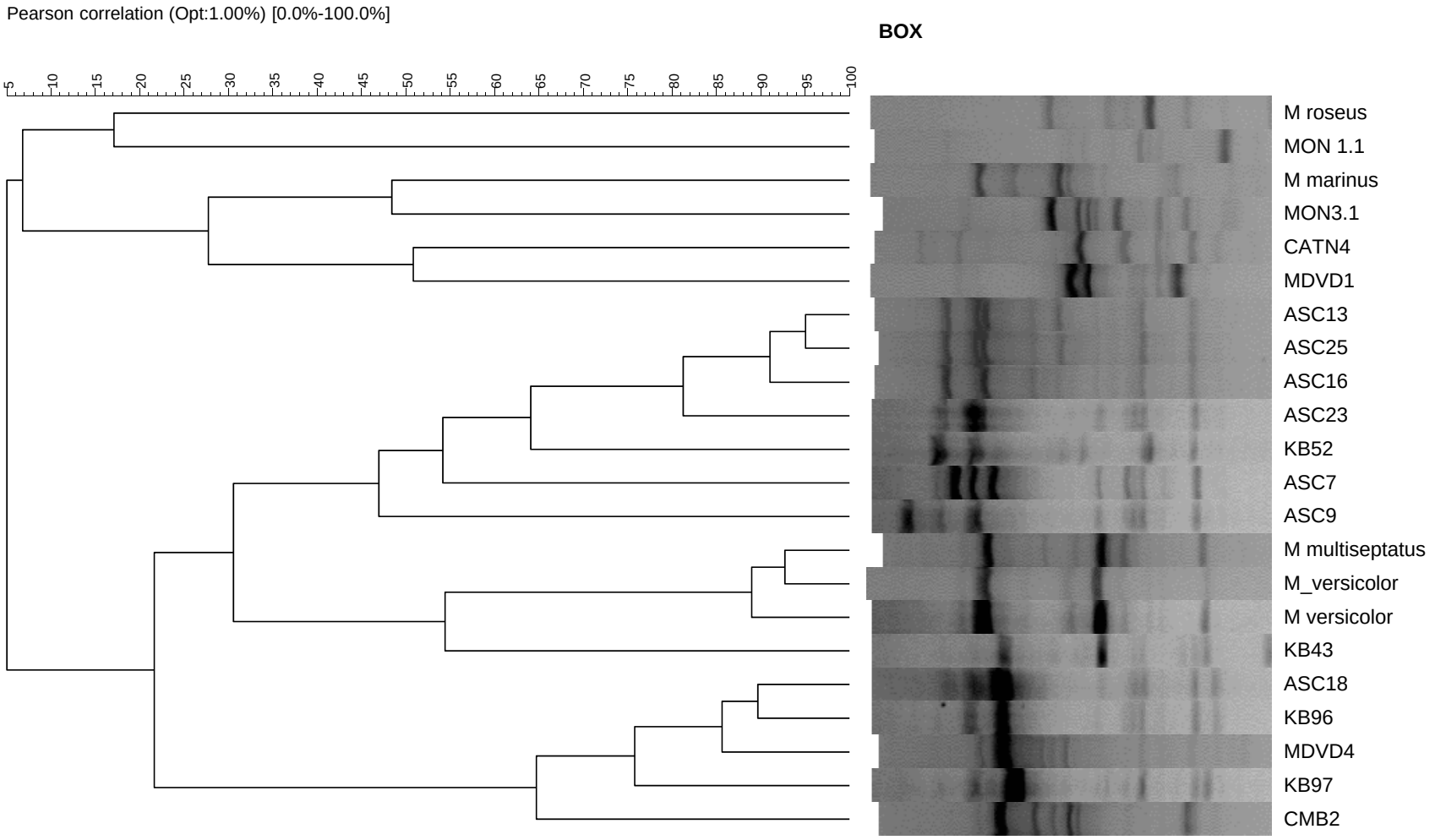


Figure SX. BOX-PCR profiles of the new isolates and related *Modestobacter* type strains.


Pearson correlation (Opt:1.00%) [0.0%-100.0%]
BOX
5
10
15
20
25
30
35
40
45
50
55
60
65
70
75
80
85
90
95
100
M roseus
MON 1.1
M marinus
MON3.1
CATN4
MDVD1
ASC13
ASC25
ASC16
ASC23
KB52
ASC7
ASC9
M multiseptatus
M_versicolor
M versicolor
KB43
ASC18
KB96
MDVD4
KB97
CMB2