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Nocardiopsis deserti sp. nov., isolated from a high altitude Atacama Desert soil

Mipeshwaree Devi Asem^{1,2†}, Nimaichand Salam^{1†}, Hamidah Idris^{3,4†}, Xiao-Tong Zhang¹, Alan T. Bull⁵, Wen-Jun Li^{1,*} and Michael Goodfellow^{3,*}

Abstract

The taxonomic status of a *Nocardiopsis* strain, designated H13^T, isolated from a high altitude Atacama Desert soil, was established by using a polyphasic approach. The strain was found to have chemotaxonomic, cultural and morphological characteristics consistent with its classification within the genus *Nocardiopsis* and formed a well-supported clade in the *Nocardiopsis* phylogenomic tree together with the type strains of *Nocardiopsis alborubida*, *Nocardiopsis dassonvillei* and *Nocardiopsis synnematoformans*. Strain H13^T was distinguished from its closest relatives by low average nucleotide identity (93.2–94.9%) and *in silico* DNA–DNA hybridization (52.5–62.4%) values calculated from draft genome assemblies and by a range of phenotypic properties. On the basis of these results, it is proposed that the isolate be assigned to the genus *Nocardiopsis* as *Nocardiopsis deserti* sp. nov. with isolate H13^T (=CGMCC 4.7585^T=KCTC 49249^T) as the type strain.

The actinobacterial genus *Nocardiopsis* [1], which belongs to the family *Nocardiopsaceae* [2] of the order *Streptosporangiales* [3], currently contains 53 validly named species (<https://www.namesforlife.com/10.1601/tx.7526>). This genus encompasses aerobic, Gram-stain-positive, non-acid-fast, non-motile, catalase-positive bacteria which form extensively branched substrate hyphae which may fragment into coccoid and bacillary elements, sparse to abundant aerial hyphae which may fragment into smooth surfaced spores; whole cell hydrolysates contain *meso*-diaminopimelic acid but lack diagnostic sugars, the muramic acid of the peptidoglycan is *N*-acetylated, the predominant menaquinones are variously hydrogenated with ten isoprene units, fatty acid profiles are rich in iso-, anteiso- and 10 methyl branched components but lack mycolic acids; major polar lipids include phosphatidylcholine, phosphatidylmethylethanolamine and phosphatidylinositol, the G+C content of genomic DNA falls within the range 64–69 mol% [4] while representative strains form a distinct clade in 16S rRNA gene trees [5, 6]. *Nocardiopsis* strains are widely distributed in the environment though the main reservoir appears

to be soil, notably alkaline [7], desert [8, 9] and hyper-saline [10, 11] soils. Some members of the genus are a source of antimicrobial and antitumour compounds [5, 12] whereas others have been implicated as opportunistic pathogens [13, 14].

During a taxonomic survey of filamentous actinobacteria present in Atacama Desert soils, an isolate was found to be closely related to *Nocardiopsis dassonvillei*, the type species of the genus. The strain, isolate H13^T, was compared with its closest phylogenetic neighbours in a polyphasic study and found to be a new species of *Nocardiopsis*, for which the name *Nocardiopsis deserti* sp. nov. is proposed.

It should be noted that Yassin *et al.* [15] established *Nocardiopsis alborubida* corrig Grund and Kroppenstedt 1990 emend Nouioui *et al.* 2018 as a later subjective synonym of *Nocardiopsis dassonvillei* Brocq-Rosseau 1904 (Approved Lists 1980) based on a wet DDH experiment. Later, this species was reclassified as a subspecies of *N. dassonvillei* by Evtushenko *et al.* [16] on the basis of having differentiating phenotypic and chemotaxonomic characteristics including MK-10 as

Author affiliations: ¹State Key Laboratory of Biocontrol and Guangdong Provincial Key Laboratory of Plant Resources, Sun Yat-sen University, Guangzhou, 510275, PR China; ²Department of Biochemistry, Manipur University, Canchipur, 795003, India; ³School of Natural and Environmental Sciences, Newcastle University, Newcastle upon Tyne NE1 7RU, UK; ⁴Biology Department, Sultan Idris Education University, 35900, Malaysia; ⁵School of Biosciences, University of Kent, Canterbury, Kent CT2 1NJ, UK.

***Correspondence:** Michael Goodfellow, michael.goodfellow@ncl.ac.uk; Wen-Jun Li, liwenjun3@mail.sysu.edu.cn

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Abbreviations: ANI, Average Nucleotide Identity; COG, Clusters of Orthologous Groups of proteins; dDDH, digital DNA–DNA Hybridization; KO, KEGG Orthology; MUSCLE, Multiple Sequence Comparison by Log-Expectation.

The whole-genome shotgun data of strain H13^T, *Nocardiopsis sinuspersici* DSM 45277^T and *Nocardiopsis quinghaiensis* YIM 28A4^T have been deposited in the DDBJ/ENA/GenBank under accession numbers VVVS000000000 and VVVT000000000 and VVVU000000000, respectively

†These authors contributed equally to this work

Two supplementary figures are available with the online version of this article.

major menaquinone; subsequently the name *Nocardiopsis dassonvillei* subsp. *albirubida* Grund and Kroppenstedt 1990 Evtushenko et al. 2000 was proposed [16]. Nouioui et al. [17] in a subsequent genome-based taxonomic study of the phylum *Actinobacteria* argued that *Nocardiopsis alborubida* corrig Grund and Kroppenstedt 1990. emend Nouioui et al. 2018 should not be considered as a heterotypic synonym of *Nocardiopsis dassonvillei* and proposed to adopt the taxonomy of Grund and Kroppenstedt [18].

Isolate H13^T was recovered from a wet gravelly, subsurface soil sample (30 cm depth) collected by one of us (MG) at 5046 metres above sea level on Carro Chajnantor (23° 00'49" S, 67° 45'31" W), east of San Pedro de Atacama, Chile, using the dilution plate procedure described by Okoro et al. [19]. The strain was isolated on oligotrophic agar [20] supplemented with cycloheximide and nystatin (each of 25 µg ml⁻¹) following incubation at 28 °C for 3 weeks; the isolation plate had been dried in a laminar flow hood prior to isolation, as recommended by Vickers and Williams [21]. Primary characterization of the isolate by sequencing a 16S rRNA amplicon indicated that it was closely related to the type strains of *Nocardiopsis alborubida* [17, 18], *Nocardiopsis dassonvillei* [1, 22] and *Nocardiopsis synnematoformans* [15]. Working cultures of the isolate were maintained on yeast extract – malt extract agar (International *Streptomyces* Project (ISP) medium 2 [23]) slopes and for long-term storage as lyophilized cells.

The isolate was examined for chemotaxonomic, cultural and morphological properties found to be of value in the systematics of *Nocardiopsis* [4]. Spore chain arrangement and spore-surface ornamentation were sought following growth on trypticase soy agar (TSA) after 10 days at 28 °C using a scanning electron microscope (Quanta 400FEG, FEI) as previously described [24]. Chemical features of isolate H13^T were determined using standard procedures, as exemplified by the detection of diaminopimelic acid isomers and whole cell sugars [25] and cell wall muramic acid type [26]. Biomass for the remaining chemotaxonomic studies was harvested from trypticase soy broth (TSB) cultures after 10 days at 28 °C washed in distilled water and freeze dried. Isoprenoid quinones extracted after Collins et al. [27] were analysed by HPLC [28], mycolic acids were extracted and analysed using the procedure described by Minnikin et al. [29] while extracted polar lipids were identified by two-dimensional chromatography on silica gel plates, as described previously [30, 31]. Fatty acids were extracted from TSA cultures after 10 days at 28 °C (i.e. at late logarithmic phase), methylated and analysed using the protocol of the Sherlock Microbial Identification (MIDI) system, version 6.1 and the resultant peaks identified using the TSBA6 database [32].

Strain H13^T formed a yellowish white substrate mycelium and an abundant white aerial spore mass but no diffusible pigments on oatmeal agar. Aerial hyphae differentiate into *rectiflexibiles* spore chains (Fig. 1). Whole-organism hydrolysates of the isolate contained *meso*-diaminopimelic acid, arabinose and galactose, muramic acid moieties were *N*-acetylated, the menaquinone profile consisted of MK-7 (37%), MK-6

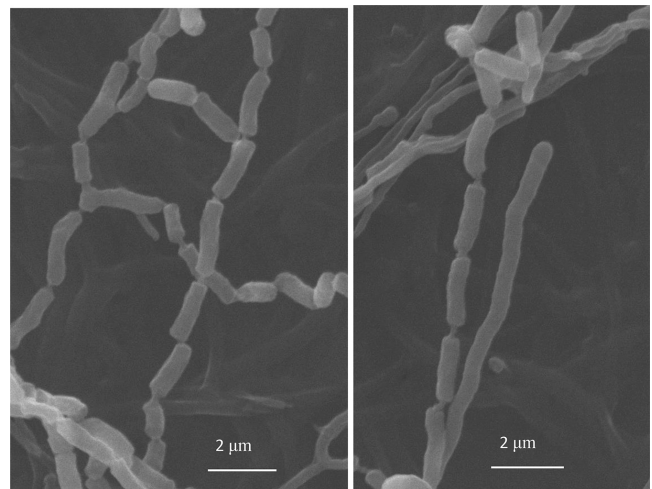


Fig. 1. Scanning electron micrographs of isolate H13^T from cultures grown on trypticase soy agar for 10 days at 28 °C showing Bars, 2 µm.

(35%), MK-10 (11%) and MK-9(H₈) (10%) while the polar lipid pattern showed the presence of diphosphatidylglycerol, phosphatidylcholine, phosphatidylinositol, phosphatidylmethylethanolamine and an unidentified phospholipid (Fig. S1, available in the online version of this article), mycolic acids were not detected. The fatty acid profile was composed of iso-C_{16:0} (28.2%), C_{18:0} 10-methyl TBSA (20.1%), anteiso-C_{17:0} (16.1%), iso-C_{18:0} (6.9%), C_{18:0} (6.7%), C_{17:0} 10-methyl (5.8%), C_{12:0} (2.4%), C_{18:1} ω8c (1.6%), C_{17:0} (1.6%), summed feature 9 (C_{16:0} 10-methyl and/or iso-C_{17:1} ω9c, 1.5%), C_{16:0} (1.5%), iso-C_{14:0} (1.1%), anteiso-C_{15:0} (1.1%) and minor contents (<1%) of iso-C_{10:0}, iso-C_{12:0}, anteiso-C_{13:0}, iso-C_{15:0}, C_{14:0} 2OH, iso-C_{17:0}, C_{17:1} ω8c, anteiso-C_{19:0}, summed feature 7 (C_{19:1} ω7c and/or C_{19:1} ω6c), C_{19:0}, C_{18:1} 2OH and iso-C_{20:0}.

Growth at 4, 15, 20, 28, 37, 45, 55, 60 and 65 °C and from pH 4 to 13.0 (at intervals of 1.0 pH unit) were observed by culturing in TSB for 7 and 14 days; the pH of the media were maintained as described by Asem et al. [33]. Salt tolerance was observed by supplementing TSA with up to 20% (w/v) NaCl. Gram-reaction was tested by using a Gram Stain Solution Kit (Shanghai Yeasen Biotechnology), and verified by a non-staining method [34] and motility checked by inoculating the strain in a tube containing a semi-solid medium [35]. Oxidase activity was determined by assessing the oxidation of 1% (w/v) tetramethyl-*p*-phenylenediamine [36] and catalase activity by looking for the formation of bubbles on addition of a drop of 3% (v/v) H₂O₂ to colonies. Degradation of cellulose, gelatin, starch and Tweens (20, 40, 60 and 80), urea hydrolysis, H₂S production, milk coagulation and peptonization, and nitrate reduction were determined as previously described [37, 38]. Utilization of sole nitrogen sources was determined as described by Li et al. [39]. The remaining phenotypic characteristics were determined using API ZYM (bioMérieux) and API 20NE (bioMérieux) kits and the GEN III Micro-Plate (Biolog) system in each case following the instructions provided by the manufacturers.

Isolate H13^T was non-motile, grew from 15–37 °C (optimally at 28 °C), from pH 7–9 (optimally at, pH 7) and in the presence of up to 12% NaCl (w/v). It tested positive for catalase activity, but was negative for the oxidase, H₂S production, milk coagulation and peptonization, and nitrate reduction tests. The isolate hydrolysed starch and urea but was negative for cellulose, gelatin and Tweens 20, 40, 60, 80 degradation; L-alanine, L-arginine, L-asparagine, L-methionine and potassium nitrate were used as sole nitrogen sources.

Genomic DNA was extracted from harvested biomass (cultivated in TSB for 7 days at 28 °C) of isolate H13^T and sequenced at Novogene Co. Ltd. (Beijing, China) using a HiSeq PE150 sequencer (Illumina, San Diego, CA, USA). The reads from each of the data sets were filtered, and high quality paired-end reads assembled into contigs using SPADES [40] and the completeness, any contamination of the genomes checked by using CheckM [41]. The G+C content (mol%) was calculated from the genome sequence. Coding sequences (CDS) of genome scaffolds over 500 bp were predicted using Prodigal [42] with default settings. Predicted CDS were annotated using KOALA (KEGG Orthology and Links Annotation) [43], HMMSCAN online version (<https://www.ebi.ac.uk/Tools/hmmer/search/hmmscan>) [44] and eggno-mapper v1 online server [45]. The 16S rRNA gene sequence of isolate H13^T was retrieved from the assembled draft genome using RNAmmer [46] and pairwise sequence comparison performed using the EzBioCloud server [47]. A 16S rRNA gene-based phylogeny was generated using the neighbour joining method [48] in the MEGA X program [49], following alignments of the 16S rRNA gene sequences using CLUSTAL X v.2.1 [50]. Evolutionary distances were calculated by the Kimura two-parameter model [51] and the topology of the tree was evaluated by bootstrap analysis of 1000 replications [52].

The phylogenomic tree was based on 30 protein marker genes (*dnaG*, *frr*, *infC*, *nusA*, *pyrG*, *rplA*, *rplB*, *rplC*, *rplD*, *rplE*, *rplF*, *rplK*, *rplL*, *rplM*, *rplN*, *rplP*, *rplS*, *rplT*, *rpmA*, *rpoB*, *rpsC*, *rpsB*, *rpsE*, *rpsI*, *rpsJ*, *rpsK*, *rpsM*, *rpsS*, *smfB*, *tsf*) selected from the AMPHORA2 database [53]. The marker genes were extracted from the draft genomes of strain H13^T and from available genomes of *Nocardiopsis* type strains. The sequences of each of the marker genes were aligned separately using MUSCLE [54] and poorly aligned regions removed from the datasets using Gblocks [55]. Cleaned alignments were concatenated by using perl script (<https://github.com/nylander/catfasta2phymml>), prior to generation of the phylogenomic tree using the RAxML method [56] with the default parameters, and visualized using the online Tree of Life program v.4.2 [57]. For further investigation of phylogenetic relationships, Average Nucleotide Identity (ANI) values between the draft genome of isolate H13^T and those of the type strains of its three closest phylogenomic neighbours were calculated using online JSpeciesWS software (<http://jspecies.ribohost.com/jspeciesws/>) [58], and digital DNA–DNA hybridization (dDDH) analysis using the DSMZ Genome-to-Genome Distance Calculator platform (<http://ggdc.dsmz.de/distcalc2.php>) [59].

The draft assembly of the genome sequence of strain H13^T (GenBank accession number: VWVS00000000) was composed of 59 contigs with a total genome size of 7251616 bp and with a N₅₀ length of 398233 bp. The checkM analysis indicated that the genome completeness was 100% with little contamination (0.55%) and no strain heterogeneity. The digital DNA G+C content of strain H13^T is 71.94 mol%. The number of predicted genes was 6236 with a total length of 5949129 bp (82% of the total genome). The number of CDS assigned to the KO, Pfam and COG databases were 2282, 4937 and 4857, respectively. Isolate H13^T showed high 16S rRNA gene sequence identities to *Nocardiopsis dassonvillei* DSM 43111^T (99.66%), *Nocardiopsis alborubida* NBRC 13392^T (99.38%), *Nocardiopsis synnematoformans* DSM 44143^T (99.31%), *Nocardiopsis lucentensis* DSM 44048^T (98.83%) and *Nocardiopsis aegyptia* DSM 44442^T (98.76%), but less than 98.65% similarities to other members of the genus *Nocardiopsis*. It can be seen from Fig. 2 that isolate H13^T formed a well-supported phyletic line in the *Nocardiopsis* phylogenomic tree together with the type strains of *N. alborubida*, *N. dassonvillei* and *N. synnematoformans* [1, 15, 17, 18, 22]. A similar relationship is also shown with the above three type strains in the 16S rRNA gene tree for isolate H13^T (Fig. S2). It can be seen from Table 1 that isolate H13^T shares dDDH similarities with its closest phylogenomic neighbours that are well below the 70% cut-off point recommended for the circumscription of prokaryotic species [60]. In turn, the ANI values are below the 95–96% threshold used to assign closely related strains to the same species [61–63].

It can be seen from Table 2 that a broad range of phenotypic properties can be weighted to distinguish between isolate H13^T and its closest phylogenomic neighbours though all of these strains shared common features. In particular, the isolate can be distinguished from the type strain of *N. alborubida*, its nearest phylogenomic neighbour (see Table 1), by a range of properties, as exemplified by its ability to hydrolyse urea, utilize D-arabitol, D-galactose, D-sorbitol, L-alanine, citric acid and α -keto-glutaric acid as sole carbon sources and its ability to grow at 15 °C. In contrast, the *N. alborubida* strain unlike the isolate, reduces nitrate, uses myo-inositol, D-salicin, sucrose and L-lactic acid as sole carbon sources and shows much greater activity in the API and/or Biolog GEN III tests. Similar combinations of phenotypic properties distinguish the isolate from the type strains of *N. dassonvillei* and *N. synnematoformans*. In turn, many phenotypic features can be used to separate the type strains of *N. alborubida* and *N. dassonvillei*, as illustrated by the ability of the *N. dassonvillei* strain to hydrolyse aesculin and metabolise a much broader range of carbon sources, as shown in Table 2.

It can be concluded from the chemotaxonomic, morphological and phylogenomic data that isolate H13^T is a *bona fide* member of the genus *Nocardiopsis*. The isolate can be distinguished from the type strains of its three closest phylogenetic neighbours using a combination of phenotypic properties and by low ANI and dDDH values. Consequently, it is proposed that isolate H13^T represents a new *Nocardiopsis* species. The

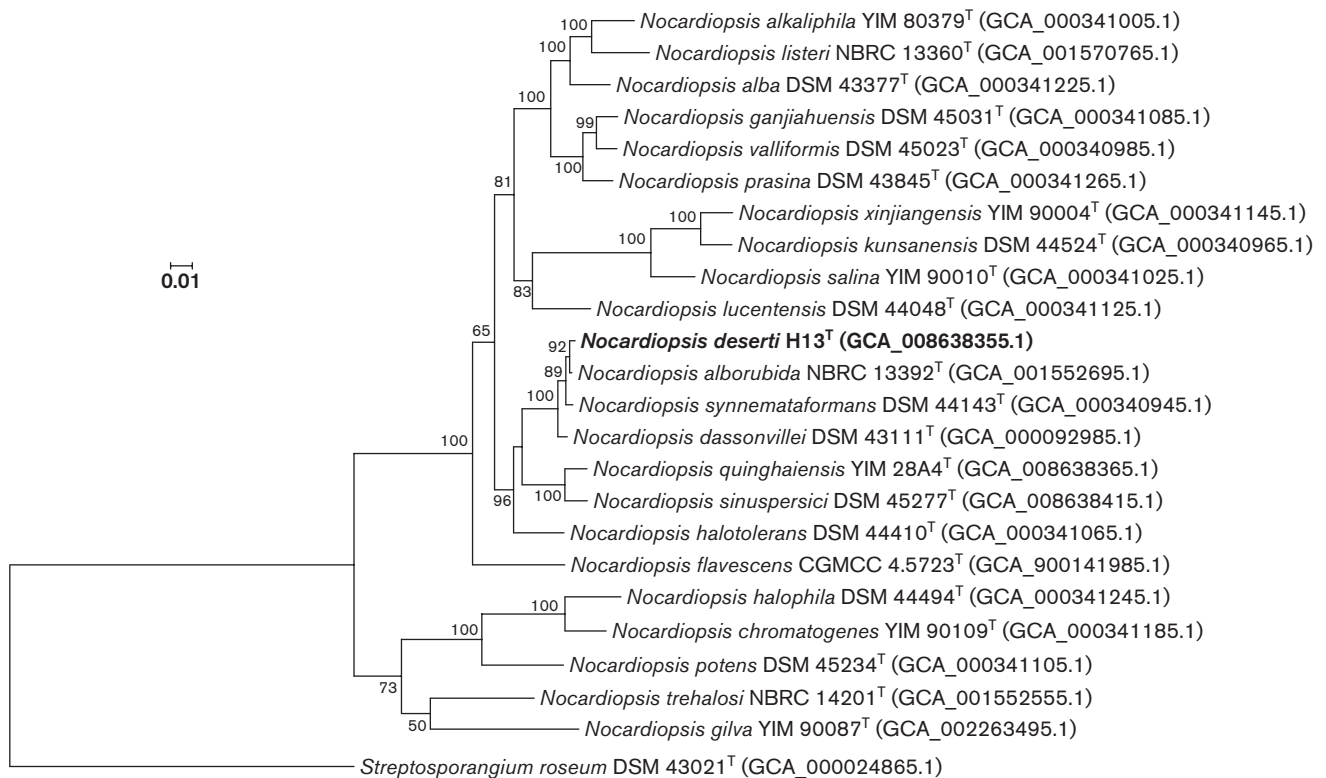


Fig. 2. RAxML phylogenomic tree showing the relationships between strain H13^T and related type strains of the genus *Nocardioopsis*. Values at the nodes are bootstrap supports (%). *Streptosporangium roseum* DSM 43021^T (GCA_000024865.1) was used as the outgroup. Bar, 0.01 substitutions per nucleotide position.

name chosen for this new species is *Nocardioopsis deserti* sp. nov.

Further, it is established from the distinct lineage in the genome based tree (Fig. 2) and lower overall genome relatedness indices (Table 1) that *N. alborubida* should not be considered as a synonym of *N. dassonvillei* as proposed by Yassin *et al.* [15]; a result consistent with the view of Nouioui *et al.* [17] to adopt the taxonomy of Grund and Kroppenstedt [18]. This study therefore adopts the name *Nocardioopsis alborubida*

corrig Grund and Kroppenstedt 1990. emend. Nouioui *et al.* 2018.

DESCRIPTION OF *NOCARDIOPSIS DESERTI* SP. NOV.

Nocardioopsis deserti (de.ser'ti. L. gen. n. *deserti* of or pertaining to a desert, thereby referring to the source of the isolate).

Aerobic, Gram-stain-positive, non-acid-alcohol fast, catalase-positive actinobacterium which forms a yellowish white substrate mycelium and an abundant aerial spore mass but no diffusible pigments on oatmeal agar. Long branched substrate hyphae fragment into irregular rods. Aerial hyphae differentiate into long chains of irregular spores (0.3×1.5–1.8 μm) with smooth surfaces. Growth occurs at 15–37 °C, pH 7–9 and in the presence of up to 12% NaCl. Optimal growth at 28 °C, pH 7 and in the absence of NaCl. Hydrolyses starch and urea, but not cellulose, gelatin, or Tweens. Negative for milk coagulation and peptonization, H₂S production and nitrate reduction tests. Assimilates *N*-acetyl glucosamine, *D*-glucose, malic acid, *D*-maltose, *D*-mannose and *D*-mannitol, but not *L*-arabinose, capric acid or trisodium citrate (API 20NE tests). Positive activities for alkaline phosphatase, esterase lipase (C8) and naphthol-AS-BI-phosphohydrolase but is negative for cystine arylamidase, α -fucosidase, α - and β -galactosidase,

Table 1. Comparison of the overall genomic relatedness indices of the genome of isolate H13^T and those of three closely related type strains classified in the genus *Nocardioopsis*

Taxa: 1, Isolate H13^T (GCA_008638355.1); 2, *N. synnemataformans* DSM 44143^T (GCA_000340945.1); 3, *N. alborubida* NBRC 13392^T (GCA_001552695.1); 4, *N. dassonvillei* DSM 43111^T (GCA_000092985.1).

dDDH	1	2	3	4
ANI				
1	–	58.9	62.4	52.5
2	94.08	–	58.1	53.1
3	94.87	93.95	–	53.4
4	93.22	93.63	93.54	–

Table 2. Phenotypic properties that distinguish isolate H13^T from its closest phylogenomic neighbours

1, Isolate H13^T; 2, *Nocardiopsis dassonvillei* KCTC 9190^T; 3, *Nocardiopsis alborubida* CGMCC 4.2086^T; 4, *Nocardiopsis synnematoformans* CGMCC 4.2091. +, positive; w, weak positive; -, negative.

Characteristics	1	2	3	4
Growth physiology				
Temperature range (optimum, °C)	15–37 (28)	28–37	28–37 (28)	28–37 (28)
pH range (optimum)	7–9 (7)	6–9 (7)	7–10 (7)	7–9(7)
NaCl requirement	0–12	0–13	0–13	0–12
Milk coagulation	–	+	w	w
Milk peptonization	–	+	+	w
Nitrate reduction	–	+	+	–
Hydrolysis of:				
Starch	+	+	+	–
Urea	+	–	–	–
API ZYM tests:				
Acid phosphatase	–	+	w	w
Alkaline phosphatase	+	+	w	+
α-Chymotrypsin	–	+	w	–
Esterase (C4)	–	+	w	+
Esterase lipase (C8)	+	+	w	–
α-Glucosidase	–	w	+	–
β-Glucosidase	–	+	+	–
Leucine arylamidase	–	+	+	w
Lipase (C14)	–	+	w	+
Valine arylamidase	–	+	w	w
API 20NE tests:				
β-Galactosidase	–	+	+	–
β-Glucosidase (aesculin hydrolysis)	–	+	–	–
Protease (gelatin hydrolysis)	–	–	w	+
Urease	+	–	–	–
Assimilation of:				
Phenylacetic acid	–	+	+	–
L-Arabinose	–	–	+	–
N-acetyl Glucosamine	+	–	–	–
D-Glucose	+	–	–	+
D-Maltose	+	–	+	+
D-Mannose	+	–	–	–
Potassium gluconate	+	–	+	+
Trisodium citrate	–	+	+	+
BIOLOG GEN III				

Continued

Table 2. Continued

Characteristics	1	2	3	4
Carbon source utilization assay:				
L-Aspartic acid	-	-	-	+
β -hydroxy-D,L-Butyric acid	-	+	-	+
Citric acid	+	-	-	-
Formic acid	-	+	-	-
L-Galactonic acid lactone	-	-	-	+
L-Pyroglutamic acid	+	+	+	-
α -keto-Glutamic acid	+	-	-	+
L-Lactic acid	-	+	+	-
D-Lactic acid methyl ester	-	-	-	+
L-Malic acid	-	+	+	-
p-Hydroxy-phenylacetic acid	+	+	-	-
D-Saccharic acid	+	+	+	-
D-Arabitol	+	+	-	+
Dextrin	+	+	-	+
D-Fructose	+	+	-	+
D-Fructose-6-PO ₄	-	-	-	+
D-Galactose	+	+	-	-
N-acetyl-D-Galactosamine	-	-	-	+
Gentiobiose	+	+	+	-
N-acetyl-D-Glucosamine	-	-	-	+
α -D-Glucose	+	+	-	-
D-Glucose-6-PO ₄	-	-	-	+
Glycerol	+	-	+	+
Myo-inositol	-	-	+	-
D-Maltose	+	+	-	+
D-Mannose	+	+	+	-
N-acetyl- β -D-Mannosamine	-	-	-	+
D-Melibiose	-	-	-	+
Pectin	+	-	-	-
D-Raffinose	-	-	-	+
L-Rhamnose	+	+	+	-
D-Salicin	-	+	+	+
D-Sorbitol	+	+	-	+
Sucrose	-	+	+	-
D-Trehalose	-	-	-	+
D-Turanose	-	+	-	+

Continued

Table 2. Continued

Characteristics	1	2	3	4
L-Alanine	+	+	-	+
L-Arginine	+	+	-	+
Glycyl-L-proline	+	+	-	+
L-Histidine	+	-	+	-
L-Serine	+	-	-	+
Inosine	+	+	+	-
Chemical sensitivity assay:				
Potassium tellurite	+	-	-	-
Aztreonam	-	-	-	+
Lincomycin	+	-	-	-
Nalidixic acid	+	-	-	+
G+C content (mol%)	71.9	72.7*	72.0*	72.3*

*Data from EzBioCloud database.

N-acetyl- β -glucosaminidase, α - and β -glucosidase, α -mannosidase and trypsin (API ZYM tests). Acetic acid, acetoacetic acid, citric acid, D-gluconic acid, L-glutamic acid, L-pyroglytamic acid, α -keto-glutaric acid, D-malic acid and D-saccharic acid are oxidized but not D- or L-aspartic acid, γ -amino-butyric acid, β -hydroxy-D,L-butyric acid, α -keto-butyric acid, formic acid, D-galacturonic acid, D-glucuronic acid, L-lactic acid, mucic acid, *N*-acetyl-neuraminic acid, propionic acid, quinic acid or bromo-succinic acid (organic acids). D-Arabitol, D-cellobiose, dextrin, D-fructose, D-galactose, gentiobiose, α -D-glucose, β -methyl-D-glucoside, glycerol, inosine, lactose, D-maltose, D-mannitol, D-mannose, L-rhamnose and D-sorbitol are metabolized but not D- or L-fucose, *N*-acetyl-D-galactosamine, 3-methyl-glucose, *N*-acetyl-D-glucosamine, glucuronamide, *myo*-inositol, *N*-acetyl- β -D-mannosamine, D-melibiose, methyl-pyruvate, D-raffinose, D-salicin, stachyose, sucrose, D-trehalose or D-turanose (sugars). Degrades pectin (polymer) but not Tween 40. Grows in the presence of lithium chloride, sodium butyrate, sodium lactate (1 %, w/v) and tetrazolium blue but is inhibited by sodium bromate and tetrazolium violet. Sensitive to fusidic acid, guanidine hydrochloride, rifamycin SV, troleandomycin and vancomycin. Whole-organism hydrolysates contain *meso*-diaminopimelic acid, arabinose and galactose, muramic acid moieties are *N*-acetylated, the predominant fatty acids are iso- $C_{16:0}$, $C_{18:0}$ 10-methyl TSBA and anteiso- $C_{17:0}$ and the polar lipid profile contains diphosphatidylglycerol, phosphatidylcholine, phosphatidylinositol, phosphatidyl-methylethanolamine and an unidentified phospholipid. The genome size is 7.25 Mb and the *in silico* DNA G+C content is 71.9 mol%.

The type strain, H13^T (=CGMCC 4.7585=KCTC 49249), was isolated from a high altitude Atacama Desert soil in Chile. The

DDBJ/ENA/GenBank accession number of the whole genome sequence of strain H13^T is VWVS000000000.

This is the first classification of a *Nocardiopsis* species isolated from an Atacama Desert soil though isolates with very similar colonial properties to isolate H13^T have been isolated from other high altitude soils collected from Carro Chajnantor, as have members of a putatively novel species related to *Nocardiopsis trehalosi* [64]. Idris also found that isolate H13^T inhibited the growth of several *Bacillus subtilis* reporter strains, including ones detecting the inhibition of cell envelope and cell wall synthesis. These preliminary data suggest that representatives of novel *Nocardiopsis* strains, like member of other genera isolated from Atacama Desert soils and shown to have large genome (*sensu* Baltz 2017 [65]), should feature in bioprospecting campaigns [66].

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Conflicts of interest

The authors declare that they have no conflict of interest.

References

- Meyer J. *Nocardiopsis*, a new genus of the order Actinomycetales. *Int J Syst Bacteriol* 1976;26:487–493.
- Rainey FA, Ward-Rainey N, Kroppenstedt RM, Stackebrandt E. The genus *Nocardiopsis* represents a phylogenetically coherent taxon and a distinct actinomycete lineage: Proposal of *Nocardiopsaceae* fam. nov. *Int J Syst Bacteriol* 1996;46:1088–1092.
- Goodfellow M, Order XV *Streptosporangiales* ord. nov. In: Goodfellow M, Kämpfer P, Busse H-J, Trujillo ME, Suzuki K et al. (editors). *Bergey's Manual of Systematic Bacteriology, 2nd edition: The Actinobacteria Part A and B*. Springer; 2012. pp. 1805–1806.
- Hozzein WN, Trujillo ME. *Nocardiopsis*. In: Goodfellow M, Kämpfer P, Busse H-J, Trujillo ME, Suzuki K et al. (editors). *Bergey's Manual of Systematics Bacteriology 2nd Edition: The Actinobacteria, Part A and B*. Springer; 2012. pp. 1891–1906.
- Bennur T, Kumar AR, Zinjarde S, Javdekar V. *Nocardiopsis* species: Incidence, ecological roles and adaptations. *Microbiol Res* 2015;174:33–47.
- Bouras N, Meklat A, Zitouni A, Mathieu F, Schumann P et al. *Nocardiopsis algeriensis* sp. nov., an alkalitolerant actinomycete isolated from Saharan soil. *Antonie van Leeuwenhoek* 2015;107:313–320.
- Jiang C-L, L-H X. Actinomycete diversity in unusual habitats. In: Jiang C-L, L-H Xu (editors). *Actinomycetes Research*. Yunnan: Yunnan University Press; 1993. pp. 259–270.
- Zitouni A, Boudjella H, Lamari L, Badji B, Mathieu F et al. *Nocardiopsis* and *Saccharothrix* genera in Saharan soils in Algeria: isolation, biological activities and partial characterization of antibiotics. *Res Microbiol* 2005;156:984–993.
- Meklat A, Sabaou N, Zitouni A, Mathieu F, Lebrihi A. Isolation, taxonomy, and antagonistic properties of halophilic actinomycetes in Saharan soils of Algeria. *Appl Environ Microbiol* 2011;77:6710–6714.
- Li W-J, Kroppenstedt RM, Wang D, Tang S-K, Lee J-C et al. Five novel species of the genus *Nocardiopsis* isolated from hypersaline soils and emended description of *Nocardiopsis salina* Li et al. 2004. *Int J Syst Evol Microbiol* 2006;56:1089–1096.
- Hamedi J, Mohammadipanah F, Ventosa A. Systematic and biotechnological aspects of halophilic and halotolerant actinomycetes. *Extremophiles* 2013;17:1–13.
- Bennur T, Ravi Kumar A, Zinjarde SS, Javdekar V. *Nocardiopsis* species: a potential source of bioactive compounds. *J Appl Microbiol* 2016;120:1–16.
- Ajello L, Brown J, Macdonald E, Head E. Actinomycetoma caused by *Nocardiopsis dassonvillei*. *Arch Dermatol* 1987;123:426.
- Mordarska H, Zakrzewska-Czerwińska J, Paściak M, Szponar B, Rowiński S, Rare RS. Rare, suppurative pulmonary infection caused by *Nocardiopsis dassonvillei* recognized by glycolipid markers. *FEMS Immun Med Microbiol* 1998;21:47–55.
- Yassin AF, Rainey FA, Burghardt J, Gierth D, Ungerechts J et al. Description of *Nocardiopsis synnemataformans* sp. nov., elevation of *Nocardiopsis alba* subsp. *prasina* to *Nocardiopsis prasina* comb. nov., and designation of *Nocardiopsis antarctica* and *Nocardiopsis alborubida* as later subjective synonyms of *Nocardiopsis dassonvillei*. *Int J Syst Bacteriol* 1997;47:983–988.
- Evtushenko LI, Taran VV, Akimov VN, Kroppenstedt RM, Tiedje JM et al. *Nocardiopsis tropica* sp. nov., *Nocardiopsis trehalosi* sp. nov., nom. rev. and *Nocardiopsis dassonvillei* subsp. *albirubida* subsp. nov., comb. nov. *Int J Syst Evol Microbiol* 2000;50:73–81.
- Nouioui I, Carro L, García-López M, Meier-Kolthoff JP, Woyke T et al. Genome-based taxonomic classification of the phylum Actinobacteria. *Front Microbiol* 2018;9:2007.
- Grund E, Kroppenstedt RM. Chemotaxonomy and numerical taxonomy of the genus *Nocardiopsis* Meyer 1976. *Int J Syst Bacteriol* 1990;40:5–11.
- Okoro CK, Brown R, Jones AL, Andrews BA, Asenjo JA et al. Diversity of culturable actinomycetes in hyper-arid soils of the Atacama desert, Chile. *Antonie van Leeuwenhoek* 2009;95:121–133.
- Senechkin IV, Speksnijder AGCL, Semenov AM, van Bruggen AHC, van Overbeek LS. Isolation and partial characterization of bacterial strains on low organic carbon medium from soils fertilized with different organic amendments. *Microb Ecol* 2010;60:829–839.
- Vickers JC, Williams ST. An assessment of plate inoculation procedures for the enumeration and isolation of soil streptomycetes. *Microbios Lett* 1987;35:113–117.
- Brocq-Rousseu D. Sur un *Streptothrix*. *Rev Gen Bot* 1904;16:219–230.
- Shirling EB, Gottlieb D. Methods for characterization of *Streptomyces* species. *Int J Syst Bacteriol* 1966;16:313–340.
- Ming H, Yin Y-R, Li S, Nie G-X, Yu T-T et al. *Thermus caliditerrae* sp. nov., a novel thermophilic species isolated from a geothermal area. *Int J Syst Evol Microbiol* 2014;64:650–656.
- Staneck JL, Roberts GD. Simplified approach to identification of aerobic actinomycetes by thin-layer chromatography. *Appl Microbiol* 1974;28:226–231.
- Uchida K, Kudo T, Suzuki K-I, Nakase T. A new rapid method of glycolate test by diethyl ether extraction, which is applicable to a small amount of bacterial cells of less than one milligram. *J Gen Appl Microbiol* 1999;45:49–56.
- Collins MD, Pirouz T, Goodfellow M, Minnikin DE. Distribution of menaquinones in actinomycetes and corynebacteria. *J Gen Microbiol* 1977;100:221–230.
- Tamaoka J. Analysis of bacterial menaquinone mixtures by reverse-phase high-performance liquid chromatography. *Met Enzymol* 1986;123:251–256.
- Minnikin DE, Hutchinson IG, Caldicott AB, Goodfellow M. Thin-Layer chromatography of methanolysates of mycolic acid-containing bacteria. *J Chromatogr A* 1980;188:221–233.
- Collins MD, Jones D. Lipids in the classification and identification of coryneform bacteria containing peptidoglycans based on 2, 4-diaminobutyric acid. *J Appl Bacteriol* 1980;48:459–470.
- Minnikin DE, Collins MD, Goodfellow M. Fatty acid and polar lipid composition in the classification of *Cellulomonas*, *Oerskovia* and related taxa. *J Appl Bacteriol* 1979;47:87–95.
- Sasser M. Identification of bacteria by gas chromatography of cellular fatty acids [database on the Internet]. http://www.microbialid.com/PDF/TechNote_101.pdf 2001.
- Asem MD, Salam N, Zheng W, Liao L-H, Zhang X-T et al. *Vitreimonas flagellata* gen. nov., sp. nov., a novel member of the family *Hyphomonadaceae* isolated from an activated sludge sample. *Int J Syst Evol Microbiol* 2020 [Epub ahead of print 05 Mar 2020].
- Buck JD. Nonstaining (KOH) method for determination of gram reactions of marine bacteria. *Appl Environ Microbiol* 1982;44:992–993.
- Leifson E. *Atlas of Bacterial Flagellation*. New York: Academic Press; 1960.
- Kovacs N. Identification of *Pseudomonas pyocyanea* by the oxidase reaction. *Nature* 1956;178:703–704.
- Gonzalez C, Gutierrez C, Ramirez C. *Halobacterium vallismortis* sp. nov. an amylolytic and carbohydrate-metabolizing, extremely halophilic bacterium. *Can J Microbiol* 1978;24:710–715.
- Tindall BJ, Sikorski J, Smibert RA, Krieg NR et al. Phenotypic characterization and the principles of comparative systematics. In: Reddy CA, Beveridge TJ, Breznak JA, Marzluf GA, Schmidt TM et al. (editors). *Methods for General and Molecular Microbiology*, 3rd ed. Washington, DC: American Society of Microbiology; 2007. pp. 330–393.
- Li H-W, Zhi X-Y, Yao J-C, Zhou Y, Tang S-K et al. Comparative genomic analysis of the genus *Nocardiopsis* provides new insights into its genetic mechanisms of environmental adaptability. *PLoS One* 2013;8:e61528.
- Harrison P, Strulo B. SPADES - a process algebra for discrete event simulation. *J Logic Comput* 2000;10:3–42.
- Parks DH, Imelfort M, Skennerton CT, Hugenholtz P, Tyson GW. CheckM: assessing the quality of microbial genomes

- recovered from isolates, single cells, and metagenomes. *Genome Res* 2015;25:1043–1055.
42. Hyatt D, Chen G-L, Locascio PF, Land ML, Larimer FW *et al.* Prodigal: prokaryotic gene recognition and translation initiation site identification. *BMC Bioinform* 2010;11:119.
 43. Kanehisa M, Sato Y, Morishima K. BlastKOALA and GhostKOALA: KEGG tools for functional characterization of genome and metagenome sequences. *J Mol Biol* 2016;428:726–731.
 44. Potter SC, Luciani A, Eddy SR, Park Y, Lopez R *et al.* HMMER web server: 2018 update. *Nucleic Acids Res* 2018;46:W200–W204.
 45. Huerta-Cepas J, Szklarczyk D, Forsslund K, Cook H, Heller D *et al.* eggNOG 4.5: a hierarchical orthology framework with improved functional annotations for eukaryotic, prokaryotic and viral sequences. *Nucleic Acids Res* 2016;44:D286–D293.
 46. Lagesen K, Hallin P, Rødland EA, Staerfeldt H-H, Rognes T *et al.* RNAmmer: consistent and rapid annotation of ribosomal RNA genes. *Nucleic Acids Res* 2007;35:3100–3108.
 47. Yoon S-H, Ha S-M, Kwon S, Lim J, Kim Y *et al.* Introducing EzBioCloud: a taxonomically United database of 16S rRNA gene sequences and whole-genome assemblies. *Int J Syst Evol Microbiol* 2017;67:1613–1617.
 48. Saitou N, Nei M. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* 1987;4:406–425.
 49. Kumar S, Stecher G, Li M, Knyaz C, Tamura K. MEGA: molecular evolutionary genetics analysis across computing platforms. *Mol Biol Evol* 2018;35:1547–1549.
 50. Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA *et al.* Clustal W and Clustal X version 2.0. *Bioinformatics* 2007;23:2947–2948.
 51. Kimura M. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol* 1980;16:111–120.
 52. Felsenstein J. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 1985;39:783–791.
 53. Wu M, Scott AJ. Phylogenomic analysis of bacterial and archaeal sequences with AMPHORA2. *Bioinformatics* 2012;28:1033–1034.
 54. Edgar RC. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res* 2004;32:1792–1797.
 55. Castresana J. Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. *Mol Biol Evol* 2000;17:540–552.
 56. Stamatakis A. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 2014;30:1312–1313.
 57. Letunic I, Bork P. Interactive tree of life (iTOL) V4: recent updates and new developments. *Nucleic Acids Res* 2019;47:W256–W259.
 58. Richter M, Rosselló-Móra R, Oliver Glöckner F, Peplies J. JSpeciesWS: a web server for prokaryotic species circumscription based on pairwise genome comparison. *Bioinformatics* 2016;32:929–931.
 59. Meier-Kolthoff JP, Auch AF, Klenk H-P, Göker M. Genome sequence-based species delimitation with confidence intervals and improved distance functions. *BMC Bioinformatics* 2013;14:60.
 60. Wayne LG, Moore WEC, Stackebrandt E, Kandler O, Colwell RR, Brenner DJ, Grimont PAD *et al.* Report of the *ad hoc* Committee on reconciliation of approaches to bacterial Systematics. *Int J Syst Evol Microbiol* 1987;37:463–464.
 61. Goris J, Konstantinidis KT, Klappenbach JA, Coenye T, Vandamme P *et al.* DNA–DNA hybridization values and their relationship to whole-genome sequence similarities. *Int J Syst Evol Microbiol* 2007;57:81–91.
 62. Richter M, Rosselló-Móra R. Shifting the genomic gold standard for the prokaryotic species definition. *Proc Natl Acad Sci U S A* 2009;106:19126–19131.
 63. Chun J, Rainey FA. Integrating genomics into the taxonomy and systematics of the bacteria and archaea. *Int J Syst Evol Microbiol* 2014;64:316–324.
 64. Idris H. *Actinobacterial Diversity in Atacama Desert Habitats as a Road Map to Biodiscovery*. UK; PhD thesis: Newcastle University; 2016.
 65. Baltz RH. Gifted microbes for genome mining and natural product discovery. *J Ind Microbiol Biotechnol* 2017;44:573–588.
 66. Bull AT, Goodfellow M. Dark, rare and inspirational microbial matter in the extremobiosphere: 16 000 M of bioprospecting campaigns. *Microbiology* 2019;165:1252–1264.

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