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Micromonospora parastrephiae sp. nov. and *Micromonospora tarensis* sp. nov., isolated from the rhizosphere of a *Parastrephia quadrangularis* plant growing in the Salar de Tara region of the Central Andes in Chile

Valeria Razmilic[1,2], Imen Nouioui[1,3], Andrey Karlyshev[4], Rana Jawad[4], Martha E. Trujillo[5], Jose M. Igual[6], Barbara A. Andrews[2], Juan A. Asenjo[2], Lorena Carro[1,5] and Michael Goodfellow[1,*]

Abstract

Two novel *Micromonospora* strains, STR1-7^T and STR1S-6^T, were isolated from the rhizosphere of a *Parastrephia quadrangularis* plant growing in the Salar de Tara region of the Atacama Desert, Chile. Chemotaxonomic, cultural and phenotypic features confirmed that the isolates belonged to the genus *Micromonospora*. They grew from 20 to 37°C, from pH7 to 8 and in the presence of up to 3%, w/v NaCl. The isolates formed distinct branches in *Micromonospora* gene trees based on 16S rRNA gene sequences and on a multi-locus sequence analysis of conserved house-keeping genes. A phylogenomic tree generated from the draft genomes of the isolates and their closest phylogenetic neighbours showed that isolate STR1-7^T is most closely related to *Micromonospora orduensis* S2509^T, and isolate STR1S-6 ^{T} forms a distinct branch that is most closely related to 12 validly named *Micromonospora* species, including *Micromonospora saelicesensis* the earliest proposed member of the group. The isolates were separated from one another and from their closest phylogenomic neighbours using a combination of chemotaxonomic, genomic and phenotypic features, and by low average nucleotide index and digital DNA–DNA hybridization values. Consequently, it is proposed that isolates STR1-7^T and STR1S-6^T be recognized as representing new species in the genus *Micromonospora*, namely as *Micromonospora parastrephiae* sp. nov. and *Micromonospora tarensis* sp. nov.; the type strains are STR1-7^T (=CECT 9665^T=LMG 30768^T) and STR1S-6^T (=CECT 9666^T=LMG 30770^T), respectively. Genome mining showed that the isolates have the capacity to produce novel specialized metabolites, notably antibiotics and compounds that promote plant growth, as well as a broad-range of stress-related genes that provide an insight into how they cope with harsh abiotic conditions that prevail in high-altitude Atacama Desert soils.

INTRODUCTION

Members of the phylum *Actinomycetota* [1], formerly *Actinobacteria sensu* Goodfellow [2], remain the most promising source of new natural products [3, 4]. Novel filamentous actinomycetes, notably streptomycetes, from Atacama Desert soils are an especially rich source of new specialized (secondary) metabolites and bioactive compounds [5, 6], thereby providing evidence that harsh abiotic conditions in extreme habitats select for novel actinomycetes with new chemistry needed for innovative biotechnology [7, 8]. Culture-dependent and culture-independent surveys show that small numbers of rare and novel, taxonomically diverse filamentous actinomycetes other than streptomycetes are a feature of the Atacama Desert landscape [7–11], as exemplified by validly published *Amycolatopsis* [12], *Micromonospora* [13, 14] and *Pseudonocardia* [15] species. Representative species of the

Author affiliations: [1]School of Natural and Environmental Sciences, Newcastle University, Newcastle-upon Tyne, UK; [2]Centre for Biotechnology and Bioengineering (CeBiB), Department of Chemical Engineering, Biotechnology and Materials, University of Chile, Beauchef 851, Santiago, Chile; [3]Department of Microorganisms, Leibniz Institute DSMZ–German Collection of Microorganisms and Cell Cultures, 38124, Braunschweig, Germany; [4]Department of Biomolecular Sciences, School of Life Sciences, Pharmacy and Chemistry, Faculty of Health, Science, Social Care and Education, Kingston University London, Kingston upon Thames, KT1 2EE, UK; [5]Microbiology and Genetics Department, University of Salamanca, Salamanca, Spain; [6]Instituto de Recursos Naturales y Agrobiología de Salamanca, Consejo Superior de Investigaciones Científicas (IRNASA-CSIC), c/Cordel de Merinas 40-52, 37008 Salamanca, Spain.
***Correspondence:** Michael Goodfellow, m.goodfellow@ncl.ac.uk
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The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene and the draft whole genome sequences for strains STR1-7^T and STR1S-6^T are MG725921 and JAEVHM000000000, and MG725922 and JAEVHL000000000, respectively.
Eight supplementary tables and four supplementary figures are available with the online version of this article.
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genera *Amycolatopsis* and *Micromonospora* have genomes that contain taxon specific biosynthetic gene clusters (BGCs) with the potential to encode for new chemical entities [13, 16–18], a discovery which opens up the prospect of selecting extremely gifted strains for natural product drug discovery [3, 4]. Similarly, analyses of stress-related genes in the genomes of Atacama Desert actinomycetes provide valuable insights into how they have adapted to the harsh environmental conditions of this biome [13, 19–21].

Micromonospora, the nomenclatural type genus of the family *Micromonosporaceae* [22, 23], was proposed by Ørskov in 1923 [24], emended by Gao *et al.* [25] and then by Nouioui *et al.* [26] in extensive phylogenomic analyses of actinomycetes. The genus typically contains aerobic and microaerophilic, Gram-stain-positive, non-acid-fast actinomycetes which form single, non-motile spores on substrate mycelia, lack aerial hyphae, produce whole-organism hydrolysates rich in *meso*- and/or dihydroxy- diaminopimelic acid (A_2pm)/ and xylose with either mannose or galactose and glucose, *N*-glycolated muramic acid moieties, phosphatidylethanolamine as the characteristic polar lipid, and complex mixtures of fatty acids and menaquinones with a DNA G+C composition within the range 65–75 mol% [26, 27]. Micromonosporae can be distinguished from strains belonging to other genera classified in the family *Micromonosporaceae* using chemotaxonomic, morphological, phylogenetic and phylogenomic data [26, 28]. At the time of writing the genus encompasses 127 validly published species (https://lpsn.dsmz.de/genus/micromonospora) which form a monophyletic group composed of four well supported lineages [17], the members of which can be distinguished using chemotaxonomic, morphological and phenotypic criteria [13, 28]. Micromonosporae are widely distributed in natural habitats including rhizosphere soil [29, 30], plant tissues [31–33] and, notably, in nitrogen-fixing root nodules [33–38].

The application of innovative selective isolation and associated taxonomic methods show that small numbers of bioactive, phylogenetically diverse *Micromonospora* strains are present in Atacama Desert habitats, especially in the rhizosphere of *Parastrephia quadrangularis*, a leguminous plant found in the Central Andes [30] and in extreme hyper-arid soil from Lomas Bayas, one of the driest regions of the desert. Five isolates from Lomas Bayas soil were found to have genomes rich in novel BGCs, two of the isolates were proposed as new species [13], thereby providing further evidence that the genus *Micromonospora* is underspeciated. The present study was designed to build upon these earlier studies [13, 30] by establishing the taxonomic status and biological properties of strains isolated from the rhizosphere of *P. quadrangularis*. The outcome of this genome-based study shows that the isolates merit recognition as novel *Micromonospora* species. Analysis of the genome sequences of the isolates revealed the presence of genes and gene clusters related to specialized metabolite biosynthesis, plant growth promoting compounds, and stress-responses associated with properties of ecological significance [10, 11].

HABITAT AND ISOLATION

Strains STR1S-6^T and STR1-7^T were isolated from the rhizosphere of a *P. quadrangularis* plant growing at 4300 m above sea level in the Salar de Tara region of the Atacama Desert in Chile (23° 03′ 97″ S 67° 18′ 87″ W). The rhizosphere sample was collected by one of us (M.G.) on 05.10.2016. A sample of the arid soil (0.5 g) was heated at 120°C for 15 min, sprinkled over plates of humic acid–vitamin (HV) agar [39] supplemented with cycloheximide and nalidixic acid (each at 50 µg ml^{-1}) and incubated at 28°C for 14 days. In parallel, 1 g soil was diluted in 0.85% sodium chloride supplemented with 1.5% phenol [40], shaken for 1 h, incubated at 70°C for 40 min in a water bath [41], shaken again for 2 h at room temperature, serial dilutions prepared with a saline solution, and the resultant preparations shaken at room temperature prior to spreading aliquots (100 µl) of each dilution over HV agar [39], and Zhang's starch-soil extract agar [42] supplemented with cycloheximide and nalidixic acid (each at 50 ug ml^{-1}); in each case the inoculated plates were incubated at 28°C for 14 days.

Branching substrate hyphae characteristic of *Micromonospora* colonies detected on the isolation plates using a stereoscopic microscope were transferred to HV agar plates that were incubated at 28°C for 14 days. Fourteen isolates, taken from the HV agar plates [39] inoculated with preheated soil particles and from plates inoculated using the serial dilutions, were maintained on M65 agar (DSMZ medium No 65) and in 20% v/v glycerol at −80°C for long-term preservation. These strains included isolates STR1S-6 ^{T} and STR1-7 ^{T} which were recovered from the HV and starch–soil extract agar plates, respectively. Biomass for the chemotaxonomic, molecular systematic and whole-genome sequencing studies carried out on each of the isolates was prepared in shake flasks (130 rpm) of yeast extract–malt extract broth [International *Streptomyces* Project (ISP) medium 2 [43]] following incubation at 28°C for 14 days. Harvested cells were washed three times in sterile distilled water and those for the chemotaxonomic studies were freeze-dried and the biomass kept at room temperature.

GENOMIC CHARACTERIZATION

Genomic DNA was extracted from wet biomass of single colonies of the isolates, which had been grown on ISP 2 agar for 7 days at 28°C, using a DNeasy UltraClean Microbial Kit (Qiagen Cat. No. 12224-50) following the manufacturer's instructions. The whole-genome sequences were generated using an Ion Torrent PGM sequencing instrument. DNA fragmentation and library preparation were achieved using a NEBNext Fast DNA Fragmentation and Library Preparation kit for the Ion Torrent instrument by following the protocol of the manufacturer. Adapter ligated fragments of approximated 500 base pair (bp) size were

selected using the E-Gel system (SYBR Gold II), Polymerase Chain Reaction (PCR) amplified and checked using the Agilent BioAnalyser. The template was prepared by using the OT2 system and the Ion PGM HiQ View OT2 Kit. Template positive Ion Sphere Particles were isolated using the OT2 ES system. Sequencing was performed on the Ion Torrent PGM using the Ion PGM Hi-Q View Sequencing Kit and IonTorrent 316v2 chips. The assembly was conducted using SPAdes system version 3.1.0. Whole-genome sequences were annotated using the NCBI Prokaryotic Genome Annotation Pipepline version 5.0 and the RAST server [44–46]. The reads obtained for isolate STR1S-6^T were assembled into 532 contigs giving a genome size of 7.4 million base pairs (Mbp) with a digital Guanine(G)+Cytocine (C) content of 71.4mol%, the corresponding values for isolate STR1-7^T were 417 contigs, a genome size of 6.5 Mbp and a digital G+C content of 71.5mol%. The genomes of strains STR1S-6^T and STR1-7^T contained 7172 and 6246 coding sequences, respectively; the corresponding L50 and N50 values were 85 and 223 597 and 65 and 28 516. The Whole Genome Shotgun data for isolates STR1S-6^T and STR1-7^T have been deposited at DDBJ/ENA/GenBank under accession numbers JAEVHL000000000 and JAEVHM000000000, the corresponding versions described in this article are JAEVHL010000000 and JAEVHM010000000.

PHYLOGENY

The complete 16S rRNA gene sequences obtained from the whole genome sequences of isolates STR1S-6^T and STR1-7^T were compared with corresponding sequences of the type strains of *Micromonospora* species using the EzBioCloud database [47]. Phylogenetic trees were inferred using the neighbour-joining [48], maximum-likelihood [49] and maximum-parsimony [50] algorithms drawn from MEGA XI software [51]. The topologies of the trees were evaluated in boostrap analyses [52] using 1000 replicates. The trees were rooted using *Catellatospora citrea* DSM 44097^T (GenBank accession No. X93197).

The isolates formed distinct phyletic lines in the 16S rRNA neighbour-joining, maximum-parsimony and maximum-likelihood trees that were loosely associated with the type strain of six *Micromonospora* species (Fig. 1), notably with that of *Micromonospora chokoriensis* [53], the earliest named species in the lineage. The isolates shared a 16S rRNA sequence of 98.98%, a value which corresponded to 23 nucleotide (nt) differences. In turn, isolates STR1-7^T and STR1S-6^T were most closely related to the type strains of *Micromonospora orduensis* [54] and *M. chokoriensis* [53], respectively, they shared 16S rRNA sequences similarities of 99.37%, a value equivalent to 9 nt differences. The *M. chokoriensis* clade was most closely related to a lineage defined by Trujillo *et. al.* [55] that encompassed eight *Micromonospora* species, including the earliest proposed species, *Micromonospora saelicesensis*. The composition of the *M. chokoriensis* lineage in the full *Micromonospora* 16S rRNA gene tree (Fig. S1, available in the online version of this article) was almost identical to clades delineated in earlier studies [13, 26, 36, 37].

Multilocus sequence analyses (MLSA) were carried out based on 16S rRNA, *atpD*, *gyrB*, *recA* and *rpoB* sequences drawn from the draft genomes of the isolates held in GenBank, as described previously [34]. The MLSA trees were generated from 9262 nt using the three algorithms cited previously, and the resultant trees evaluated in bootstrap analyses based on 1000 replicates. It can be seen from Fig. 2 that the isolates formed distinct branches in a well-supported clade which encompassed the type strains of 21 *Micromonospora* species including those of *M. chokoriensis* and *M. saelicesensis*, as was the case in the full *Micromonospora* tree (Fig. S2). It is also evident from the tree that isolate STR1-7^T formed a well supported lineage with the type strain of *M. orduensis* and isolate STR1S-6^T a corresponding lineage with the type strains of *M. chokoriensis*, *M. taraxaci* and *M. violae*. These results add to the wealth of evidence which show that micromonosporal phylogenies based on concatenated sequences of housekeeping genes give greater resolution between closely related strains than corresponding trees generated from 16S rRNA gene sequences [13, 14, 34]. The MLSA distances between isolates STR1S-6^T and STR1-7^T and closely related strains were above the 0.007 threshold used to distinguish between bacterial species [56] (Table S1), confirming that they belong to different species. Further, on this basis isolate STR1-7^T was most closely related to the type strain of *M. orduensis*, and isolate STR1S-6^T to those of *M. alfalfae* and *M. ureilytica*. The *M. chokoriensis* lineage in the full *Micromonospora* MLSA tree corresponded to previously defined clades [14, 17] which equates to *Micromonospora* subcluster Iva delineated in the genome-based classification of Carro *et al.* [17].

The draft genome sequences of isolates STR1S-6^T and STR1-7^T were uploaded onto the Type (Strain) Genome Server (TYGS) [57] and compared with the genomes of their closest phylogenomic neighbours available in the TYGS database using the MASH algorithm for fast approximation of intergenomic relatedness between strains [58]. Precise distances were calculated using the Genome BLAST Distance Phylogeny (GBDP), the algorithm 'coverage' and the distance formula d_5 [59]. The resulting intergenomic distances were used to infer a balanced minimum-evolution tree with branch support via FastME 2.1.4, including SPR post-processing [60]. Branch support was inferred from 100 *pseudo*-bootstrap replicates.

Greater confidence can be placed in relationships inferred in phylogenomic trees than in corresponding 16S rRNA and MLSA phylogenies as the former are generated from millions, not hundreds or thousands, of unit characters [26]. Figure 3 not only shows that the isolates formed distinct branches in the phylogenomic tree but confirmed that strain STR1-7^T is most closely related to the type strain of *M. orduensis*. In contrast, isolate STR1S-6^T formed a distinct phyletic line which was most closely related to a well-supported lineage which encompassed *M. alfalfae* MED01^T, *M. arida* LB32^T, *M. inaquosa* LB39^T, *M. foliorum* PSH25^T, *M. hortensis* NIE111^T, *M. noduli* GUI43^T, *M. saelicesensis* DSM 44871^T, *M. salmantinae* PSH03^T, *M. trifolii* NIE79^T, *M. ureilytica* LB19^T, *M. vinacea* DSM 101695^T and *M. violae* NEAU-zh8 ^{T}. Isolate STR1S-6^T is also loosely associated with a lineage that included the

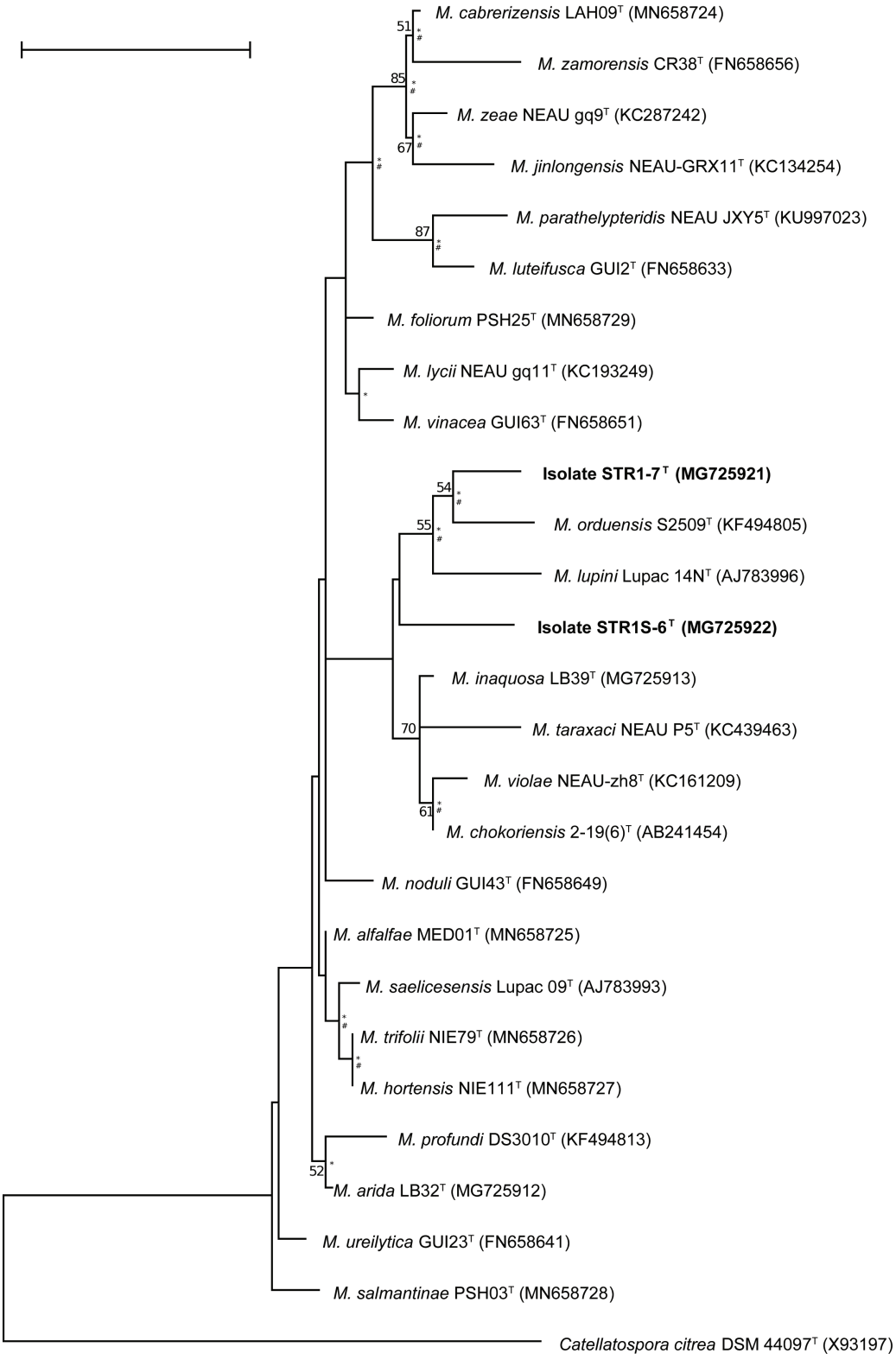

Fig. 1. Neighbour-joining phylogenetic tree based on almost-complete 16S rRNA gene sequences showing relationships between isolates STR1S-6^T and STR1-7^T and between them and closely related *Micromonospora* type strains. Numbers at the nodes indicate levels of bootstrap support (%), only values above 50% are shown. Asterisks and hashes indicate branches of the tree that were also recovered in the maximum-likelihood and maximum-parsimony trees, respectively. *Catellatospora citrea* was used as the outgroup. Bar, 0.01 substitutions per nucleotide position.

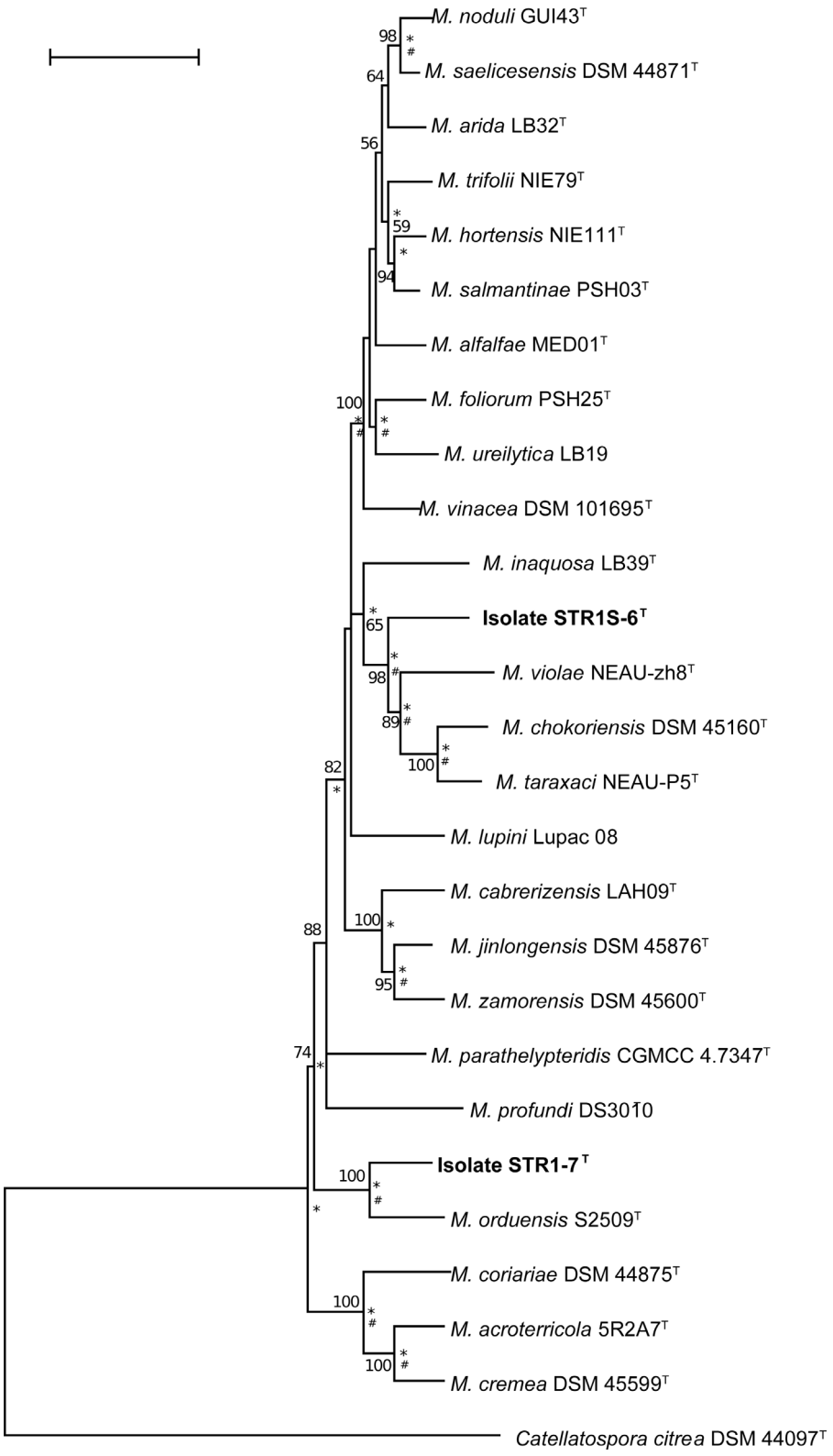

Fig. 2. Neighbour-joining phylogenetic tree based on multilocus analysis of 16S rRNA, *gyrB*, *rpoB*, *atpD* and *recA* gene sequences showing relationships between isolates STR1S-6^T and STR1-7^T and between them and closely related *Micromonospora* type strains. Numbers at the nodes indicate levels of bootstrap support (%), only values above 50% are shown. Asterisks and hashes indicate branches of the tree that were also recovered in the maximum-likelihood and maximum-parsimony trees, respectively. *Catellatospora citrea* was used as the outgroup. Bar, 0.02 substitutions per nucleotide position.

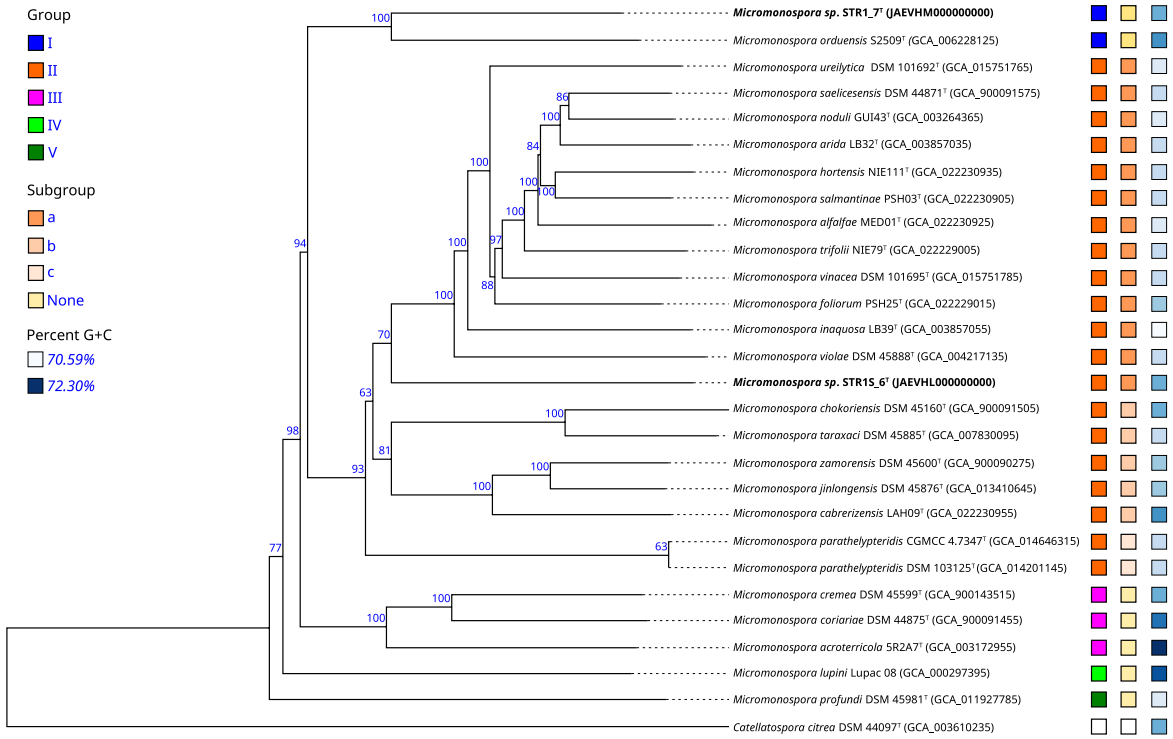

Fig. 3. Whole-genome phylogenomic tree generated with TYGS showing relationships between the strains STR1-6^T and STR1-7^T, and between them and closely related type strains with available genomes. The tree was inferred with FastME from GBDP distances calculated from the genome sequences. Branch lengths were scaled using the GBDP distance formula d5 [59]. Numbers above branches indicate GBDP *pseudo*-bootstrap support values from 100 replications. *Catellatospora citrea* was used as the outgroup.

type strains of *M. cabrerizensis*, *M. chokoriensis* and *M. taraxaci*. It is interesting that isolate STR1S-6^T belongs to genomic clade IVa which encompasses the type strains of *Micromonospora* species isolated from ecto- and endo-rhizospheres [17].

COMPARATIVE GENOMICS

Average nucleotide identity (ANI) and digital DNA–DNA hybridization (dDDH) similarities were determined between the genomes of isolates STR1S-6^T and STR1-7^T, and between them and their closest phylogenomic neighbours, using the ANI [61] and Genome-to-Genome Distance Calculator [59] web server, respectively. The recommended ANI and DDH values used to assign closely related strains to different species are 95–96 and 70%, respectively [62–64].It is apparent from Table 1 that on this basis the isolates should be recognized as representing novel *Micromonospora* species. The metrics also showed that isolate STR1-7^T is most closely related to *M. orduensis* S2509^T, a strain isolated from Black Sea sediment [54], and isolate STR1S-6^T to the type strain of *M. foliorum* which was isolated from the phylloplane of *Pisum sativum* [33].

CHEMOTAXONOMY

The isolates were examined for chemotaxonomic properties known to be characteristic of *Micromonospora* strains [27, 30]. Standard chromatographic procedures were used to detect A_{2pm} isomers [65], whole-cell sugars [66], polar lipids [67, 68] and isoprenoid quinones [69]. Fatty acids were extracted, methylated and analysed by gas chromatography (model 7890A GC System, Agilent Technology) and analysed using the protocol of the Sherlock Microbial Identification (MIDI) system, version 5 and the resultant peaks identified using the ACTINO 6 database [70]. The chemotaxonomic profiles of the isolates are consistent with their classification in the genus *Micromonospora* [27, 30]. The diamino acid of the wall peptidoglycan of the isolates is *meso*-A_{2pm}, the cellular sugars, arabinose, galactose, glucose, mannose, rhamnose and xylose, and the polar lipids, diphosphatidylglycerol, phosphatidylethanolamine (diagnostic phospholipid), phosphatidylinositol and phosphatidylinositol mannosides and several unidentified components (phospholipid pattern type 2 [71]), as shown in Fig. S4. The fatty acid profiles are rich in saturated, unsaturated and branched-chain components, and the predominant isoprenoid quinones are MK-10(H_4) and MK-(H_6).

Table 1. Average nucleotide identities (ANI) and digital DNA:DNA hybridization (dDDH) similarities between the genomes of isolates STR1-7^T (1), STR1S-6^T (2) and their closest phylogenomic neighbours

Strain	ANI		dDDH	
	1	2	1	2
1, STR1-7^T		88.97		37.3
2, STR1S-6^T	88.97		37.3	
M. acroterricola 5R2A7^T	89.61	88.53	39.2	36.5
M. alfalfae MED01^T	88.81	90.08	36.7	40.7
M. arida LB32^T	89.03	90.38	37.5	41.6
M. cabrerizensis LAH09^T	89.14	89.7	37.6	39.2
M. chokoriensis DSM 45160^T	88.34	89.19	35.8	37.9
M. coriariae DSM 44875^T	89.49	88.39	38.4	35.9
M. cremea DSM 45599^T	89.65	88.22	39	35.9
M. foliorum PSH25^T	89.37	90.72	38.5	42.6
M. hortensis NIE111^T	88.69	90.23	36.7	40.7
M. jinlongensis DSM 45876^T	89.22	89.81	36.8	40.7
M. inaquosa LB39^T	88.79	90.1	37.7	39.5
M. lupini Lupac 08	89.03	87.76	37.6	34.8
M. noduli GUI43^T	89.01	90.44	37.3	41.4
M. orduensis S2509^T	92.24	88.62	47.7	36.7
M. parathelypteridis CGMCC 4.7347^T	89.2	89.54	37.7	39.3
M. profundi DS3010^T	87.82	87	34.5	33
M. saelicesensis DSM 44871^T	89.18	90.44	37.5	41.6
M. salmantinae PSH03^T	89.04	90.41	37.2	41.5
M. taraxaci NEAU-P5^T	88.5	89.52	35.9	38.4
M. trifolii NIE79^T	88.97	90.31	37	41.2
M. ureilytica LB19	88.98	90.35	37.2	41.4
M. vinacea DSM 101695^T	89.11	90.36	37.4	41.5
M. violae NEAU-zh8^T	88.73	90.16	36.5	40.4
M. zamorensis DSM 45600^T	89.12	89.79	37.5	39.2

CULTURAL, MORPHOLOGICAL, CHEMOTAXONOMIC, AND PHYSIOLOGICAL FEATURES

The isolates were examined for cellular morphological features and motility by phase contrast microscopy (Leica, CTR, MIC) and Gram-stain reaction after Doetsch *et al.* [72]. Their ability to grow and produce substrate mycelial pigments on tryptone–yeast extract, yeast extract–malt extract, oatmeal, inorganic salts–starch, glycerol–asparagine, peptone–yeast extract, iron and tyrosine agar (ISP 1–7 [43]) was recorded following incubation at 28°C for 14 days. Pigment colours were recorded by comparison against colour charts [73]. The isolates were also tested for their capacity to grow over a range of adjusted pH (pH 4.5, 5.5, 6.5, 8.0 and 9.0) using phosphate buffers and temperatures (4, 10, 20, 28, 37 and 45°C), and in the presence of various concentrations of NaCl (1, 3, 5, 7 and 9%, w/v) using ISP 2 agar [43], as described previously. The morphological properties of STR1-7^T and STR1S-6^T were determined by scanning electron microscopy after 2 and 20 weeks growth on ISP 2 agar, respectively, and their enzymatic profiles recorded using API ZYM strips (bioMérieux) following the protocol of the manufacturer. In addition, the isolates were tested for their ability to oxidize diverse sole carbon and sole nitrogen sources and to grow in the presence of inhibitory compounds using GEN III MicroPlates in an OMNILOG device (Biolog Inc). The MicroPlates were inoculated, in duplicate, with suspensions of the isolates in inoculating fluid (IFC solution) provided by the manufacturer, at a cell density of 80% transmittance with a run time of 7 days at 28°C in phenotypic array mode. The resultant data were analysed using the opm package for R version 1.0.6 [74, 75].

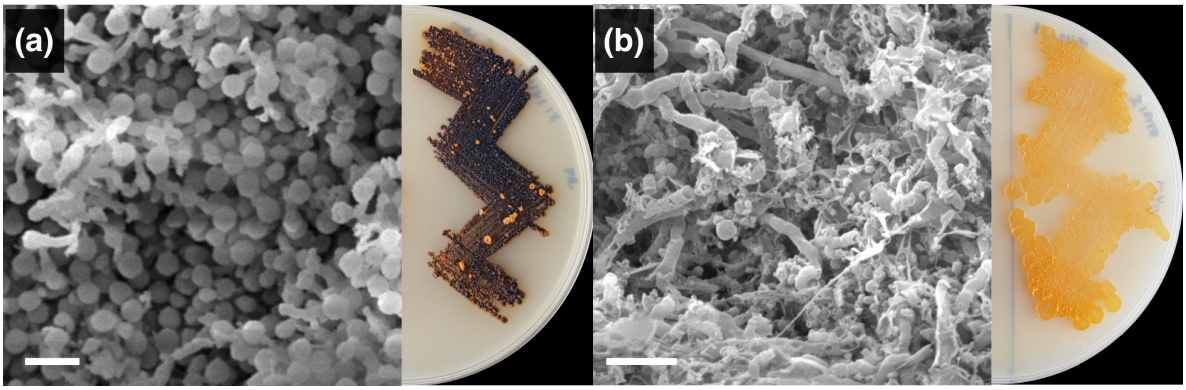

Fig. 4. Scanning electron microscope images of isolates STR1-7^T (a) and STR1S-6^T (b), showing the presence of sessile, smooth spores on substrate hyphae after growth on ISP 2 agar for 2 and 30 weeks, respectively and associated growth phenotypes. Bar, 2 µm.

The isolates were found to be aerobic, Gram-stain-positive actinomycetes which formed extensively branched, stable, substrate hyphae that carried single, non-motile, sessile spores. They grew on all of the ISP media, notably on ISP 2 agar, mainly producing orange substrate hyphae (Table S2, Fig. S3), and from 20 to 37°C, optimally at 28°C, from pH 7.0 to 8.0, optimally at pH 7.0, and in the presence of 3% w/v NaCl. In contrast, they can be distinguished using chemotaxonomic, morphological and physiological properties from related species. Isolate STR1-7^T formed warty ornamented spores (0.8×1.0 µm) and isolate STR1S-6^T elongated spores (0.5×1.0 µm) with smooth surfaces (Fig. 4). Table 2 shows that the isolates gave differential responses to some of the API-ZYM tests. In addition, isolate STR1S-6^T was found to be more metabolically active than isolate STR1-7^T as it oxidized D-arabitol, D-fucose, glycerol, D-mannitol, L-rhamnose, trehalose, *N*-acetyl-D-galactosamine, *N*-acetyl-D-glucosamine, *N*-acetyl-*β*-D-mannosamine and 3-*O*-methyl-D-glucose; it also grew in the presence of lincomycin, sodium formate and sodium lactate (1%, w/v), as shown in Table S3. Additional differential GEN III MicroPlate test data are shown in Table S3 together with results that were positive for each of the strains.

The isolates showed differences in some of the chemotaxonomic tests; notably qualitative and quantitative differences in fatty acid composition (Table S4), as exemplified by higher proportions of iso-$C_{16:0}$, iso-$C_{17:0}$ and anteiso-$C_{17:0}$ in isolate STR1-7^T. Similar conclusions can be drawn from the menaquinone data as isolate STR1S-6^T contained MK10(H_4) (44%), MK10(H_6) (12%) and MK9(H_6) (11%) whereas the corresponding profile of isolate STR1-7^T was composed of major proportions of MK10(H_4) (39%), MK10(H_6) (31%) and MK10(H_8) (17%). The isolates can also be distinguished by their polar lipid profiles as only isolate STR1S-6^T gave spots corresponding to an aminolipid, a glycophospholipid and a phosphoaminolipid (Fig. S4).

It is also apparent from Table 2 that the isolates can be distinguished from their immediate phylogenomic neighbours using combinations of phenotypic properties. Isolate STR1S-6^T can be separated from its closest phylogenomic neighbours by an ability to use a range of sole carbon and nitrogen sources, as exemplified by its capacity to use aspartic acid, D-serine, glycerol and amino- *n*-butyric, citric, malic, mucic and quinic acids, and by its ability to grow on tetrazolium blue. In turn, isolate STR1-7^T can be distinguished from *M. orduensis*, its closest phylogenomic relative, by its ability to degrade casein and produce warty ornamented spores. In contrast, the *M. orduensis* strain, unlike the isolate, grew at pH5 and 40°C. Similarly, combinations of phenotypic features can be weighted to distinguish between the reference type strains.

SPECIALIZED METABOLITES – BGCS

The draft genomes of the isolates were mined for NP -BGCs using antiSMASH [76]. This programme predicts BGCs and potential natural products based on the percentage of genes from the closest-known bioclusters showing BLAST hits to the genomes under consideration. The genomes of isolates STR1-7^T and STR1S-6^T contained 57 and 72 BGCs, respectively, though these numbers are likely to be an overestimate as some of the bioclusters represent large BGCs, as shown in Table S5.

Like other micromonosporae, the isolates were found to have the genetic capacity to synthesise a broad range of specialized compounds, including lantipeptides, non-ribosomal peptide synthases (NRPS), polyketide synthases (PKS), siderophores and terpenes [13, 17], albeit with low levels of gene sequence identity (Table S6). Some bioclusters were discontinuously distributed between the isolates though the genomes of both isolates contained bioclusters characteristic of micromonosporae [17], including ones predicted to encode for the siderophore desferrioxamine B, the glycosylated carotenoid sioxanthin, the lantipeptide, SapB and alkyl-O-dihydrogeranyl-methoxyhydroquinone, a type III PKS. It is particularly interesting that the genomes of the isolates contained seven bioclusters that have been found only in the genomes of micromonoporae isolated from the Atacama Desert [13], namely ones related to the synthesis of coumermycin A1, an aminocoumarin antibiotic, aclacinomycin, and chlorizidine

Table 2. Phenotypic properties which distinguish the isolates from one another and from their closest phylogenomic neighbours

Strains: 1, STR1S-6^T; 2, *Micromonospora violae* NEAU-Zh8^T; 3, *Micromonospora inaquosa* LB39; 4, *Micromonospora ureilytica* GUI23^T; 5, *foliorumMicromonospora* PSH25^T; 6, *Micromonospora vinacea* GUI63^T; 7, *trifoliiMicromonospora* NIE79^T; 8, *alfalfaeMicromonospora* MED01^T; 9, *hortensisMicromonospora* NIE11^T; 10, *salmantinaeMicromonospora* PSH03^T; 11, *Micromonospora arida* LB32^T; 12, *Micromonospora noduli* GUI43^T; 13, *Micromonospora saelicesensis* DSM 44871^T; 14, STR1-7^T; 15, *Micromonospora orduensis* S2509^T. API ZYM data for isolates STR1S-6^T, STR1-7^T, *M. orduensis* S2509^T and *M. violae* NEUA-Zh8^T were obtained in this work. The API-ZYM data for the remaining type strains of the *Micromonospora* species were taken from references [13, 33, 36]. All the strains were positive for alkaline phosphatase, cystine arylamidase, esterase C4, esterase lipase C8, *β*-galactosidase, *N*-acetyl-*β*-glucosaminidase and naphthol-AS-BI-phosphohydrolase. +, Positive; –, negative; w, weakly positive; ND, not determined.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
API ZYM tests:															
Acid phosphatase	–	+	+	–	+	–	+	+	+	+	–	–	+	+	+
α-Chymotrypsin	–	+	+	+	+	+	+	+	+	w	–	+	+	–	+
α-Fucosidase	–	–	–	–	–	–	–	–	–	–	–	–	+	+	–
α-Galactosidase	–	+	+	–	+	+	+	+	+	+	+	+	+	+	+
α-Glucosidase	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+
β-Glucosidase	+	+	+	–	w	+	+	+	+	+	+	–	+	+	+
β-Glucuronidase	+	–	+	–	+	–	w	–	–	–	+	–	–	+	–
Leucine arylamidase	+	–	+	+	+	+	+	+	+	+	+	+	+	–	+
Lipase (C14)	+	+	+	–	–	–	+	+	–	–	+	–	–	+	–
α-Mannosidase	+	+	+	–	–	–	–	–	–	–	–	–	+	+	–
Degradation of:															
Casein	+	–	+	+	+	+	+	+	+	+	+	+	+	+	–
Tolerance tests:															
Temperature range (°C)	20–37	12–37	12–37	20–37	4–37	12–37	18–37	18–37	18–37	4–37	20–37	12–37	12–45	20–37	28–40
pH range	7–8	6–9	6–8	7–8	4.5–9	7–9	6–8.5	6–9	6–9	4.5–9	6–8	7–9	7–9	7–8	5–11
Growth in the presence of:															
NaCl (% w/v)	3	2	3	1	1	3	3	3	3	3	1	3	1	3	3
Spore surface:															
Smooth	+	+	ND	+	–	+	+	–	–	–	ND	+	+	–	+
Warty	–	–	ND	–	+	–	–	+	+	+	ND	–	–	+	–

A, anticancer agents [77, 78], ecosides, antimicrobial compounds, fengycin, an antifungal antibiotic, polysaccharide B, and svaricin, although only 6–20% of the biosynthetic genes were found to show similarity to these bioclusters (Table S6). In addition, each isolate had bioclusters predicted to encode for the type II PKS pradimicin (12% sequence identity) and the terpene, phosphonoglycan (3% sequence identity).

Isolate STR1S-6^T was shown to have a greater genetic capacity to produce novel compounds than isolate STR1-7^T as its genome included bioclusters predicted to express for bottromycin A2 (9% sequence identity), cetoniacytone A (12% sequence identity), cystothiazole A (12% sequence identity), tetronomycin (9% sequence identity), the NRPS compound tubulysin (9% sequence identity), the type I PKS, ebelactone (70% sequence identity) and oligomycin (38% sequence identity). It is especially interesting that this isolate contained bioclusters associated with the production of abyssomicin-like compounds (10 and 17% sequence identity). The abyssomicins were initially isolated from the type strain of *Verrucosispora maris* [79], now classified as *Micromonospora maris* [26], and shown to have unique chemical structures and modes of action, as well as antibiotic, anticancer and anti-HIV properties [8]. In contrast, only the genome of isolate STR1-7^T harboured bioclusters associated with the synthesis of the type I PKS compound, neocarzilin (35% sequence identity) and the NRPS agent,bleomycin (9% sequence identity).

The genome mining data along with those from earlier studies [13, 14, 17] show that the genomes of novel *Micromonspora* species isolated from the Atacama Desert soils have the potential to synthesise new specialized metabolites, notably antibiotics. Consequently, gifted micromonosporae such as these should be considered as prime candidates in the search for new bioactive compounds needed to treat infections caused by multi-drug resistance microbial pathogens.

GENES ASSOCIATED WITH PLANT GROWTH

Micromonospora strains isolated from rhizosphere soil and plant material are a promising source of metabolites that promote plant growth [33, 80, 81]. The genomes of the isolates were rich in genes associated with direct (eg. phosphate solubilization, phytohormone production) and indirect (eg. ability to synthesise siderophores) ways of enhancing the health of plants [82], as shown in Table S7. Micro-organisms with the capacity to solubilize inorganic phosphorus play a significant role in releasing this element which is essential for the growth of plants [82]. The isolates contained genes associated with phosphate regulation and metabolism, as exemplified by genes *pp*X–*gpp*A, which encode for the solubilization of inorganic phosphate [83], and *pst*S that does likewise for the production of the phosphate binding protein PstS, which is involved in the production of the phosphate ABC transporter [84].

In turn, phytohormones, such as ethylene and indol-3-acetic acid (IAA), have a decisive role in the growth and development of plants [85]. The isolates were found to have the capacity to produce anthranilate phosphoribosyltransferase (gene *trpD*), anthranilate synthase (genes *trpE* and *trpG*) and tryptophan synthase (genes *trpA* and *trpB*) which are intermediates in the tryptophan biosynthetic pathway that is involved in the production of IAA [86]. The isolates also have the genetic capacity to synthesise siderophores, Fe^{3+} chelators that have an important role in scavenging iron form iron-binding proteins produced by plants [87]. In contrast, neither of the isolates were found to have genes encoding for nitrogen fixation. The genome of isolate STR1-7^T contained a gene predicted to encode for 1-aminocyclopropane-1-carboxylate deaminase, a plant growth-promoter that reduces ethylene-induced physiological damage in plants caused by biotic and abiotic stress, including high salinity conditions [88, 89].

These genome-mining studies provide further evidence that micromonoporae, like other actinomycete taxa associated with plants, have genomes equipped with genes predicted to encode for the synthesis of phytohormones, as well as ones functionally linked to inorganic phosphate solubilization and siderophore production [13, 17, 33, 82, 90]. Micromonosporae may also prove to be a source of metallophores which protect plants against the toxic effect of heavy metals [91].

STRESS-RELATED GENES

The genomes of isolates STR1S-6^T and STR1-7^T contained 71 and 72 stress-related genes, notably ones associated with carbon starvation, DNA repair, heat shock response and oxidative stress (Table S8). Both isolates, for example, harboured genes belonging to the CspA family which are involved in cold shock responses [92], genes belonging to the *uvr* ABC DNA repair system implicated in resistance to UV light [93] and *rex* and *osmcl* genes which encode for redox-sensitive transcriptional regulators that have a role in protection against oxidative stress [94]. The isolates also contained a gene associated with the detoxification of NO, namely *hmo*, which converts NO to nitrate as an inducible response to nitrosative stress [95]. These results are in agreement with those reported in previous studies on micromonosporae isolated from Atacama Desert soils [13, 14, 17] and provide an indication of how micromonosporae adapt to the harsh environmental conditions characteristic of the Atacama landscape [10, 30, 96].

CONCLUSION

It is evident from the wealth of genomic, genotypic, phenotypic and phylogenomic data that the isolates from the rhizosphere of *P. quadran*gularis merit recognition as representing novel *Micromonospora* species. It is, therefore, proposed that isolates STR7-7^T

and STR1S-6^T be recognised as the type strains of *Micromonospora parasrephidae* sp. nov. and *Micromonospora tarensis* sp. nov., respectively.

DESCRIPTION OF *MICROMONOSPORA PARASTREPHIAE* SP. NOV.

Micromonospora parastrephiae (pa.ra.stre'phi.ae N.L. gen. n. *parastrephiae* of *Parastrephia*, referring to the isolation of the strain from the rhizosphere of *Parastrephia quadrangularis*).

Aerobic, Gram-stain-positive, chemoorganotrophic actinomycete which forms non-motile, sessile, single warty ornamented spores (0.8–1 µm) on extensively branched, non-fragmenting substrate hyphae. Does not produce aerial hyphae. Colonies are orange on yeast extract - malt extract agar turning brown on sporulation. Grows from 20–37°C, from pH 7 to 8, and in the presence of 3% w/v NaCl. Grows well on ISP 1, ISP 2, ISP 3 and ISP 4, and poorly on ISP 5, ISP 6 and ISP 7 agar. Utilizes cellobiose, D-fructose, D-galactose, D-glucose, D-glucose-6-phosphate, lactose, maltose, D-mannose, melibiose, raffinose, D-salicin, D-sorbitol, sucrose and trehalose as carbon sources, but not on D-arabitol, L-fucose, 3-*O*-methyl-D-glucose, D-mannitol, L-rhamnose or D-serine. Sensitive to lincomycin, troleandomycin and vancomycin. Additional phenotypic properties are cited in the text and Table 2. Whole-cell hydrolysates contain *meso*-A_2pm, arabinose, galactose, glucose, mannose, rhamnose and xylose. The major fatty acids are iso-$C_{15:0}$, iso-$C_{16:0}$ and $C_{17:1}$ ω8*c* and the predominant isoprenoid quinones are MK10 (H4, H6, H8). The polar lipid profile consists of diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol, phosphatidylinositol mannosides, and an unknown lipid. Genome size is 6.5Mbp and DNA G+C content is 71.5mol%.

The type strain, STR1-7^T (=CECT 9665^T=LMG 30768^T), was isolated from the rhizosphere of a *P. quadrangularis* plant growing in the Salar de Tara region of the Atacama Desert, Chile. The DDBJ/EMBL/GenBank accession numbers for the 16S rRNA gene and genome sequences are MG725921 and JAEVHM000000000, respectively.

DESCRIPTION OF *MICROMONOSPORA TARENSIS* SP. NOV.

Micromonospora tarensis (tar.en'sis N.L. fem. adj. *tarensis* belonging to Tara, the name of the salt flat, the source of the isolate).

Aerobic, Gram-stain-positive, chemoorganotrophic actinomycete which forms non-motile, single spores (0.5–1 µm) with smooth surfaces on extensively branched non-fragmenting substrate hyphae. Does not produce aerial hyphae. Raised and folded, vividly orange-coloured colonies are formed on yeast extract–malt extract agar. Grows from 20 to 37°C, from pH 7.0 to 8.0 and in the presence of up to 1%, w/v of NaCl. A light pink pigment is produced in tyrosine agar (ISP 7). Catalase and oxidase positive. Degrades casein and starch. Tolerant to UVC radiation. Utilizes D-arabitol, cellobiose, dextrin, L-fucose, D-fructose, D-galactose, D-glucose, D-glucose-6-phosphate, 3-*O*-methyl-D-glucose, lactose, maltose, D-mannitol, D-mannose, melibiose, raffinose, L-rhamnose, D- salicin, D-sorbitol, sucrose, and trehalose as carbon sources, but not D-serine. Sensitive to troleandomycin and vancomycin. Additional phenotypic data are cited in the text and Table 2. Whole cell hydrolysates contain *meso*-A_2pm, arabinose, galactose, glucose, mannose, rhamnose and xylose, the predominant isoprenoid quinones are MK9 (H6) and MK10 (H4, H6). The major fatty acids are iso-$C_{15:0}$, $C_{17:0}$ and $C_{17:1}$ ω8*c*, and the polar lipid profile contains diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol, phosphatidylinositol mannosides, and an aminolipid, a glycophospholipid and phosphoaminolipid. Genome size is 7.4 Mbp and DNA G+C content is 71.4mol%.

The type strain, STR1S-6^T (=CECT 96660^T=LMG 30770^T), was isolated from the rhizosphere of a *P. quadrangularis* plant growing in the Salar de Tara region of the Atacama Desert, Chile. The DDBJ/EMBL/GenBank accession numbers for the 16S rRNA gene and genome sequences are MG725922 and JAEVHL000000000, respectively.

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Conflicts of interest
The authors declare that there are no conflicts of interest.

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