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TAXONOMIC DESCRIPTION

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Micromonospora tarapacensis sp. nov., a bacterium isolated from a hypersaline lake

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Abstract

Strain Llam7^T was isolated from microbial mat samples from the hypersaline lake Salar de Llamará, located in Taracapá region in the hyper-arid core of the Atacama Desert (Chile). Phenotypic, chemotaxonomic and genomic traits were studied. Phylogenetic analyses based on 16S rRNA gene sequences assigned the strain to the family *Micromonosporaceae* with affiliation to the genera *Micromonospora* and *Salinispora*. Major fatty acids were $C_{17:1}\omega 8c$, iso-$C_{15:0}$, iso-$C_{16:0}$ and anteiso-$C_{17:0}$. The cell walls contained *meso*-diaminopimelic acid and LL-2,6 diaminopimelic acid (LL-DAP), while major whole-cell sugars were glucose, mannose, xylose and ribose. The major menaquinones were MK-9(H_4) and MK-9(H_6). As polar lipids phosphatidylglycerol, phosphatidylethanolamine, diphosphatidylglycerol and several unidentified lipids, i.e. two glycolipids, one aminolipid, three phospholipids, one aminoglycolipid and one phosphoglycolipid, were detected. Genome sequencing revealed a genome size of 6.894 Mb and a DNA G+C content of 71.4 mol%. Phylogenetic analyses with complete genome sequences positioned strain Llam7^T within the family *Micromonosporaceae* forming a distinct cluster with *Micromonospora* (former *Xiangella*) *phaseoli* DSM 45730^T. This cluster is related to *Micromonospora pelagivivens* KJ-029^T, *Micromonospora craterilacus* NA12^T, and *Micromonospora craniellae* LHW63014^T as well as to all members of the former genera *Verrucosispora* and *Jishengella*, which were re-classified as members of the genus *Micromonospora*, forming a clade distinct from the genus *Salinispora*. Pairwise whole genome average nucleotide identity (ANI) values, digital DNA–DNA hybridization (dDDH) values, the presence of the diamino acid LL-DAP, and the composition of whole sugars and polar lipids indicate that Llam7^T represents a novel species, for which the name *Micromonospora tarapacensis* sp. nov. is proposed, with Llam7^T (=DSM 109510^T,=LMG 31023^T) as the type strain.

The genus *Micromonospora* is the type genus of the family *Micromonosporaceae* [1]. The genus was established by Ørskov [2] and since then it has undergone extensive revisions based on chemotaxonomic characteristics [3], gene marker sequence analyses [4, 5] and recently whole-genome based phylogenomic analyses [6]. The genus *Micromonospora* currently consists of 106 species with validly published names [7]. Most of the members of the genus have xylose in their whole-sugar content and *meso*-diaminopimelic acid and/or 3-OH-diaminopimelic acid as a diamino acid. The use of whole-genome sequences in microbial taxonomy improved the resolution of the taxa within the family compared with single gene analyses and has led to the emendation of genera such as *Verrucosispora*, *Xiangella* and *Jishengella* and merging them into the genus *Micromonospora* as far as genomic data were available [6]. Representatives of the genus *Micromonospora* have been isolated from diverse extreme environments, such as *Micromonospora acroterricola* 5R2A7^T isolated from a soil sample collected at high altitude in the Atacama Desert [8], which is the oldest and continuously driest non-polar temperate desert on Earth [9].

In this study, we identified the taxonomic status of strain Llam7^T, isolated from the hypersaline lake Salar de Llamará in the Atacama Desert. The chemotaxonomic and genomic features of the strain were compared with those of type strains of closely related species of the genus *Micromonospora*.

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Keywords: *Actinobacteria*; Atacama; hypersaline lake; *Micromonospora tarapacensis*; polyphasic taxonomy.

Abbreviations: BGC, biosynthetic gene cluster; dDDH, digital DNA–DNA hybridization; orthoANI, average nucleotide identity by orthology.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequence and the genome sequences of Llam7^T are MK088238 and JAHCDI000000000, respectively.

One supplementary figure and one supplementary table are available with the online version of this article.

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Fig. 1. Salar de Llamará, the isolation source of strain Llam7^T.

ISOLATION AND ECOLOGY

Llam7^T was isolated from Salar de Llamará (Fig. 1), a small hypersaline lake located in the Atacama Desert (Chile). This hypersaline lake has been characterized by moderately alkaline pH (around 8), temperatures around 25–30 °C, and high concentrations of total ions of arsenic, chlorine, sodium, sulphate, potassium, magnesium and calcium [10]. A microbial mat sample was collected from the geographic coordinates 21° 16.087′ S, 069° 37.094′ W. The sample was diluted in Ringer 1/4 buffer (0.12 g $CaCl_2.2H_2O$, 0.11 g KCl, 0.05 g $NaHCO_3$ and 2.25 g NaCl in 1 l deionized water) to 10^{-1}, 10^{-2} and 10^{-3}, to select spore-forming actinobacteria the samples were heated in a water bath at 56 °C for 10 min. 100 µl of each dilution was spread on starch–yeast-extract–peptone (SYP) medium containing 2 g starch, 1 g yeast extract, 0.5 g soy peptone, supplemented with 25 µg ml^{-1} nalidixic acid, 50 µg ml^{-1} cycloheximide, 20 g Tropic Marine Salt (Dr Biener), 12 g gellan gum and 2 g $CaCl_2.2H_2O$ in 1 l deionized water, and incubated at 26 °C for 21 days. Llam7^T was isolated and maintained in SYP medium at 26 °C and cryopreserved using the Cryobank System (MAST DIAGNOSTIC) at −20 °C and −80 °C.

16S rRNA GENE PHYLOGENY

For the genomic DNA preparation, cells of Llam7^T were grown in 100 ml SYP medium for 2 weeks at 26 °C. DNA was extracted using a DNeasy Blood and Tissue Kit (QIAGEN). The quantity and quality of the extracted DNA was evaluated by agarose gel electrophoresis. First amplification of the 16S rRNA gene was made with DreamTaqGreen PCR MasterMix (Fisher Scientific) using the primers 27 f (5′-AGAGTTTGATCMTGGCTCAG-3′) and 1492 r (5′-TACG GYTACCTTGTTACGACTT-3′). As the obtained 16S rRNA gene sequence had a length of only 1320 bp, further analyses were carried out with the complete 16S rRNA gene sequence (1517 bp, MK088238) obtained from the genomic data.

The 16S rRNA gene sequence similarities between Llam7^T and related type strains were obtained from EzTaxon e-server [11]. The closest relatives (approximately 50 strains) and *Catellatospora citrea* subsp. *citrea* IMSNU 22008^T as the outgroup, were aligned using SINA aligner (v1.2.11) [12]. Phylogenetic trees were reconstructed using neighbour-joining method [13] including bootstrap analysis with 1000 repeats using the MEGA software version 10.2.4 [14]. NJplot was used to draw the phylogenetic trees expressed in the Newick phylogenetic tree format [15].

The complete 16S rRNA gene sequence of Llam7^T showed high similarity values with members of the family *Micromonosporaceae* in the range of 98.2–99.1%, showing the highest 16S rRNA gene sequence similarity values with *Micromonospora craniellae* LHW63014^T (99.10%) [16], *Micromonospora craterilacus* NA12^T (98.96%) [17], *Micromonospora zingiberis* PLAI 1-1^T (98.86%) [18], *Micromonospora phaseoli* CGMCC 4.7038^T (98.75%) [19], *Salinispora pacifica* CNR-114^T (98.68%) [20] and *Salinispora goodfellowii* CNY-666^T (98.68%) [21]. However, the neighbour-joining tree (Fig. 2) indicated that Llam7^T formed a distinct phylogenetic line with low bootstrap values next to *Verrucosispora endophytica* A-T 7972^T (98.04%) [22] and *Micromonospora deserti* 13K206^T (98.61%) [23]. It appears more closely related to species of the genus *Salinispora* species than to the bulk of species of the genus *Micromonospora*. Members of the genus *Verrucosispora* were affiliated to the genus *Micromonospora* by Nouioui *et al*. [6], but *V. endophytica* A-T 7972^T and also '*Verrucosispora rhizosphaerae*' 2603PH03 and *Verrucosispora sonchi* NEAU-QY3^T were not included in that study, because there was no genome data available at this time.

GENOME FEATURES

Llam7^T was grown aerobically on SYP modified with double strength agar plates (10 g starch, 4 g yeast extract, 2 g soy peptone, 18 g agar and 20 g Tropic Marine Salt in 1 l deionized water) at 30 °C for 2 weeks. Colonies of Llam7^T were used for the DNA extraction applying the Fast DNA SPIN Kit for Soil with LysingMatrixE tubes (MP Biomedicals), following the standard protocol of the manufacturer. DNA was cleaned-up from the agarose gel using an Ultra Clean DNA Purification Kit (MoBio). The extracted DNA had a concentration of 110 ng µl^{-1}. The quality of the DNA was checked with NanoDrop (A_{260}:A_{280} ratio of >1.8, A_{260}:A_{230} ratio of <1.8).

The Llam7^T genome was sequenced with MiSeq using Reagent Kit v3 (600 cycles) (Illumina), and MinION nanopore technology (Oxford Nanopore Technologies) using the MinION Flow-Cell (catalogue number FLO_FLG001) with the Flow Cell Priming Kit (catalogue number EXP-FLP002) and the Rapid Sequencing Kit (catalogue number SQK-RAD004), following the protocols of the manufacturer. The raw Illumina data were trimmed and filtered using Trimmomatic [24]. The high accuracy model of Guppy (Oxford Nanopore Technologies. Version 4.4.0+3a263d4) was used for base calling of the MinION reads. Initially, the MinION data were assembled using Flye [25], then the trimmed Illumina reads were used to improve this assembly using Pilon [26] in four consecutive runs. The annotation was prepared using Prokka and barrnap

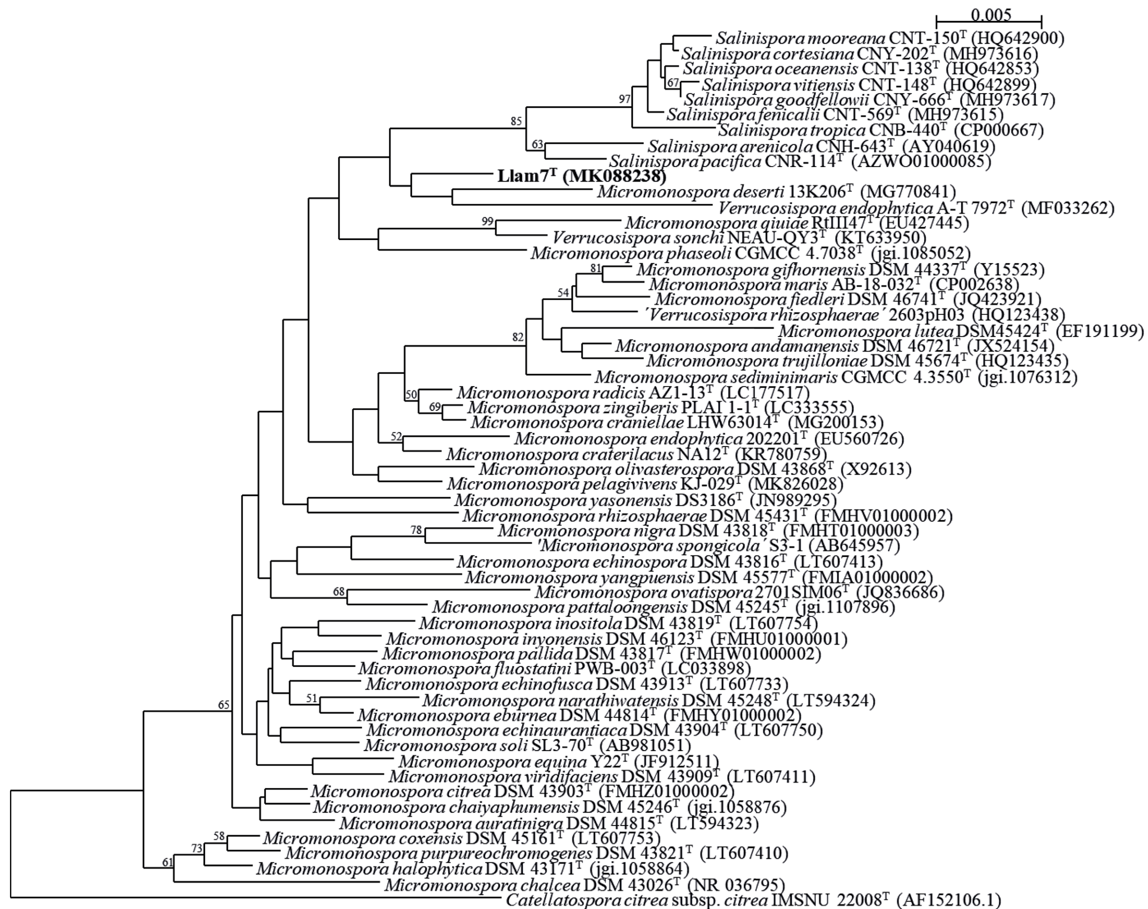

Fig. 2. Neighbour-joining tree of Llam7^T and related strains based on 16S rRNA gene sequences. *Catellatospora citrea* subsp. *citrea* IMSNU 22008^T was used as an outgroup. Only bootstrap values above 50% are given at the branches (1000 replications). Bar, evolutionary distance of 0.005.

[27]. Putative biosynthetic gene clusters (BGC) were identified using antiSMASH 6.0.1 [28].

The size of the genome of Llam7^T was 6.894 Mbs and the DNA G+C content of the genome was 71.4 mol%. Llam7^T has 6524 coding sequences (CDS), 67 tRNA and 9 rRNA genes. Llam7^T genome encodes 18 putative biosynthetic gene clusters (BGCs, Table S1, available in the online version of this article). One of the clusters encodes non-ribosomal peptide synthetase and type I polyketide synthase for the biosynthesis of the apoptosis inducer polyoxypeptin [29], sharing only 56% of the total genes of this pathway. Additionally, another cluster that contains multiple BGC types shared 55% of total genes with the biosynthetic pathway for the antibiotic kendomycin [30]. Llam7^T has one non-ribosomal peptide synthetase cluster encoding enzymes responsible for the production of fluorine-containing metabolites [31]. A type III polyketide synthase exhibits 71% similarity with the alkyl-*O*-dihydrogeranyl-methoxyhydroquinones biosynthetic pathway which is commonly found in all strains of species of the genus *Micromonospora* [32].

An initial phylogenomic tree was calculated using the Genome Taxonomy Database toolkit (GTDB-Tk) pipeline [33]. To improve the relationship, type strains of species related to Llam7^T were selected from the GTDB-Tk and also those that revealed close relations according to the 16S rRNA gene phylogenetic tree (Fig. 2). The type strains of the closest relatives according to Eztaxon services were also included. A phylogenomic tree was reconstructed with the GTDB-Tk pipeline, the type species of the genus *Micromonospora, Micromonospora chalcea* DSM 43026^T and *Catellatospora citrea* DSM 44097^T as the outgroup (Fig. 3). The tree showed that Llam7^T clusters closer to type strains of members of the genus *Micromonospora* than to members of the genus *Salinispora*, specifically to *M. phaseoli* CGMCC 4.7038^T with a 100% bootstrap score. This is supported by digital DNA–DNA hybridization (dDDH) values obtained by using the Genome-to-Genome Distance Calculator (GGDC) [34]. The dDDH value between Llam7^T and *M. phaseoli* CGMCC 4.7038^T (38.40%) is higher in comparison to *M. deserti* 13K206^T (27.40%), clustering with Llam7^T in the 16S rRNA gene sequence phylogenetic tree (Fig. 2), which is not

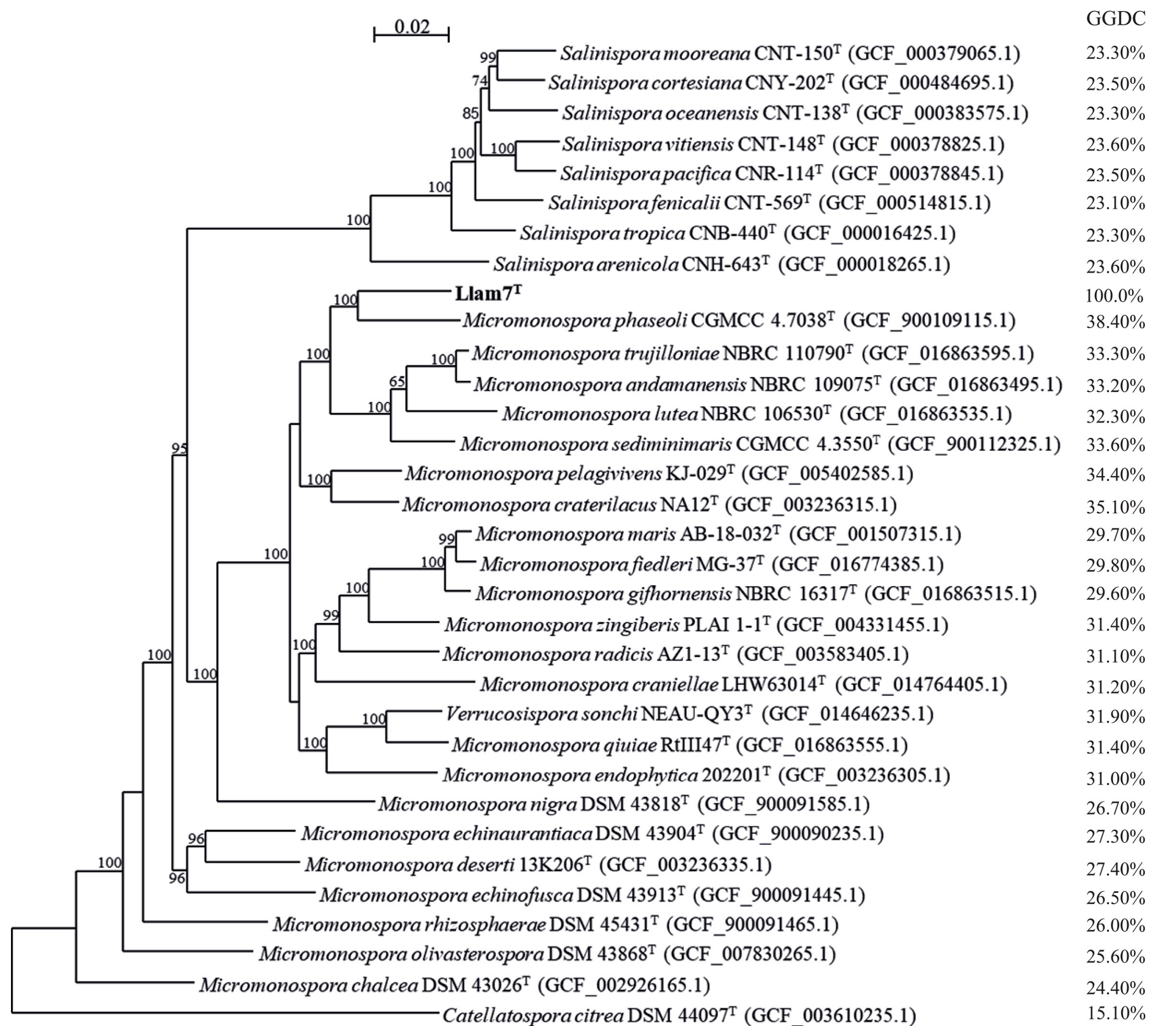

Fig. 3. Phylogenomic tree based on whole genome sequence data of Llam7^T and 31 closely related strains reconstructed using the GTDB-Tk pipeline. Numbers at the nodes indicate bootstrap values. Percentage values to the right of the type strain names and accession number indicate the GGDC (dDDH) values obtained in comparison with Llam7^T. Bar, 0.02 substitutions per nucleotide position.

supported by a bootstrap value exceeding 50%, and to *M. craniellae* LHW63014^T (31.20%), the closest relative according to 16S rRNA gene sequence similarity. The range of dDDH values of Llam7^T against type strains of species of the genus *Salinispora* (23.10–23.60%) and of the genus *Micromonospora* (24.40–38.40%) is below the boundary of 70% [35], indicating that the isolate represents a novel species affiliated to the genus *Micromonospora*. This is also supported by average nucleotide identity by orthology (orthoANI) values that were calculated according to the method of Lee *et al.* [36]. The orthoANI values between Llam7^T and the type strains e.g. *M. phaseoli* CGMCC 4.7038^T (89.31%), *M. deserti* 13K206^T (83.43%), and *M. craniellae* LHW63014^T (85.85%) with an overall range of 79–89% being lower than the suggested boundary (95–96%) for species delineation [36].

The cluster comprising strain Llam7^T, the type strains of *Micromonospora* (formerly *Xiangella*) *phaseoli. Micromonospora pelagivivens*, *Micromonospora craterilacus* and *M. craniellae* as well as *Micromonospora* (formerly *Jishengella*) *zingiberis*, *Micromonospora* (formerly *Jishengella*) *endophytica*, *Micromonospora* (formerly *Verrucosispora*) *andamanensis, Micromonospora* (formerly *Verrucosispora*) *fiedleri*, *Micromonospora* (formerly *Verrucosispora*) *gifhornensis, Micromonospora* (formerly *Verrucosispora*) *lutea, Micromonospora* (formerly *Verrucosispora*) *maris*, *Micromonospora* (formerly *Verrucosispora*) *qiuiae*, *Micromonospora* (formerly *Verrucosispora) sediminis, Micromonospora trujilloniae* (formerly *Verrucosispora wenchangensis*), *Verrucosispora endophytica, Verrucosispora rhizosphaerae* and *Verrucosispora sonchi*, branched from *Micromonospora nigra* (Fig. 2). Interestingly this cluster is separated from other species of the genus *Micromonospora* species together with the group of type strains from the genus *Salinispora*.

As phages are of great importance for bacteria by providing ecological and evolutionary benefits e.g. as agents of horizontal gene transfer, as sources of genetic variation and as

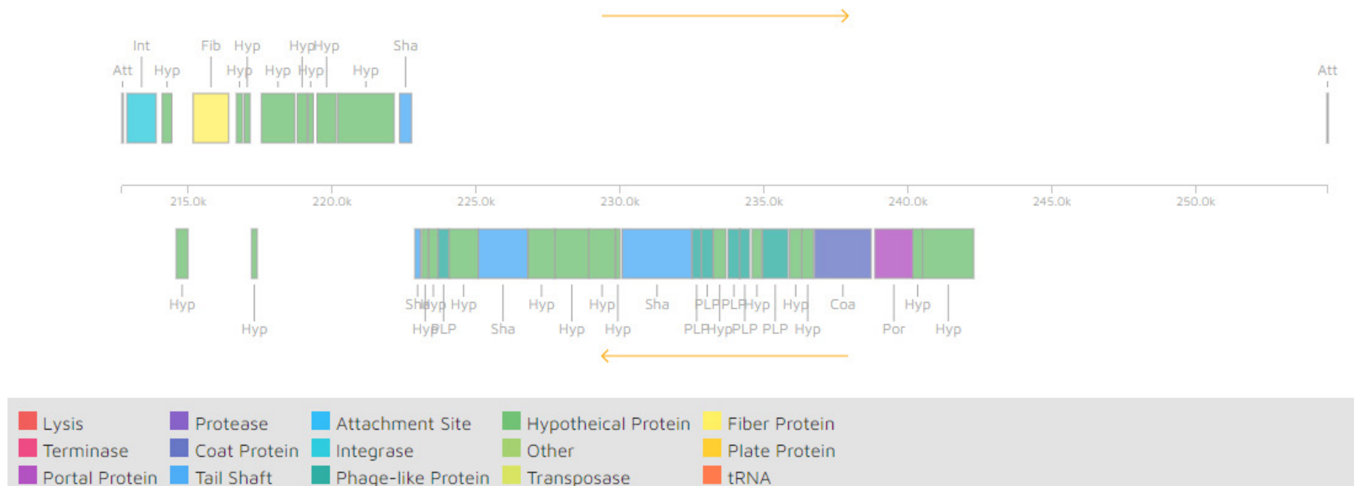

Fig. 4. Detailed graphical representation of phage regions in the genome of Llam7^T with individual loci coloured according to annotations, generated by PHASTER. Arrows indicate strand direction. ATT, attachment site; Int, integrase; Hyp, hyphothetical protein; Fib, fibre protein; Sha, tail protein, PLP, phage-like protein; Coa, head protein, Por, portal protein.

weapons of bacterial competition [37], the presence of phages in the genome of Llam7^T was examined. PHAge Search Tool Enhanced (PHASTER) [38] and Prophage Hunter [39] (with similarity matching) were used to detect and annotate prophage sequences within the genome assembly. Identified intact prophage regions annotated as coding for hypothetical proteins were additionally classified with machine-learning algorithms using the ensemble predictor STEP3 [40]. VIRFAM [41] was used for additional homology detection of viral protein superfamilies, complementing the classification and viral proteomic tree generation for the top 200 related phages with VipTree [42]. One intact prophage region was identified. This region comprised 41.8 kb and contained two attachment sites and 37 protein coding sequences of which 14 could be reliably annotated as integrase, terminase, phage major capsid, tail and portal proteins, as well as other unannotated protein coding sequences from closely related temperate phage genomes infecting members of the genus *Streptomyces* (Fig. 4). Of the remaining 23 hypothetical proteins, 13 could be additionally assigned to other tail protein components of the phage machinery, albeit with low sequence similarity to distantly related phages. On the basis of the classification of head–neck–tail proteins and genomic similarity to phages infecting members of the genus *Streptomyces*, we can assume this is an inducible dsDNA prophage of the family *Siphoviridae* of Type 1 (Cluster 3).

PHYSIOLOGY AND CHEMOTAXONOMY

A broad range of phenotypic properties of Llam7^T was studied, including morphological and physiological characteristics and the ability to adapt to ranges of pH, temperature and salinity. Cells grown in SYP liquid medium for 5 days at 26 °C with shaking at 120 r.p.m. were used for Gram-staining by applying Colour Gram 2 (bioMérieux) according to the manufacturer's instructions. Motility was studied by light microscopy. The cell morphology was examined using light and scanning electron microscopy. For scanning electron microscopy Llam7^T was grown for 21 days on SYP agar plates at 26 °C. Three colonies were cut from the agar plate, dehydrated with an ascending ethanol series (50, 70, 90% and three times at 100% for 10 min each) [43] and critical-point dried with carbon dioxide and sputter-coated with Au/Pb.

Colonies on solid SYP medium grown for 14 days at 28 °C were elevated, orange, hard, wrinkled and dry. Old colonies grown for more than 6 weeks could penetrate into the agar, while young ones (1–2 week) could be easily removed from the agar surface. The diameter of the colonies was 2–4 mm. Cells obtained from these colonies were Gram-staining-positive filaments ranging from 8 to 25 µm in length and from 0.2 to 0.3 µm in diameter. Cells cultured on SYP agar plates observed with SEM were long filaments from 10 to 30 µm in length and 0.2 to 0.3 µm in width. In addition, spherical spores were formed, 0.7 to 1.4 µm in diameter (Fig. 5).

Growth responses to temperature and pH variation were tested on SYP medium for 30 days. The temperature for growth ranged from 15 to35 °C with an optimum of 30–35 °C, no growth was observed above 35 °C. The pH range for growth of Llam7^T was from pH 6 to pH 12 with an optimum at 7 to 8. Salt-depended growth was investigated after incubation at 26 °C for 30 days on SYP agar medium, supplemented with NaCl and Tropic Marine Salt, respectively, at concentrations of 0, 1, 2.5, 5, 7.5 and 10%. Llam7^T grew with a range from 0–5% NaCl and Tropic Marine Salt with an optimum at 1% NaCl and 1–2.5% Tropic Marine Salt. Llam7^T showed weak growth in the absence of NaCl (0%) and Tropic Marine Salt during long periods of incubation (up to 30 days).

Physiological characteristics of Llam7^T including enzymatic activities were tested using API 20E (bioMérieux), including the oxidase assay, and API ZYM (bioMérieux) according to

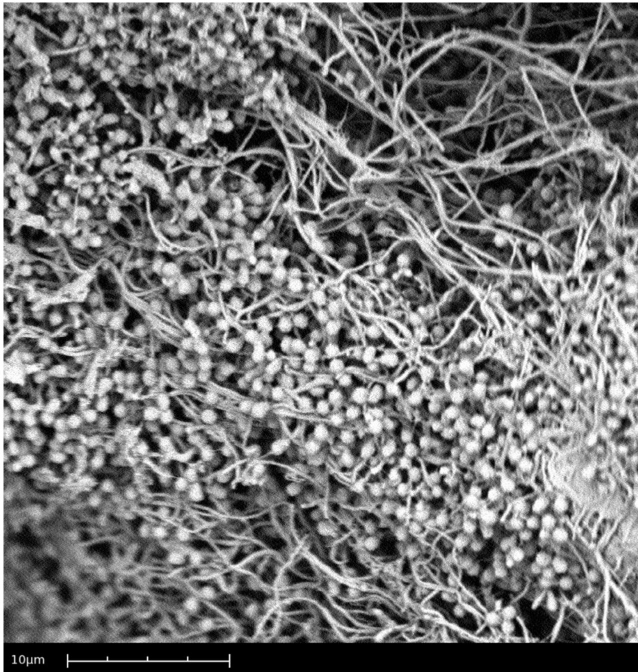

Fig. 5. Scanning electron micrograph of Llam7^T after cultivation on SYP agar medium for 21 days plates at 26 °C. The spherical spores exhibited a diameter up to four times higher than that of the cells. Bar, 10 μm

the manufacturer's instructions. Carbon source utilization tests were prepared using minimal medium supplemented independently with starch, trehalose, mannitol, glucose, and *N*-acetylglucosamine (1 g $NaNO_3$, 1 g K_2HPO_4, 1 g KCl, 0.5 g $MgSO_4.7H_2O$, 15 g Tropic Marine Salt, 15 g Bacto-Agar and 5 g carbon source in 1 l deionized water). All tests were made in duplicate. The metabolic characteristics of Llam7^T showed positive activity in the tests for alkaline phosphatase, esterase (C 4), esterase lipase (C 8), lipase (C 14), leucine arylamidase, valine arylamidase, acid phosphatase, naphthol-AS-Bl-phosphohydrolase, Voges–Proskauer test (production of acetoin) and gelatinase but were negative for activities of α-galactosidase, β-glucoronidase, α-glucosidase, α-fucosidase, β-galactosidase, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, citrate utilization, sulphide production, tryptophan deaminase and indole production. Llam7^T grew using starch and trehalose as carbon sources.

The profile of the cellular fatty acids was studied using GC-analysis according to the Microbial Identification System (MIDI) [44]. The determination of the respiratory quinones and polar lipids followed the procedure as described by Tindall [45, 46] and Tindall *et al.* [47], respectively. The identification of diamino acids was performed according to the protocol of Rhuland *et al.* [48]. The whole-cell sugars were identified according to the method of Staneck and Roberts [49]. These experiments were carried out by the German Collection of Microorganisms and Cell Cultures (DSMZ, Braunschweig, Germany). Components of the fatty acid profile are shown

Table 1. Fatty acid profile of strain Llam7^T

Fatty acid	Content (%)
Saturated:	
$C_{15:0}$	1.3
$C_{16:0}$	2.2
$C_{17:0}$	6.6
$C_{17:0}$ 10-methyl	1.1
$C_{18:0}$	2.1
Branched-chain:	
iso-$C_{14:0}$	0.5
iso-$C_{15:1}$G	1.5
iso-$C_{15:0}$	13.6
anteiso-$C_{15:0}$	7.8
iso-$C_{16:1}$G	1.9
iso-$C_{16:0}$	12.6
anteiso-$C_{17:1}$A	2.5
iso-$C_{17:0}$	4.6
anteiso-$C_{17:0}$	11.5
Monounsaturated:	
$C_{17:1}$iso ω9*c*	2.6
$C_{17:1}$ω8*c*	18.2
$C_{18:1}$ ω9*c*	6.1
Others	
Summed feature 3	2.5

*Summed features represent two or three fatty acids that cannot be separated by the Microbial Identification System. Summed feature 3 represents iso-$C_{15:1}$ 2-OH and/or $C_{16:1}ω7c$.

in Table 1. The dominant fatty acids present in Llam7^T were $C_{17:1}$ ω8*c* (17.2%), iso-$C_{15:0}$ (13.6%), iso-$C_{16:0}$ (12.6%), and anteiso-$C_{17:0}$ (11.5%) which amounted to approximately 59.5% of the total fatty acids. The major respiratory quinones were MK-9(H_6) with 52% and MK-9(H_4) with 42%. As polar lipids diphosphatidylglycerol (DPG), phosphatidylethanolamine (PE), phosphatidylglycerol, together with eight unidentified lipids, i.e. one aminolipid, two glycolipids, one aminoglycolipid, one phosphoglycolipid, and three phospholipids, were detected (Fig. S1). The diamino acids present in the peptidoglycan layer are *meso*-DAP and LL-DAP. The whole cells of Llam7^T contained glucose, mannose, xylose and ribose as major sugars.

The chemotaxonomic features of Llam7^T were compared with those of closely related species (Table 2). Llam7^T exhibited characteristics different from those of other species of the genus *Micromonospora*. Most significantly, it was the only strain containing LL-DAP in the cell wall in addition to

Table 2. Differential characteristics of Llam7ᵀ and the type strains of closely related species of the genus *Micromonospora*

Strains: 1, Llam7ᵀ; 2, *Micromonospora phaseoli* CGMCC 4.7038ᵀ; 3, *Micromonospora craniellae* LHW63014ᵀ; 4, *Micromonospora deserti* 13K206ᵀ.

Characteristic	1	2	3	4
Isolation source	Hypersaline lake	Plant root	Marine sponge	Desert soil
Genome size	6.9 Mb	6.5 Mb	6.8 Mb	6.6 Mb
DNA G+C content (%)	71.39	71.33	70.91	72.44
Diamino acid(s)	*m*-DAP, LL-DAP	*m*-DAP	*m*-DAP	*m*-DAP, 3-OH-DAP
Whole-cell sugars*	Glc, Man, Xyl, Rib	Gal, Man, Glc	Glc, Xyl, Rib, Man	Ara, Gal, Glc, Man, Rib, Rha, Xyl
Major fatty acids	$C_{17:1}$ω8*c*, iso-$C_{15:0}$, iso-$C_{16:0}$, anteiso-$C_{17:0}$	$C_{16:0}$, $C_{18:0}$, $C_{17:1}$ω7*c*, iso-$C_{15:0}$, $C_{17:0}$	iso-$C_{16:0}$, iso-$C_{15:0}$, $C_{17:1}$ω8*c*, $C_{18:1}$ω9*c*	iso-$C_{16:0}$
Major menanquinone(s)	MK-9(H4) MK-9(H6)	MK-9(H4) MK-9(H6)	MK-9(H4)	MK-9(H4) MK-9(H6) MK-9(H2).
Polar lipids†	PG, PE, DPG, GL, AL, PL, GNL, PGL	PME, PE, PC, PI, PIM	PE, DPG, PI, PL, PIM, GL, PL, L	DPG, PE, PG, PI
Reference	This study	Wang *et al.* 2013 [19]	Li *et al.* 2019 [16]	Saygin *et al.* 2020 [23]

*Glc, glucose; Man, mannose; Xyl, xylose; Rib, ribose; Gal, galactose; Ara, arabinose; Rha, rhamnose.
†PG, phosphatidylglycerol; PE, phosphatidylethanolamine; DPG, diphosphatidylglycerol; GL, glycolipid; AL, aminolipid; PL, phospholipid; GNL, aminoglycolipid; PGL, phosphoglycolipid; PME, phosphatidylmethylethanolamine; PC, phosphatidylcholine; PI, phosphatidylinositol; PIM, phosphatidylinositol mannoside; L, lipid.

meso-DAP. Furthermore, Llam7ᵀ showed different whole-cell sugars, fatty acids and the polar lipid pattern compared with *M. phaseoli* CGMCC 4.7038ᵀ, *M. craniellae* LHW63014ᵀ and *M. deserti* 13K206ᵀ.

On the basis of chemotaxonomic characteristics, genomic data and phylogenomic analyses strain Llam7ᵀ is proposed to represent a novel species of the genus *Micromonospora*, for which the name *Micromonospora tarapacensis* sp. nov. is proposed.

DESCRIPTION OF *MICROMONOSPORA TARAPACENSIS* SP. NOV.

Micromonospora tarapacensis (ta.ra.pa.cen'sis. N.L. fem. adj. *tarapacensis*, pertaining to Tarapacá, a region in Chile where Salar de Llamará is located).

Cells grown in liquid medium are long filaments from 10 to 30 µm in length and 0.2 to 0.3 µm in width. Single non-motile spherical spores with a diameter between 0.7 to 1.4 µm are formed. Growth optima are at 30–35 °C and at pH 7–8. Growth range is from 15 to 35 °C and from pH 6 to pH 12 and from 0 to 5% of NaCl and Tropic Marine Salt with growth optima at 1% and 1–2.5% respectively. Cells grow aerobically, using starch and trehalose, and produce trypsin, *N*-acetyl-β-glucosaminidase, α-mannosidase, alkaline phosphatase, esterase (C 4), esterase lipase (C 8), lipase (C 14), leucine arylamidase, valine arylamidase, acid phosphatase, naphthol-AS-Bl-phosphohydrolase, gelatinase and acetoin. The peptidoglycan contains *meso*-diaminopimelic acid and LL-2,6 diaminopimelic acid, while the whole-cell sugars present are glucose, mannose, xylose and ribose. The polar lipids are phosphatidylglycerol, phosphatidylethanolamine, diphosphatidylglycerol and unidentified lipids belonging to the glycolipids, aminolipids, phospholipids, aminoglycolipids and phosphoglycolipids. The predominant menaquinones are MK-9(H_6) and MK-9(H_4). Major cellular fatty acids are $C_{17:1}$ω8*c*, iso-$C_{15:0}$, iso-$C_{16:0}$ and anteiso-$C_{17:0}$.

The type strain Llam7ᵀ (=DSM 109510ᵀ=LMG 31023ᵀ) was isolated from the hypersaline Salar de Llamará, Chile. The DNA G+C content of the DNA of the type strain is 71.39 mol% and the genome size is 6.9 Mb. The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequence and the genome sequence of Llam7ᵀ are MK088238 and JAHCDI000000000, respectively.

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Author contributions
A.S.V.: Conceptualization, formal analyses (microbiology, bioinformatics), investigation, writing original draft; J.W.: Conceptualization, validation, reviewing and editing, E.B.: Formal analyses (genomics),

validation; T.R.: Formal analysis (microbiology); B.M.S.: Formal analyses (assembling, data curation), validation; L.X.S.: Formal analyses (genomics); S.K.: Formal analyses (sequencing); J.F.I.: Conceptualization, supervision, revision; C.D.: Resources. All authors were involved in the processes of writing and reviewing.

Conflicts of interest

The authors declare that there are no conflicts of interest.

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