### Title

Six novel species of the obligate marine actinobacterium *Salinispora*, *Salinispora cortesiana* sp. nov., *Salinispora fenicalii* sp. nov., *Salinispora goodfellowii* sp. nov., *Salinispora mooreana* sp. nov., *Salinispora oceanensis* sp. nov. and *Salinispora v*...




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### Authors

Román-Ponce, Brenda
Millán-Aguiñaga, Natalie
Guillen-Matus, Dulce
et al.



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TAXONOMIC DESCRIPTION

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# Six novel species of the obligate marine actinobacterium *Salinispora*, *Salinispora cortesiana* sp. nov., *Salinispora fenicalii* sp. nov., *Salinispora goodfellowii* sp. nov., *Salinispora mooreana* sp. nov., *Salinispora oceanensis* sp. nov. and *Salinispora vitiensis* sp. nov., and emended description of the genus *Salinispora*

Brenda Román-Ponce[1], Natalie Millán-Aguiñaga[2], Dulce Guillen-Matus[3], Alexander B. Chase[3], Joape G.M. Ginigini[4], Katy Soapi[4], Klaus D. Feussner[4], Paul R. Jensen[3] and Martha E. Trujillo[1,*]

**Abstract**

Ten representative actinobacterial strains isolated from marine sediments collected worldwide were studied to determine their taxonomic status. The strains were previously identified as members of the genus *Salinispora* and shared >99% 16S rRNA gene sequence similarity to the three currently recognized *Salinispora* species. Comparative genomic analyses resulted in the delineation of six new species based on averagenucleotide identity and digital DNA–DNAhybridization values below 95 and 70 %, respectively. The species status of the six new groups was supported by a core-genome phylogeny reconstructed from 2106 orthologs detected in 118 publicly available *Salinispora* genomes. Chemotaxonomic and physiological studies were used to complete the phenotypic characterization of the strains. The fatty acid profiles contained the major components iso-$C_{16:0}$, $C_{15:0}$, iso-$_{17:0}$ and anteiso $C_{17:0}$. Galactose and xylose were common in all whole-sugar patterns but differences were found between the six groups of strains. Polar lipid compositions were also unique for each species. Distinguishable physiological and biochemical characteristics were also recorded. The names proposed are *Salinispora cortesiana* sp. nov., CNY-202$^T$ (=DSM 108615$^T$=CECT 9739$^T$); *Salinispora fenicalii* sp. nov., CNT-569$^T$ (=DSM 108614$^T$=CECT 9740$^T$); *Salinispora goodfellowii* sp. nov., CNY-666$^T$ (=DSM 108616$^T$=CECT 9738$^T$); *Salinispora mooreana* sp. nov., CNT-150$^T$ (=DSM 45549$^T$=CECT 9741$^T$); *Salinispora oceanensis* sp. nov., CNT-138$^T$ (=DSM 45547$^T$=CECT 9742$^T$); and *Salinispora vitiensis* sp. nov., CNT-148$^T$ (=DSM 45548$^T$=CECT 9743$^T$).

## INTRODUCTION

The family *Micromonosporaceae* is a member of the order *Micromonosporales* [1] in the phylum *Actinobacteria*. This taxon consists of bacteria that stain Gram-positive and form non-fragmenting, branched and septate substrate hyphae; aerial mycelium being absent or scant. Members of *Micromonosporaceae* typically produce spores which may be motile or non-motile. These bacteria are further defined as aerobic, non-acid fast and mesophilic. Colonies have flat to elevated morphologies with smooth or wrinkled surfaces and show a variety of pigmentation. Many strains produce carotenoid mycelial pigments giving the colonies an orange to red appearance [2]. The type genus of the family is *Micromonospora* [1].

The genus *Salinispora* was placed in the family *Micromonosporaceae* as part of the description of *Salinispora arenicola* and *Salinispora tropica* [3]. Subsequently, a third species (*Salinispora pacifica*) was named [4]. Salinisporae were recognized as unique among actinobacteria in that strains failed to grow when seawater was replaced with deionized water in the

**Author affiliations:** [1]Departamento de Microbiología y Genética, Campus Miguel de Unamuno, Universidad de Salamanca, 37007 Salamanca, Spain; [2]Universidad Autónoma de Baja California, Facultad de Ciencias Marinas, Ensenada, Baja California, Mexico; [3]Center for Marine Biotechnology and Biomedicine, Scripps Institution of Oceanography, University of California San Diego, La Jolla, California, USA; [4]The University of South Pacific, Faculty of Science, Technology and Environment, Institute of Applied Sciences, Suva, Fiji.
***Correspondence:** Martha E. Trujillo, mett@usal.es
**Keywords:** Salinispora; obligate marine; Actinobacteria.
The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequence and draft whole genome sequences for strains CNY-202$^T$, CNT-569$^T$, CNY-666$^T$, CNT-150$^T$, CNT-138$^T$ and CNT-148$^T$ are: MH973616, MH973615, MH973617, HQ642900, HQ642853 and HQ642899 and NZ_AXVR00000000, NZ_AZWQ00000000, jgi_2563366532, NZ_AQZW0000000, NZ_ARTO00000000 and NZ_AQZE00000000, respectively.
Eight supplementary figures and five supplementary tables are available with the online version of this article.

growth medium [5]. Further investigations provided evidence for the genetic underpinnings of this phenotype, linking the loss of the mechanosensitive channel of large conductance gene (*mscL*) to the lack of growth following transfer to low osmotic strength media [6, 7]. Subsequent studies defined specific ions required for growth [8]. Members of the genus have been cultured from marine sediments collected around the globe [9], marine sponges [10] and other marine organisms [11]. Notably, salinisporae are a rich source of secondary metabolites and have become model organisms in the study of natural product biosynthesis and the development of new methods for natural product discovery [12].

Within the family *Micromonosporaceae*, *Salinispora* is closely related to *Micromonospora*, a bacterial genus that has been reported from both terrestrial habitats [13] and marine samples [3]. While *Salinispora* fail to grow when seawater is replaced with deionized water in the growth medium, *Micromonospora* strains with this physiological trait have yet to be reported, although some strains can tolerate up to 7% (w/v) NaCl [14]. Apart from this important physiological distinction, the two micro-organisms share many commonalities, including morphological and chemotaxonomic traits, and thus cannot be easily distinguished based on phenotype features alone [1]. At the genetic level, *Micromonospora* and *Salinispora* strains also share high 16S rRNA gene sequence similarity, thus creating challenges in resolving their phylogenetic relationships using this conserved marker. However, a recent classification of the family *Micromonosporaceae* based on whole-genome sequencing provided support for the separation of the two genera [15]. Moreover, subsequent phylogenomic analyses using additional *Micromonospora* type strains and *Salinispora* [13] further supported the conclusions reached by Carro and colleagues and revealed that *Micromonospora pattaloongensis* and *Micromonospra pisi*, which clade outside of the *Micromonospora*/*Salinispora* lineage, may need to be reclassified as a third genus.

Following precedent for the application of whole-genome sequencing to resolve phylogenetic relatedness among closely related strains, we sought to investigate species delineations within the genus *Salinispora*. Recent studies have suggested using >95% average nucleotide identity (ANI) values to demarcate species boundaries [16, 17]. Indeed, a comparative genomic analysis of 119 *Salinispora* strains confirmed the established species designations of *S. arenicola* and *S. tropica*; whereas the larger clade encompassing *S. pacifica* seemingly provided an underestimation of species relationships [18]. Therefore, the aim of this study was to employ polyphasic approaches to establish the taxonomic status within *Salinispora* by connecting fine-scale genetic diversity to phenotypic differences. Our results indicate that the members of the genus *Salinispora* analyzed here comprise nine species, including *S. arenicola* and *S. tropica*, and the reclassification of the larger clade that includes *S. pacifica* into six additional species.

## HOME HABITAT AND ISOLATION

Ten representative actinobacterial strains previously identified as members of the genus *Salinispora* were selected for this study based on the results of a prior comparative genomic analysis [18]. The strains were originally recovered from marine sediment samples collected from the Island of Palau in 2004 (CNS-237 and CNR-942), Fiji in 2006 (CNS-801, CNT-029, CNT-138$^T$, CNT-148$^T$, CNT-150$^T$) and 2008 (CNT-569$^T$), the Sea of Cortez in 2008 (CNY-202$^T$), and the Madeira Islands in 2012 (CNY-666$^T$) (Table 1). The strains were isolated using either dry/stamping or heat shock methods followed by plating on medium A1 (1% soluble starch, 0.4% yeast extract, 0.2% peptone, 1.6%

**Table 1.** Origin and geographic coordinates of the marine sediments from which the strains were derived

| Strain | Sample no. | Origin | Isolation date | Isolation medium* | GPS coordinates |
|---|---|---|---|---|---|
| CNT-138 (DSM 45547)$^T$ | FJ06_138 #7 | Fiji | July 2006 | SWA | 18° 45.667 S 178° 01.089 E |
| CNT-029 | FJ06_30 #5 | Fiji | July 2006 | SWA | 18° 24.374 S 178° 09.494 E |
| CNT-148 (DSM 45548)$^T$ | FJ06_154 #9 | Fiji | July 2006 | A1 | 18° 47.151 S 178° 33.155 E |
| CNS-801 | FJ06-84 #1 | Fiji | July 2006 | A1 | 18° 42.806 S 178° 29.438 E |
| CNT-150 (DSM 45549)$^T$ | FJ06_32 #1 | Fiji | July 2006 | SWA | 18° 25.301 S 178° 08.453 E |
| CNS-237 | PL04-118 #2A | Palau | March 2004 | 10% A1 | NA |
| CNT-569$^T$ | FJ08-173 #2 | Fiji | February 2008 | SWA | 18° 15.26 S 178° 05.10 E |
| CNR-942 | PL04-003 #1A | Palau | March 2004 | 10% A1 | 07° 16 N 134° 28 E |
| CNY-202$^T$ | AMS-301 | Sea of Cortez, Mexico | July 2008 | NA | 25.9503217 N 111.306283 W |
| CNY-666$^T$ | MD12-107A | Madeira Island, Portugal | June 2012 | 50% A1 | 32° 38.901 N 16° 49.365 W |

*SWA, seawater agar (natural seawater, 1.6% agar); M1, medium 1 [46]; A1 (peptone 0.2%, yeast extract 0.4%, starch 1%, Instant Ocean or natural seawater, agar 1.6%). NA, not available.

agar, 100% natural seawater) or SWA agar (1.6% agar, 100% natural seawater) and incubation for up to 16 weeks at room temperature as previously described [4, 19, 20]. Colonies were repeatedly streaked onto A1 medium until pure cultures were achieved as evidenced by uniform colony morphology. Purified strains were maintained at −80 °C in medium A1 (without agar) supplemented with 10% glycerol as cryoprotectant.

## 16S rRNA GENE PHYLOGENY

To confirm affiliation with the genus *Salinispora*, we assessed the 16S rRNA gene of the 10 strains. Genomic DNA was obtained with the REDExtract-N-Amp Plant PCR kit (Sigma), amplified and sequenced as described previously [21]. The sequences for strains CNS-237, CNT-148$^T$ and CNT-029 were previously determined [20, 22] and directly downloaded from GenBank. Pairwise similarities between the 10 *Salinispora* strains and the type strains representing the three currently recognized *Salinispora* species (*S. pacifica* CNR-114$^T$, *S. tropica* CNB-440$^T$ and *S. arenicola* CNH-643$^T$) were calculated using EzTaxon-e (http://eztaxon-e.ezbiocloud.net/) and aligned with CLUSTAL X (2.0) software [23]. Evolutionary distances were calculated according to Kimura's two-parameter model [24] and phylogenetic trees reconstructed using the neighbour-joining [25] and maximum-likelihood methods [26] using the MEGA7 platform [27].

The 10 isolates shared >99% 16S rRNA gene sequence similarity to the three *Salinispora* type strains and the highest sequence similarity (99.8–99.9%) to *S. pacifica* CNR-114$^T$ (Table S1, available in the online version of this article). A maximum-likelihood phylogeny reveals that the three *Salinispora* type strains form a well-supported clade that is distinct from representatives of the most closely related genera in the family *Micromonosporaceae* (Fig. 1). In this topology, all 10 strains belong to a large clade that includes *S. pacifica* CNR-114$^T$; however, the phylogenetic relationships among these strains remain poorly resolved. A similar topology was observed using the neighbour-joining algorithm to reconstruct the tree (not shown).

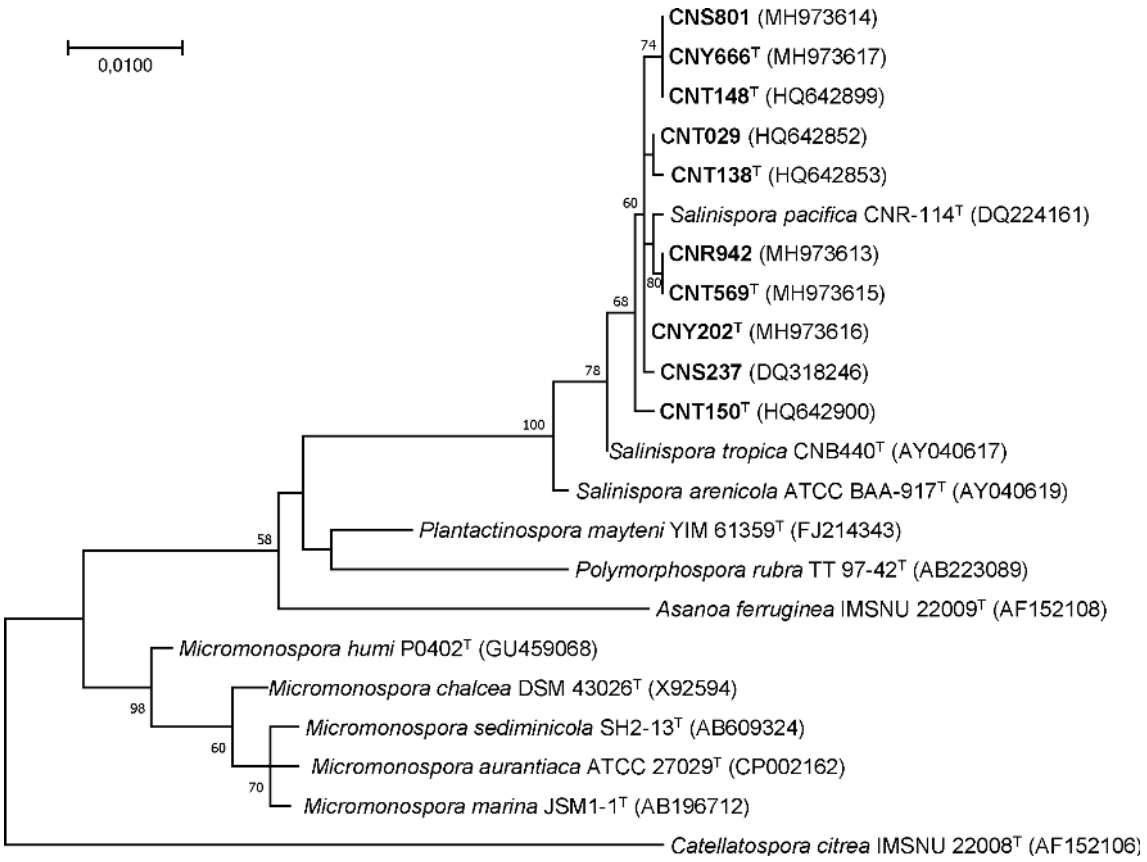


**Fig. 1.** Maximum-likelihood tree based on 16S rRNA gene sequences showing the relationships between the 10 *Salinispora* strains examined in this study, the currently described *Salinispora* species and select representatives of the family *Micromonosporaceae*. The Kimura two-parameter method was used to calculate distances. A total of 1323 positions were used following the elimination of gaps and missing data. Evolutionary analyses were conducted in MEGA 7. Bootstrap values above 50% are shown at branching points. Bar, 0.01 substitutions per nucleotide position.

**Table 2.** Genome characteristics of the *Salinispora* strains studied here and the *Salinispora arenicola*, *Salinispora pacifica* and *Salinispora tropica* type strains

| Strain | Genome size (Mb) | No. of Contigs | CDS | G+C content (mol%) | Sequencing depth | N50 value | Accession numbers | Reference |
|---|---|---|---|---|---|---|---|---|
| *Salinispora arenicola* CNH-643$^T$ | 6.61 | 85 | 5056 | 69.4 | 289× | NA | NZ_VFOL00000000 | [47] |
| *Salinispora tropica* CNB-440$^T$ | 5.18 | 1 | 4664 | 69.5 | NA | 5183331 | NC_009380 | [30] |
| *Salinispora pacifica* CNR-114$^T$ | 5.87 | 101 | 5521 | 69.7 | NA | 137332 | NZ_AZWO00000000 | [18] |
| CNT-138$^T$ | 5.45 | 15 | 4981 | 69.7 | NA | 1212913 | NZ_ARTO00000000 | [18] |
| CNT-029 | 5.24 | 45 | 4847 | 69.7 | NA | 326700 | NZ_AZWB00000000 | [18] |
| CNT-148$^T$ | 5.10 | 10 | 4888 | 69.9 | NA | 1829387 | NZ_AQZE00000000 | [18] |
| CNS-801 | 4.97 | 39 | 4668 | 69.9 | 321× | NA | jgi_2561511036 | [18] |
| CNT-150$^T$ | 5.20 | 37 | 5060 | 69.3 | NA | 512538 | NZ_AQZW0000000 | [18] |
| CNS-237 | 5.24 | 77 | 4865 | 69.4 | NA | 235599 | NZ_AUGH00000000 | [18] |
| CNT-569$^T$ | 5.23 | 48 | 4856 | 69.2 | NA | 229397 | NZ_AZWQ00000000 | [18] |
| CNR-942 | 5.47 | 60 | 5086 | 69.1 | NA | 180850 | NZ_ARGG00000000 | [18] |
| CNY-202$^T$ | 5.18 | 204 | 5016 | 69.6 | NA | 70206 | NZ_AXVR00000000 | [18] |
| CNY-666$^T$ | 5.73 | 106 | 5323 | 70.0 | 287× | NA | jgi_2563366532 | [18] |

NA, Not available.

## OVERALL GENOMIC RELATEDNESS INDICES AND PHYLOGENOMICS

Overall genomic relatedness indices [28] were used to assess the species level relationships among the *Salinispora* strains following the recommendation to use genome data for the taxonomy of prokaryotes [29]. Genome sequencing, assembly and annotation were performed as described previously [18, 30]. The genome sizes ranged from 4.97 Mb (CNS-801) to 6.61 Mb (*S. arenicola* CNH-643$^T$) with an average genome size of~5.5 Mb. The number of coding DNA regions (CDS) varied from 4668 (strain CNS-801) to 5521 (strain CNR-114$^T$) (Table 2). G+C content was obtained directly from the genome sequence data output and ranged from 69.1 to 70.0 mol%.

Digital DNA–DNA hybridization (dDDH) values were calculated with the Genome-to-Genome Distance Calculator (GGDC) version 2.0 available at https://ggdc.dsmz.de/ggdc.php following the settings previously recommended [31] and used to create a dDDH heatmap with the ComplexHeatmap R package version 1.17.1 [32]. The dDDH values provide initial support for the delineation of new *Salinispora* species (Fig. 2), specifically the delineation of the large clade that includes *S. pacifica* CNR-114$^T$ into six additional species. These new species were most closely related to *S. pacifica* CNR-114$^T$, with dDDH values between 45.5–56.4%, followed by *S. tropica* CNB-440$^T$(41.4–44.5%) and *S. arenicola* CNH-643$^T$(32.2–33.4%) (Table S2). The G+C content for the 10 strains ranged from 69.1 to 70.0 mol%, which is well within the range reported for the three currently recognized species (*S. arenicola*, *S. tropica* and *S. pacifica*).

To support our classifications of the six proposed *Salinispora* species, we collated all publicly available *Salinispora* genomes (*N*=118), including the 10 strains assessed in this study. We first extracted the full-length 16S rRNA gene sequences from the genomes using Barrnap (https://github.com/tseemann/barrnap) and aligned each sequence with SINA [33]. Pairwise comparisons of the fully aligned 16S rRNA gene region revealed high sequence similarity (>99%) across all genomes, highlighting the difficulty in resolving fine-scale genetic diversity using conserved marker genes [34] . For instance, when we reconstructed a 16S rRNA gene phylogeny using RAxML version 8.0.0 [35], we found little taxonomic support and limited genetic resolution separating strains within *Salinispora* (Fig. 3a).

We sought to use whole-genome sequence data to resolve taxonomic relatedness within *Salinispora*. First, we assessed the core genome of 118 *Salinispora* strains by identifying all orthologous protein-coding genes. Orthologs were predicted using ROARY [36] with a minimum sequence identity of 85%. The resulting 2106 orthologs were aligned with CLUSTAL_O version 1.2.3 [37] and screened for complete codon reading frames (i.e. multiple of 3 bp). The resulting 2011 orthologs were concatenated to create a 2.1 Mbp core genome for phylogenetic analysis with RAxML version 8.2.10 [35] using a general time reversal model with a gamma distribution for 100 replicates. The core genome phylogeny (Fig. 3b) clearly

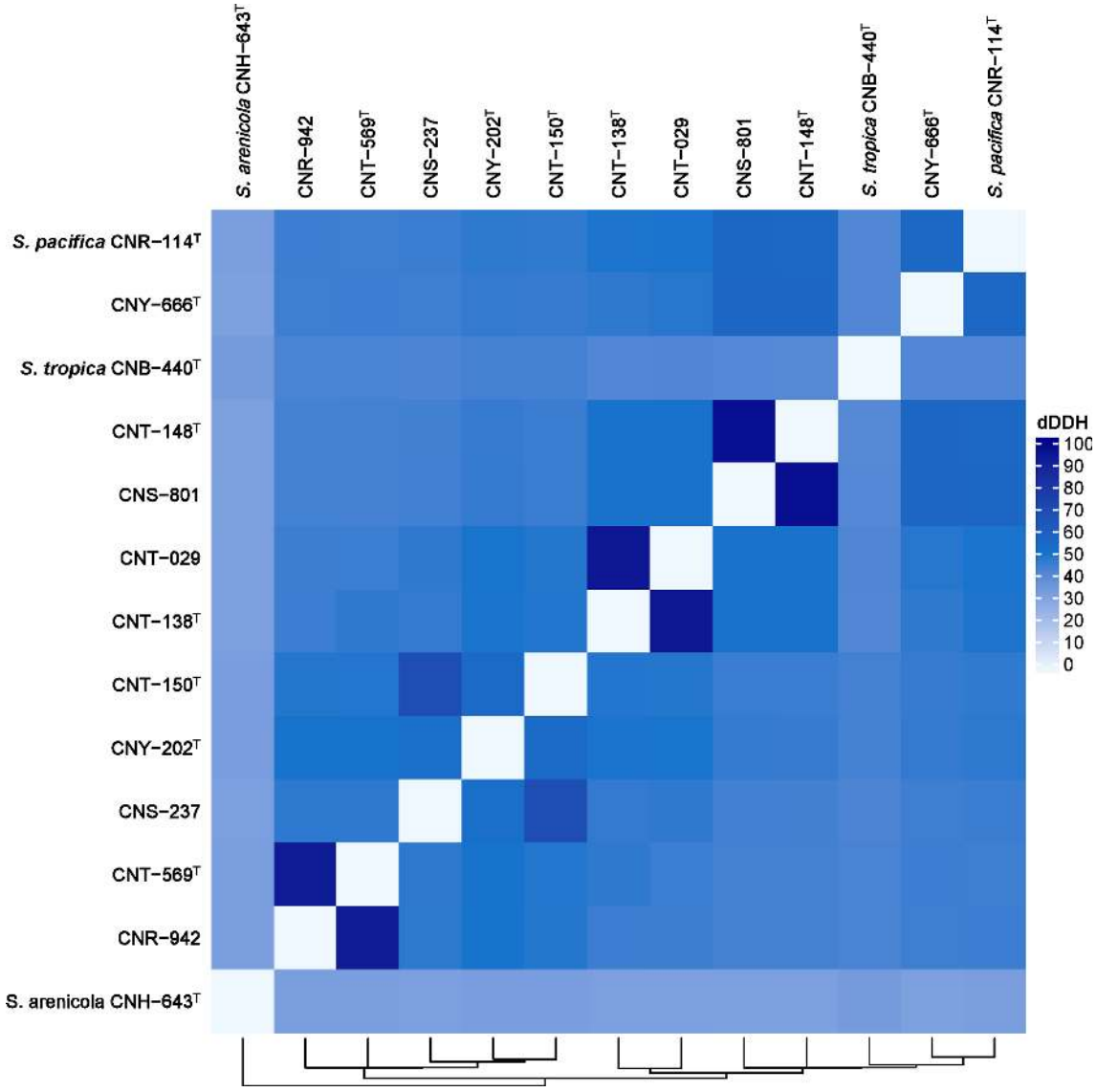


**Fig. 2.** Digital DNA–DNAhybridization pairwise comparison between the 10 *Salinispora* strains studied here and *Salinispora arenicola* CNH-643$^T$, *Salinispora tropica* CNB-440$^T$ and *Salinispora pacifica* CNR-114$^T$.

delineates *Salinispora* into nine well-supported lineages, including the separation of the large clade that includes *S. pacifica* CNR-114$^T$ into a total of seven clades, of which one includes the type strain.

Pairwise whole-genome ANI values were calculated across all 118 genomes using the enveomics package [38]. We observed a strong genetic discontinuity around the 95% ANI value (Fig. 3c), the suggested boundary for species delineation in bacteria [17]. Pairwise comparisons <90% ANI represent the divergence between the *S. arenicola* and *S. tropica-pacifica* clades (first green peak, Fig. 3c) and between *S. tropica* and the broader *S. pacifica* clade (second green peak). Notably, pairwise comparisons between the six new species proposed here all fall below the 95% ANI threshold (red peaks, Fig. 3c), providing further genomic support for their proposed taxonomic status. When adjusted for the newly proposed species designations, all intra-species relationships fall above the 95% ANI boundary (blue peaks, Fig. 3c). Pairwise ANI values of the three *Salinispora* type strains and the ten strains studied here are given in Table S3.

## CHEMOTAXONOMIC AND PHENOTYPIC CHARACTERIZATION

For morphological, physiological and biochemical characterization, the *Salinispora* strains were compared under the

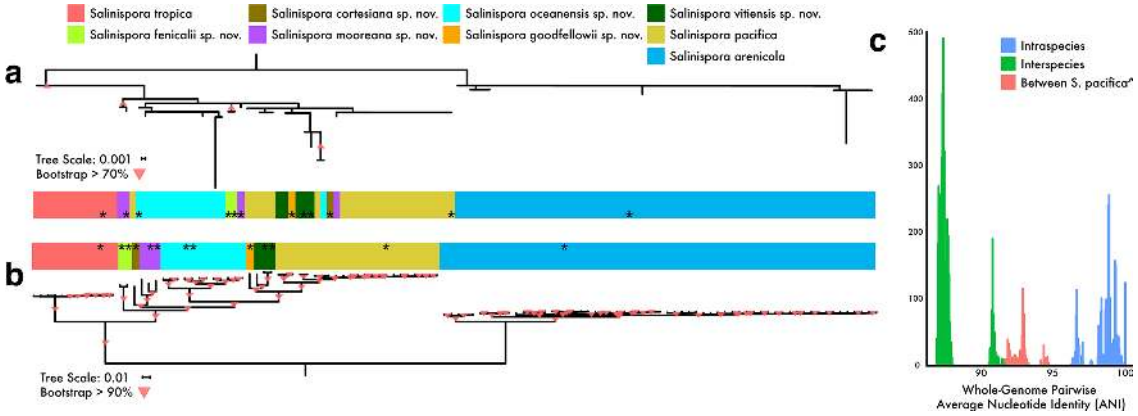


**Fig. 3.** Maximum-likelihood *Salinispora* phylogenies. (a) 16S rRNA gene phylogeny and (b) core genome phylogeny (*N*=2011 genes, 118 strains) reconstructed using a GTR+GAMMA distribution with 100 bootstraps. The trees are coloured by proposed species designations with red triangles representing nodes with at least 70 and 90% support, for the 16S and genomic trees, respectively. *Denotes strains analysed in this study. (c) Distribution of pairwise whole-genome average nucleotide identity (ANI) values. Comparisons are coloured coded as intra- or inter-species using the proposed species designations. The first set of green peaks corresponds to comparisons between *S. arenicola* and the previously described species *S. tropica* and *S. pacifica*; the second set of green peaks denotes the relationship between *S. tropica* and the major clade that includes the *S. pacifica* type strain. The 'between *S. pacifica*' (red) comparisons represent inter-species ANI values between strains of the six new species proposed here and *S. pacifica* CNR-114$^T$.

same laboratory conditions unless otherwise indicated. The type strains for the three currently named species are included for comparison. All media were prepared using 75% artificial seawater. Morphological and cultural characteristics were recorded on A1 agar and International *Streptomyces* Project media (ISP1–ISP7) [39] after 3 weeks of incubation at 28 °C. Growth at different temperatures (18, 22, 28, 37 and 44 °C) and pH (pH 6–9.5, at 0.5 pH unit intervals) was evaluated on A1 agar. Tolerance to NaCl was determined on ISP1 agar prepared with 75% artificial seawater and supplemented with various NaCl concentrations (1–9%, at intervals of 2%). Utilization of several carbon sources was determined as previously described [40]. Production of oxidase, catalase and nitrate reductase, degradation of several compounds, and antibiotic resistance were evaluated following established methods [41]. All tests were done in triplicate and incubated at 28 °C (except temperature measurements) and recorded after 3 weeks. Other enzymatic activities were evaluated using API ZYM strips (bioMérieux) following the manufacturer's instructions, with inocula prepared as previously described [4]. Additionally, the *Salinispora* genomes were screened for genes coding for proteins related with the biochemical and physiological assays carried out in the laboratory (Table 3).

For chemotaxonomic analyses, biomass was obtained by growing strains in M1 broth (DSMZ medium 1065: 1% soluble starch, 0.4% yeast extract, 0.2% peptone, 2% NaCl, 100% natural seawater; www.dsmz.de/microorganisms/medium/pdf/DSMZ_Medium1065.pdf) at 28 °C for 10–14 days under shaking (120 r.p.m.) conditions. Strains CNT-138$^T$, CNT-148$^T$, CNT-150$^T$, CNT-569$^T$, CNY-202$^T$ and CNY-666$^T$ were selected to determine whole-cell sugar, polar lipid and fatty acid profiles using standard procedures [4, 42–44]. Fatty acids were analysed by GLC using the Microbial Identification System (MIDI) with the Microbial Identification software package (Sherlock version 4.5) and peaks were named using the ACTINO database (http://www.actinobase.in/).

The strains grew as non-fragmenting, branched substrate hyphae and without production of aerial mycelia. They showed moderate to abundant growth on all ISP media except ISP6, where only scant growth was observed for strains CNS-801 and CNS-237. On these media, the colour of the substrate mycelium ranged from cream, light orange and orange, turning to dark brown on sporulation (Table S4, Fig. S1). Five strains (*S. arenicola* CNH-643$^T$, *S. tropica* CNB-440$^T$, CNT-138$^T$, CNT-029 and CNS-237) produced a brown diffusible pigment on ISP1 after 2 weeks incubation; strain CNY-666$^T$ showed a brown diffusible pigment on ISP5 and ISP7 media after 3 weeks of growth.

*Salinispora* strains are mesophilic with an optimum growth temperature between 28–37 °C, while at temperatures below 20 °C growth is variable. Neutral to lightly alkaline pH supports good growth of salinisporae while at pH 6 only 50% of the strains showed evidence of growth. All strains studied are halophiles considering that all were tolerant to at least 1% (w/v) NaCl, which was added to the basal medium already containing 75% artificial seawater (ASW). Several strains showed weak growth at 7 and 9% (w/v) NaCl addition to the 75% ASW (Table 3). Seven of the 16 carbon sources tested were assimilated by all *Salinispora* strains (L-alanine, L-glutamic acid, arbutin, lactose, *myo*-inositol, D-sorbitol and trehalose) while variable results were found for the remaining 11 sources tested. All strains studied produced catalase but were variable for the production oxidase. Nitrate

**Table 3.** Phenotypic features of putative novel species and the current *Salinispora* type strains

| Characteristics‡ | *Salinispora arenicola* CNH-643$^T$ | *Salinispora tropica* CNB-440$^T$ | *Salinispora pacifica* CNR-114$^T$ | CNT-138$^T$ | CNT-029 | CNT-148$^T$ | CNS-801 | CNT-150$^T$ | CNS-237 | CNT-569$^T$ | CNR-942 | CNY-202$^T$ | CNY-666$^T$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Environmental parameters** | | | | | | | | | | | | | |
| Optimum temperature for growth (°C) | 28–37 | 28 | 28 | 28 | 28 | 28–37 | 28–37 | 28 | 28–37 | 28 | 28 | 28–37 | 28 |
| Growth at 18 °C | – | + | – | – | – | – | – | – | – | + | + | + | – |
| Growth at 37 °C | + | + | + | + | + | + | + | – | + | + | + | + | – |
| Optimum pH for growth | 8.0 | 8.0 | 8.0 | 7.5–9.5 | 6.5–7.5 | 8.0–9.0 | 7.0–9.5 | 8 | 7.5–9.5 | 8.0 | 7.5–8.0 | 7.5–9.0 | 8–9.5 |
| Growth at pH 6.0 | – | + | + | + | + | – | + | – | + | – | + | – | – |
| Growth with NaCl (%) | 3 | 5* | 5† | 3 | 5* | 5† | 5* | 5† | 5* | 5* | 1 | 3 | 5† |
| **Carbon sources** | | | | | | | | | | | | | |
| L-Glutamic acid | + | + | w | + | + | + | w | + | w | + | w | w | + |
| Arbutin | w | + | w | w | + | + | + | + | + | w | w | + | + |
| Cellobiose | + | – | + | w | w | + | – | + | w | w | + | – | + |
| D-Fructose | + | – | + | w | – | + | + | w | w | w | w | w | w |
| Galactose | + | – | + | w | + | + | + | w | w | + | – | + | + |
| myo-Inositol | + | + | + | + | + | + | + | + | + | + | w | w | w |
| D-Mannose | + | – | + | + | + | + | + | – | – | w | – | + | + |
| Melezitose | + | + | + | + | + | + | + | + | + | + | – | + | + |
| L-Rhamnose | + | + | + | + | + | + | – | + | + | + | – | + | + |
| Trehalose | + | + | w | + | + | + | + | w | + | + | + | + | + |
| D-Salicin | + | w | + | + | – | + | + | + | + | + | + | – | + |
| Sorbose | w | + | – | – | + | + | w | w | w | – | – | – | w |
| Xylose | + | + | + | + | – | + | – | + | + | + | – | w | w |
| **API ZYM tests** | | | | | | | | | | | | | |
| Alkaline phosphatase | + | + | + | + | + | + | + | + | + | w | – | w | + |
| Esterase (C4) | + | – | + | + | + | + | + | + | + | w | + | + | – |
| Esterase lipase (C8) | + | – | + | + | + | + | + | + | + | w | + | + | – |
| Cystine arylamidase | + | + | – | w | – | + | w | + | + | + | + | + | + |

Continued

**Table 3.** Continued

| Characteristics‡ | *Salinispora arenicola* CNH-643$^T$ | *Salinispora tropica* CNB-440$^T$ | *Salinispora pacifica* CNR-114$^T$ | CNT-138$^T$ | CNT-029 | CNT-148$^T$ | CNS-801 | CNT-150$^T$ | CNS-237 | CNT-569$^T$ | CNR-942 | CNY-202$^T$ | CNY-666$^T$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Trypsin | w | + | − | + | − | + | − | + | + | + | + | + | + |
| α-Chymotrypsin | − | + | − | − | − | + | − | + | + | − | w | + | w |
| Acid phosphatase | − | w | − | w | + | + | + | + | + | − | + | w | + |
| Naphthol-AS-BI-Phosphohydrolase | + | − | − | + | − | w | + | + | w | + | + | + | + |
| α-Glucosidase | + | + | + | w | w | + | w | + | + | − | + | + | + |
| β-Glucosidase | + | + | − | + | w | + | − | + | + | + | + | + | − |
| *N*-Acetyl-β-glucosaminidase | − | + | + | + | w | + | + | + | + | + | + | + | + |
| α-Mannosidase | − | + | + | w | + | + | + | − | − | − | − | − | − |
| **Degradation (w/v)** | | | | | | | | | | | | | |
| Casein 1% | + | + | + | + | + | + | + | − | + | w | − | − | + |
| Tween 20 1% | − | + | + | − | w | − | − | w | − | − | − | − | + |
| Tween 80 1% | − | + | + | + | + | + | + | + | + | + | − | − | + |
| **Resistance (µg)** | | | | | | | | | | | | | |
| Ciprofloxacin (5) | + | + | − | + | + | + | + | − | + | + | w | − | + |
| Erythromycin (2) | + | − | − | − | w | − | − | − | + | − | − | − | + |
| Gentamicin (10) | + | + | + | + | + | + | + | − | w | + | w | − | + |
| Neomicin (30) | − | − | − | − | − | − | − | − | − | − | − | − | + |
| Polymycin B (30) | + | w | + | + | + | + | + | + | + | + | w | − | + |
| Tetracycline (30) | + | − | − | − | + | − | w | − | + | − | − | − | + |
| **Other** | | | | | | | | | | | | | |
| Oxidase | − | − | − | + | + | − | − | − | − | + | − | − | − |
| Nitrate reduction | + | + | + | + | + | + | + | + | + | + | w | + | + |
| Nitrite reduction | − | − | w | w | − | − | w | w | − | − | w | + | + |

*Strains CNB-440$^T$, CNT-029, CNS-801 and CNT-569$^T$ grow weakly at 9% NaCl (w/v).
†Strains CNR-114$^T$, CNT-148$^T$, CNT-150$^T$ and CNY-666$^T$ grow weakly at 7% NaCl (w/v).
‡Positive; −, negative; w, weak. No growth observed at 44 °C.

and nitrite reduction activity was also variable. All strains also produced amylases and chitinases for the degradation of starch and colloidal chitin but were variable for the degradation of other compounds tested. The enzymes leucine and valine arylamidase were also produced by all strains while production of α- and β-galactosidase, and β-glucuronidase was not detected. Resistance to several antibiotics was also variable, but all strains grew in the presence of ampicillin (2 µg). A summary of the tests to help differentiate between all *Salinispora* species is given in Table 3. The correlation of the genome-predicted phenotypes and the results obtained in the laboratory are presented in Table S5. Good congruence was found between the *in silico* and *in vitro* data; however, in several cases the target gene was found but no functionality was detected under the laboratory conditions tested, or a positive phenotype was observed but it was not possible to link this activity to the genome sequence. Nevertheless, genomic data can be considered a good starting point to determine genotypic profiles to predict stable phenotypic features that can be useful to differentiate between species.

Indeed, an analysis into the genomic potential to degrade various carbohydrates, ranging from oligosaccharides to polymeric substrates such as chitin, correlated significantly with the proposed species designations (Mantel test; $r$=0.25, $P$ value<0.005). Specifically, we searched all *Salinispora* genomes ($N$=118) for the presence of glycoside hydrolase (GH) and carbohydrate binding module (CBM) proteins, as previously described [45]. We used a Jaccard distance matrix to compute a neighbour-joining dendrogram and visualized the abundance of GH and CBM protein families across *Salinispora* strains as a heatmap (Fig. 4). Together with the phenotypic assays, these results suggest that carbohydrate metabolism is a key functional trait that delineates the species.

The chemotaxonomic markers analysed also confirmed the affiliation of the six representative strains to the genus *Salinispora* as these were in agreement with those reported earlier [3, 4]. The fatty acid profiles (Table 4) contained the major fatty acid iso-$C_{16:0}$ (12.3–49.8%), followed by iso-$C_{15:0}$, iso-$C_{17:0}$ and anteiso-$C_{17:0}$. With respect to their whole-sugar profile, galactose and xylose were common to all strains but the presence of arabinose, glucose and ribose varied, with strains CNT-138$^T$, CNT-148$^T$, CNT-150$^T$ and CNY-666$^T$ containing all sugars while strains CNT-569$^T$ and CNY-202$^T$ lacked arabinose. Differences were also found in the polar lipid composition of the nine type strains, nevertheless the lipids diphosphatidylglycerol, phosphatidylethanolamine and phosphatidylinositol were common to all strains while the presence of phosphatidylglycerol, phosphatidylmethanolamine, phosphatidylinositol mannoside and two unidentified glycolipids varied (Figs S2–S7). A summary of the overall chemotaxonomic profiles is compiled in Table 4.

## CONCLUSIONS

The results derived from this study support the proposal of six new *Salinispora* species as shown by the genomic and phenotypic data presented. At the genomic level, ANI and dDDH values indicate that these strains should be recognized as new genomic species, while chemotaxonomic, physiological and biochemical characteristics clearly show that all strains are distinguishable (Tables 3 and 4). With the present proposal, the number of *Salinispora* species increases from three to nine. The genus is also emended based on the new data presented in this study.

## EMENDED DESCRIPTION OF THE GENUS *SALINISPORA* MALDONADO *ET AL.* 2005

The emended description of the genus is based on that given by Maldonado *et al.* [3] with the following modifications: major amounts of galactose and xylose in whole-organism hydrolysates, the presence of arabinose is variable; predominant fatty acids are iso-$C_{16:0}$ and iso-$C_{15:0}$; major polar lipids include diphosphatidylglycerol, phosphatidylethanolamine and phosphatidylinositol, presence of phosphatidylglycerol is variable; G+C content of the DNA lies within the range 69.1–70 mol% based on genome sequence.

## DESCRIPTION OF *SALINISPORA CORTESIANA* SP. NOV.

*Salinispora cortesiana* (cor.te.si.a'na. N.L. fem. adj. *cortesiana of Córtes, referring to Sea of Córtez*).

Gram-stain-positive, aerobic, non-acid-fast actinobacterium. Fine vegetative hyphae are branched and not fragmented. Colonies are pale orange on A1 medium and appear after incubation for 2 weeks. Aerial mycelium is absent. For growth, deionized water must be replaced with seawater in culture media. After 3 weeks incubation, good growth is observed on ISP1 and ISP3 media, weak growth is seen on ISP4, ISP5 and ISP7, and no growth on ISP2 or ISP6. The temperature range for growth is 18–37 °C (optimum, 28–37 °C); pH for growth ranged from pH 6.5 to 9.0. Grows in the presence of 3% NaCl. Catalase-positive and oxidase-negative. Starch and chitin are hydrolysed but not casein, Tween 20, Tween 80, L-tyrosine or urea. Nitrite and nitrate are reduced. Substrates used as carbon sources include arbutin, D-galactose, lactose, D-mannose, melezitose, L-rhamnose, D-sorbitol and trehalose. L-Alanine, D-fructose, L-glutamic acid, myo-inositol and D-xylose are utilized weakly. Substrates which cannot be used as carbon sources are cellobiose, D-salicin and sorbose. Whole-cell sugars are glucose, galactose, ribose and xylose. Diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol, phosphatidylglycerol and phosphatidylinositolmannoside are part of the polar lipid composition. The major fatty acids (≥5%) are iso-$C_{15:0}$, iso-$C_{16:0}$, $C_{16:0}$ 9-methyl, iso-$C_{17:0}$, anteiso-$C_{17:0}$, $C_{17:0}$, $C_{17:0}$ 10 methyl and summed feature 8.

The type strain is CNY-202$^T$ (=DSM 108615$^T$=CECT 9739$^T$) and was isolated from a marine sediment collected at 330 m of depth in the Sea of Cortez, Mexico. The DNA G+C content of the type strain is 69.6 mol% (WGS).

16S rRNA gene sequence accession number: MH973616.

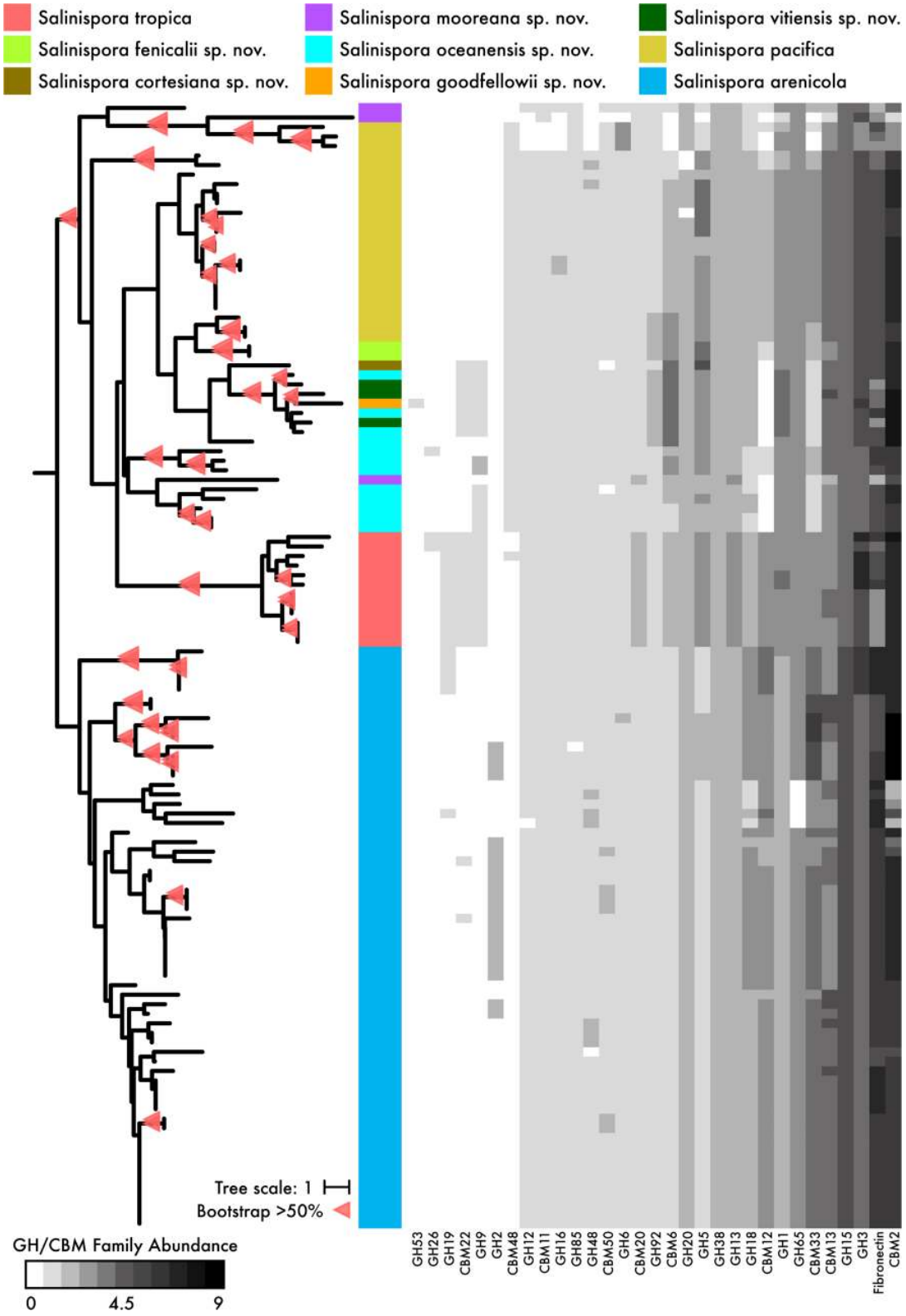


**Fig. 4.** Genomic potential to produce extracellular enzymes for carbohydrate degradation, specifically glycoside hydrolases (GH) and carbohydrate binding module (CBM) proteins. Heatmap shows abundance of GH and CBM protein families across genomes. Dendrogram shows similarity in abundance of GH/CBM family composition among strains. Red triangles indicate nodes with ≥50% support. Strains are coloured by proposed species designations.

**Table 4.** Chemotaxonomic traits of the three named *Salinispora* species and the six type strains described in this work

Strains: 1, *Salinispora arenicola* CNH-643$^T$; 2, *Salinispora tropica* CNB-440$^T$; 3, *Salinispora pacifica* CNR-114$^T$; 4, *Salinispora oceanensis* sp. nov. CNT-138$^T$; 5, *Salinispora vitiensis* sp. nov. CNT-148$^T$; 6, *Salinispora mooreana* sp. nov. CNT-150$^T$; 7, *Salinispora fenicalii* sp. nov. CNT-569$^T$; 8, *Salinispora cortesiana* sp. nov. CNY-202$^T$; 9, *Salinispora goodfellowii* sp. nov. CNY-666$^T$. Fatty acid analyses were carried out under the same conditions, cells were grown for 14 days on TSBA supplemented with 75% seawater at 28 °C. Data expressed as percentages of total fatty acids. Major components (≥5%) in bold type. Peaks that accounted for less than 2% of total fatty acid composition are not included; – not detected.

| Chemotaxonomic traits | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|---|---|---|---|---|---|---|---|---|---|
| **Fatty acid** | | | | | | | | | |
| iso-$C_{15:0}$ | **8.9** | **5.9** | **12.1** | **11.0** | **9.8** | **12.5** | **16.2** | **7.9** | **11.5** |
| anteiso-C15:0 | – | – | 2.5 | – | 2.4 | – | 4.1 | – | 9.6 |
| $C_{15:0}$ | – | – | 4.4 | **7.0** | 2.1 | – | 2.7 | – | 3.4 |
| iso-$C_{16:0}$ | **44.8** | **49.8** | **15.6** | **26.6** | **23.8** | **21.1** | **12.3** | **15.3** | **21.1** |
| $C_{16:0}$ | – | – | – | – | – | – | 2.9 | – | – |
| $C_{16:0}$ 9-methyl | 4.8 | – | 4.6 | – | – | **7.2** | 3.5 | 5.3 | – |
| iso-$C_{17:0}$ | **5.1** | 3.6 | 8.6 | 3.3 | **5.3** | **13.3** | **8.5** | **14.9** | 3.0 |
| anteiso-$C_{17:0}$ | 2.9 | 2.15 | **8.4** | 3.4 | 4.8 | **5.8** | **7.0** | **10.1** | **6.9** |
| *cis*-$C_{17:1}$ 9 | – | 3.1 | – | **9.8** | **6.8** | 4.7 | 11.4 | 3.9 | **16.7** |
| $C_{17:0}$ | – | **9.9** | – | **13.0** | **11.1** | **5.5** | **11.8** | **9.0** | **12.5** |
| $C_{16:1}$ 2-OH | **6.5** | – | – | – | – | – | – | 2.29 | – |
| $C_{17:0}$ 10-methyl | **7.2** | 2.2 | 2.9 | **6.1** | **8.5** | **5.1** | – | **7.8** | – |
| iso-$C_{18:0}$ | – | 2.4 | 2.2 | – | – | – | – | – | – |
| *cis*-$C_{18:1}$ 9 | – | – | 4.4 | – | 4.3 | 3.0 | **7.0** | – | 3.0 |
| $C_{18:0}$ | – | 3.7 | – | – | 3.2 | 2.1 | 2.9 | – | – |
| iso-$C_{18:0}$ 10-methyl | 2.9 | – | – | – | – | – | – | – | – |
| TBSA-$C_{18:0}$ 10-methyl | 2.6 | – | – | – | **5.7** | – | – | 2.2 | – |
| $C_{19:0}$ | – | – | – | – | – | – | – | 4.0 | – |
| Summed feature 8* | 0.50 | – | 3.4 | – | 3.5 | 2.8 | 2.2 | 5.3 | – |
| **Major whole-cell sugars** | | | | | | | | | |
| Arabinose | + | + | + | + | + | + | – | – | + |
| Glucose | – | – | – | + | + | + | + | + | + |
| Galactose | + | + | + | + | + | + | + | + | + |
| Ribose | – | – | – | + | + | + | + | + | + |
| Xylose | + | + | + | + | + | + | + | + | + |
| **Polar lipids†** | | | | | | | | | |
| DPG | + | + | + | + | + | + | + | + | + |
| PME | – | – | + | – | – | – | – | – | – |
| PE | + | + | + | + | + | + | + | + | + |
| PG | + | + | + | – | – | + | + | + | – |
| PI | + | + | + | + | + | + | + | + | + |
| PIM | – | – | + | + | + | + | + | + | + |
| GL1 | – | – | – | + | + | + | + | + | + |

Continued

**Table 4.** Continued

| Chemotaxonomic traits | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|---|---|---|---|---|---|---|---|---|---|
| GL2 | – | – | – | + | – | – | + | – | – |
| GL3 | – | – | – | – | + | – | + | – | + |

*Summed feature 8 consists of unidentified lipid 18.756/19:1.
†DPG, diphosphatidylglycerol; PME, diphosphatidylmethylethanolamine, PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PIM, phosphatidylinositolmannoside; GL1, GL2, GL3, unidentified mannose-containing lipids.

WGS accession number: NZ_AXVR00000000.

## DESCRIPTION OF *SALINISPORA FENICALII* SP. NOV.

*Salinispora fenicalii* (fe.ni.ca´li.i N.L. gen. n. *fenicalii,* in honor of William Fenical, an American scientist who has greatly contributed to the study of marine natural products from *Salinispora*).

Gram-stain-positive, aerobic, non-acid- fast actinobacterium. Fine vegetative non-fragmenting hyphae. Substrate mycelium color varies from bright to dark orange. Aerial mycelium is absent. Colonies are raised and folded after 2 weeks of incubation on A1 media at 28 °C. Requires at least 75% seawater for growth. After 3 weeks of incubation, good growth was observed on ISP1; moderate on ISP3 and ISP7; poor on ISP2, ISP4 and variable on ISP5 media. On ISP6 media growth is not observed. The temperature range for growth is 18–37 °C (optimum, 28 °C), whereas, the optimum pH is 8 (range, from pH 6.0 to 9.5). Grows in the presence of 5% NaCl (type strain). Catalase-positive and oxidase-negative. Starch and chitin are hydrolysed but casein and Tween 80 hydrolysis are variable. Urea, L-tyrosine and Tween 20 are not degraded. Nitrate is reduced, but nitrite reduction is variable. Substrates used as carbon sources are arbutin, cellobiose, D-fructose, L-glutamic acid, *myo*-inositol, lactose, D-salicin and trehalose but the assimilation of L-alanine, D-galactose, D-mannose, melezitose, L-rhamnose, D-sorbitol and D-xylose is variable. Sorbose cannot be used as a carbon source. Whole-cell sugars are glucose, galactose, ribose and xylose. Polar lipids composition includes diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol, phosphatidylglycerol and phosphatidylinositolmannoside. The major fatty acids (≥5%) are iso-$C_{15:0}$, iso-$C_{16:0}$, iso-$C_{17:0,}$ $C_{17:0}$ and *cis*- $C_{17:19}$. The G+C content is 69.1–69.2 mol% (WGS).

The type strain, CNT-569$^T$ (=DSM 108614$^T$=CECT 9740$^T$), and an additional representative, CNR-942, were isolated from marine sediment collected from the Islands of Fiji and Palau, respectively.

16S rRNA gene sequence accession numbers are MH973615 (strain CNT-569$^T$) and MH973613 (CNR-942).

WGS accession number: NZ_AZWQ00000000 (strain CNT-569$^T$) and NZ_ARGG00000000 (strain CNR-942).

## DESCRIPTION OF *SALINISPORA GOODFELLOWII* SP. NOV.

*Salinispora goodfellowii* (good.fel.low'i.i. N.L. gen. n. *goodfellowii,* named in honour of Michael Goodfellow for his contribution to actinobacterial systematics and description of the genus *Salinispora*).

Gram-stain-positive, aerobic, non-acid-fast actinobacterium. Fine vegetative hyphae are branched and non-fragmented. Colonies are orange and folded on A1 medium and appear after incubation for 2 weeks. Aerial mycelium is absent. For growth, deionized water must be replaced with seawater in the culture media. After 3 weeks of incubation, good growth is observed in all ISP media, with exception of ISP6. A diffusible brown pigment is observed on ISP5 and ISP7 media. Growth occurs at 28 °C (no growth at 18 and 37 °C) and pH 6.0–9.5. Grows in the presence of 5% NaCl. Catalase-positive but oxidase-negative. Starch and chitin are hydrolysed but not casein, urea, L-tyrosine, Tween 20 or Tween 80. Nitrate and nitrite are reduced. L-Alanine, arbutin, cellobiose, D-galactose, L-glutamic acid, D-mannose, melezitose, L-rhamnose, D-salicin, sorbitol and trehalose are used as carbon sources, while D-fructose, lactose, myo-inositol, sorbose and D-xylose are weakly used. Contains arabinose, glucose, galactose, ribose and xylose as whole-cell sugars. Polar lipids are diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol and phosphatidyl inositol mannoside. Main fatty acids are iso-$C_{15:0}$, anteiso-$C_{15:0}$, iso-$C_{16:0}$, anteiso-$C_{17:0,}$ $C_{17:0}$ and *cis*-$C_{17:19.}$ The G+C content is 70 mol% (WGS).

The type strain CNY-666$^T$ (=DSM 108616$^T$=CECT 9738$^T$) was isolated from a marine sediment collected from the Madeira Islands (Portugal).

16S rRNA gene sequence accession number: MH973617.

WGS accession number: jgi_2563366532.

## DESCRIPTION OF *SALINISPORA MOOREANA* SP. NOV.

*Salinispora mooreana* (moo.re.a'na. N.L. fem. adj. *mooreana,* in honor of Bradley Moore, an American scientist who has greatly contributed to the study of natural product biosynthesis in *Salinispora*).

Gram-stain-positive, aerobic, non-acid-fast actinobacterium. Fine vegetative hyphae are branched and not fragmented. On A1 medium after 2 weeks, colonies vary from light to bright orange, turning black upon sporulation. Aerial mycelium is absent. For growth, deionized water must be replaced with sea water. Good growth is observed on ISP1 and ISP3 media; moderate on ISP2. Grows at 28 °C; growth at 37 °C is variable (no growth at 18 °C). pH growth range is 6.5–9.5 (optimum, pH 8). Grows in the presence of 5% NaCl. Catalase-positive but oxidase-negative. Starch, chitin and Tween 80 are hydrolysed, while Tween 20 and casein are variable. Urea and L-tyrosine are not hydrolysed. Nitrate is reduced while nitrite reduction is variable. L-Alanine, arbutin, cellobiose, L-glutamic acid, lactose, melezitose, *myo*-inositol, L-rhamnose, D-salicin, sorbitol, trehalose and D-xylose as used as carbon sources while D-fructose, D-galactose and sorbose are weakly used. Whole-cell sugars detected are arabinose, glucose, galactose, ribose and xylose. Diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol, phosphatidylglycerol and phosphatidylinositol mannoside are main polar lipids. Major fatty acids are iso-$C_{15:0}$, iso-$C_{16:0}$, $C_{16:0}$ 9 methyl, iso-$C_{17:0}$, anteiso-$C_{17:0,}$ $C_{17:0}$ and $C_{17:0}$ 10 methyl. The G+C content is 69.3–69.4 mol% (WGS).

The type strain, CNT-150$^T$ (=DSM 45549$^T$=CECT 9741$^T$), was isolated from a marine sediment collected in Fiji, while strain CNS-237 was isolated from a marine sediment collected in Palau.

16S rRNA gene sequence accession numbers: HQ642900 (CNT-150$^T$) and DQ318246 (CNS-237).

WGS accession numbers: NZ_AQZW00000000 (strain CNT-150$^T$) and NZ_AUGH00000000 (strain CNS-237).

## DESCRIPTION OF *SALINISPORA OCEANENSIS* SP. NOV.

*Salinispora oceanensis* (o.ce.an.en'sis. L. fem. adj. *oceanensis*, of the ocean).

Gram-stain-positive, aerobic actinobacterium. Fine vegetative hyphae are branched and not fragmented. After 2 weeks, colonies are orange on A1 medium, turning brown upon sporulation. Aerial mycelium is absent. For growth, deionized water must be replaced with sea water. After 3 weeks, good growth is observed on ISP1 and ISP3, poor to moderate on ISP5 and moderate to good on ISP7 media. No growth is observed on ISP6 agar. Brown diffusible pigment is produced on ISP1 agar after 2 weeks. Growth occurs between 28–37 °C (optimum, 28 °C); pH growth range is pH 6–9.5 (optimum, pH 7.5–9.5). Grows in the presence of 3% NaCl. Catalase and oxidase are positive. Casein, chitin, starch and Tween 80 are hydrolysed; Tween 20 degradation is variable. Hydrolysis of urea and L-tyrosine are negative. Nitrate is reduced, but nitrite reduction is variable. The following substrates are used as carbon sources: arbutin, galactose, L-glutamic acid, L-alanine, myo-inositol, lactose, D-mannose, melezitose, L-rhamnose, trehalose and sorbitol. Cellobiose is utilized weakly. Variable carbon source assimilation for D-fructose, D-salicin, sorbose and D-xylose. Whole-cell sugars detected arabinose, galactose, glucose, ribose and xylose. Polar lipid composition is diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol and phosphatidylinositol mannoside. Major fatty acids are iso-$C_{15:0}$, $C_{15:0}$, iso-$C_{16:0,}$ $C_{17:0}$, *cis*-$C_{17:19}$ and $C_{17:0}$ 10 methyl. The G+C content is 69.7 mol% (WGS).

The type strain, CNT-138$^T$ (=DSM 45547$^T$=CECT 9742$^T$), and strain CNT-029 were isolated from marine sediments collected from the Fiji Islands.

16S rRNA gene sequence accession numbers: HQ642853 (strain CNT-138$^T$) and HQ642852 (strain CNT-029).

WGS accession number: NZ_ARTO00000000 (strain CNT-138$^T$) and NZ_AZWB00000000 (strain CNT-029).

## DESCRIPTION OF *SALINISPORA VITIENSIS* SP. NOV

*Salinispora vitiensis* (vi.ti.en'sis. N.L. fem. adj. *vitiensis,* from Viti Levu, native name for Fiji, where the type strain was isolated).

Gram-stain-positive, aerobic, non-acid-fast actinobacterium. Vegetative hyphae are branched and not fragmented. Colonies are dark orange or brown on A1 medium and appear after incubation for 2 weeks. Aerial mycelium is absent. For growth, necessarily deionized water must be replaced with seawater. Good growth is observed on ISP1 and ISP3, poor on ISP2, moderate on ISP4, poor to moderate on ISP5, and moderate to good on ISP7 media. The temperature range for growth is 22–37 °C (optimum, 28–37 °C). pH growth range pH 7.0–9.5 (optimum; pH 8.0–9.0, type strain). Grows in the presence of 5% NaCl. Catalase-positive and oxidase-negative. Casein, starch, chitin and Tween 80 are hydrolysed but not urea, L-tyrosine or Tween 20. Nitrate is reduced, but nitrite reduction is variable. Utilizes L- alanine, arbutin, D-fructose, D-galactose, L-glutamic acid, *myo*-inositol, lactose, D-mannose, melezitose, trehalose, D-salicin, D-sorbitol and sorbose as carbon sources; utilization of L-glutamic acid, cellobiose, L-rhamnose, sorbose and D-xylose is variable. Whole-cell sugars are arabinose, glucose, galactose, ribose and xylose. Diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol and phosphatidylinositol mannoside are the main polar lipids. Major cellular fatty acids are iso-$C_{15:0}$, iso-$C_{16:0,}$ $C_{17:0}$, iso-$C_{17:0}$ and *cis*-$C_{17:19}$, while $C_{16:0}$ 9 methyl, $C_{17:0}$ 10 methyl and *cis*-$C_{18:19}$ are variable. The G+C content is 69.9 mol%.

The type strain, CNT-148$^T$ (=DSM 45548$^T$=CECT 9743$^T$), and strain CNS-801 were isolated from marine sediments collected from the Fiji Islands.

16S rRNA gene sequence accession number: HQ642899 from CNT-148$^T$ and MH973614 from CNS-801.

WGS accession number: NZ_AQZE00000000 (strain CNT-148$^T$) and jgi_2561511036 (CNS-801).

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

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