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RESEARCH ARTICLE

Targeted search for actinomycetes from nearshore and deep-sea marine sediments

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Abstract

Sediment samples collected off the coast of San Diego were analyzed for actinomycete diversity using culture-independent techniques. Eight new operational taxonomic units (OTUs) in the *Streptomycetaceae* were identified as well as new diversity within previously cultured marine OTUs. Sequences belonging to the marine actinomycete genus *Salinispora* were also detected, despite the fact that this genus has only been reported from more tropical environments. Independent analyses of marine sediments from the Canary Basin (3814 m) and the South Pacific Gyre (5126 and 5699 m) also revealed *Salinispora* sequences providing further support for the occurrence of this genus in deep-sea sediments. Efforts to culture *Salinispora* spp. from these samples have yet to be successful. This is the first report of *Salinispora* spp. from marine sediments > 1100 m and suggests that the distribution of this genus is broader than previously believed.

Introduction

The phylum *Actinobacteria* is extraordinarily diverse (Gao & Gupta, 2012) and well represented in the marine environment (Rappé *et al.*, 1999). Of the five subclasses that comprise this phylum (the *Acidomicrobidae*, *Actinobacteridae, Coriobacteridae, Nitriliruptoridae,* and *Rubrobacteridae*), sequences belonging to the *Acidomicrobidae* are commonly observed when culture-independent techniques are applied (Jensen & Lauro, 2008). Conversely, cultured *Actinobacteria* often fall within the subclass *Actinobacteridae* and, more specifically, within the order *Actinomycetales*. These bacteria are commonly referred to as actinomycetes and have been targeted from marine samples for their ability to produce structurally novel secondary metabolites (Zotchev, 2012). While a number of marine actinomycete species and genera have been described (Tian *et al.*, 2009a; Tian *et al.*, 2009b; Zhao *et al.*, 2009; Goodfellow *et al.*, 2012) it is not clear how well these cultured strains represent the extant diversity present in the marine environment.

Culture-independent studies have revealed the presence of actinomycetes in seawater (Yoshida *et al.*, 2008) and deep-sea marine sediments (Stach *et al.*, 2003a, b). Less abundant taxa such as the marine actinomycete genus *Salinispora* (Maldonado *et al.*, 2005) have been detected when specific primers targeting this group were applied (Mincer *et al.*, 2005). Actinomycetes have also been detected in marine sponges, facilitating the selection of culture media and further increasing the diversity of isolates recovered (Webster *et al.*, 2001). In a separate study of two sponges from China, a wide difference between the genera observed using actinomycete specific primers and cultivation-based methods was observed (Xin *et al.*, 2008). Further studies on one of these sponges revealed the importance of using both culture and culture-independent methods when studying actinomycete diversity (Sun *et al.*, 2010). While all methods suffer from

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inherent biases, culture-independent techniques can help establish the occurrence of bacteria in specific environments.

In a prior study of sediment samples collected off the coast of California, culture-dependent actinomycete diversity was assessed between nearshore and offshore sites (Prieto-Davó *et al.*, 2008). The results revealed considerable, marine-specific diversity and high levels of terrestrial influence out to 125 km from shore. The present study was undertaken to provide a culture-independent assessment of the collective actinomycete diversity present in five of these samples. These studies were complimented by independent analyses of deep-sea sediment samples collected from the Canary Basin and the South Pacific Gyre (SPG).

Materials and methods

Sample collection

To further explore the diversity of actinomycetes present in marine sediments collected off the coast of California, five of eleven sediment samples previously employed for cultivation studies (Prieto-Davó *et al.*, 2008) were used to generate 16S rRNA gene clone libraries targeting the order *Actinomycetales*. All of these samples were collected using an untethered coring device designed and constructed at the Scripps Institution of Oceanography (SIO). The depths and collection sites are provided in Supporting Information Table S1. Each core was divided into five or six sections as previously described (Prieto-Davó *et al.*, 2008). Approximately 1 g of wet sediment from each section was placed in a 1.5 mL Eppendorf tube containing 1 mL of sucrose lysis buffer (50 mM Tris–HCl, 150 mM NaCl, 5 mM EDTA, 0.25 M sucrose) and immediately stored on ice for transportation to shore. Long-term storage was at −20 °C.

Samples from the Canary Basin and the SPG were analyzed specifically for the presence of *Salinispora* spp. The Canary Basin sample was collected as previously described (Stach *et al.*, 2003b). The SPG samples were collected using gravity or piston cores during the KNOX-02RR expedition (D'Hondt *et al.*, 2009). A total of 11 cores were sectioned and subsampled from 3 to 5 times at various depths from the sediment surface to the bottom of the core generating a total of 51 samples (Table S2). Subcores were taken from each section by first removing the top layer of sediment with a sterile spatula. Sterilized cut-off syringes were then pushed into the core resulting in an uncontaminated subcore. The syringe containing the subcore was stored intact in heat sealed bags at −80 °C prior to molecular analysis.

DNA extraction, PCR amplification, and cloning

Environmental DNA (eDNA) was extracted from the sediment samples collected off the coast of California using a soil DNA extraction kit (cat. No 69506) according to manufacturer's protocol (Qiagen, Valencia, CA). 16S rRNA gene primers targeting the order *Actinomycetales* (Stach *et al.*, 2003a, b) and the families *Streptomycetaceae* and *Micromonosporaceae* (Monciardini *et al.*, 2002) were used (Table S3). PCR amplification of 1–4 μL of eDNA (18–20 ng mL^{-1}) was carried out in triplicate for each sample as follows: initial denaturation at 95 °C for 10 min followed by 30 cycles of 94 °C for 45 s, 65 °C for 45 s, and 72 °C for 1 min, followed by a 10 min extension at 72 °C. Triplicate PCR products were pooled and purified using MiniElute PCR purification columns according to the manufacturer's instructions (Qiagen). Purified DNA was ligated to the plasmid vector pCR® 2.1-TOPO® and used to transform One-Shot® Mach1™ -T1® chemically competent cells using a Topo TA® cloning kit according to the manufacturer's protocol (Invitrogen, Carlsbad, CA). Transformed clones were identified using white–blue selection and inoculated into 10 mL Falcon tubes containing 3 mL of LB broth and 50 μg mL^{-1} kanamycin. Plasmid DNA was extracted using the QiaPrep® MiniPrep extraction kit according to manufacturer's instructions (Qiagen), digested with BstX I (New England BioLabs, Ipswich, MA), and run on a 1% agarose gel to confirm the presence of the correct-sized insert.

eDNA was extracted from 10 g of Canary Basin sediment using an UltraClean Mega DNA soil kit (Mo Bio Laboratories, Solana Beach, CA). The primers (Supplemental Table S3) and PCR conditions are as previously described (Stach *et al.*, 2003a, b). PCR products were purified using QIAquick gel extraction columns according to the manufacturer's instructions (Qiagen, Crawley, UK). Purified DNA was blunt-end-ligated into the plasmid vector pST-Blue-1 and used to transform NovaBlue competent cells using a Perfectly Blunt cloning kit (Novagen, Madison, WI). Cloned plasmid DNA was extracted as described above and the presence of inserts confirmed by PCR using previously described primers (Stach *et al.*, 2003a, b).

For the SPG sediments, a modified FastDNA® SPIN kit for soil protocol (Qbiogene, Irvine, CA), followed by ChromaSpin™ TE-100 columns (Clontech, Mountain View, CA), was used to ensure high eDNA yields and purity (Froschner *et al.*, 2009). The eDNA samples were subjected to whole-genome amplification using the REPLI-g® Midi kit (Qiagen) as previously described (Froschner *et al.*, 2009) and analyzed for the presence of *Salinispora* spp. using *Salinispora*-specific 16S rRNA gene PCR primers (Table S3). The reactions included 200 ng

of whole-genome amplification product and consisted of an initial 5-min denaturation step at 94 °C followed by 30 cycles of 30 s at 94 °C, 90 s at 60 °C, and 90 s at 72 °C followed by a final 10 min extension at 72 °C. The reaction products were purified using a QIAquick PCR purification kit (Qiagen) following manufacturer's guidelines and quantified using a NanoDrop Spectrophotometer (NanoDrop, Wilmington, DE). PCR amplicons were cloned using the pGEM®-T Easy Vector System (Promega, Madison, WI) at a 1 : 1 insert to vector ratio per the manufacturer's protocol. Transformed clones were grown overnight in LB broth and the plasmids extracted using Wizard® *Plus* SV Minipreps (Promega).

Terminal restriction fragment length polymorphism (T-RFLP) analysis

T-RFLP was used to probe the SPG samples for the presence of *Salinispora* spp. Whole-genome amplification products were PCR amplified as described above with the addition of a 6-FAM label to the forward primer. The *Salinispora*-specific primers were designed as part of a prior study and shown to be > 98% specific for this taxon (Mincer *et al.*, 2005). Approximately 40 ng of fluorescently labeled PCR product was digested independently with 4 U of the restriction enzymes AluI, AvaII, and EcoO109I in 10-μL reaction volumes. Reference T-RFLs were generated for the three *Salinispora* species by digesting labeled PCR products from cultured strains (Supplemental Table S5). The restriction digests were incubated at 37 °C for 16 h to ensure complete digestion prior to being deactivated at 65 °C for 20 min. The digests were precipitated and resuspended in Hi-Di formamide (Applied Biosystems, Carlsbad, CA) to a final concentration of approximately 0.6 ng μL^{-1}. About 0.5 μL GeneScan Liz 1200 size standard (Applied Biosystems) was added to each sample. The samples were denatured at 95 °C for 5 min and placed immediately on ice. The terminally labeled restriction fragments (T-RFs) were visualized with the Applied Biosystems 3130xl genetic analysis system.

Sequencing and phylogenetic analyses

Plasmid inserts from the California samples were sequenced at the UCSD Rebecca and John Moore Cancer Center using the M13 primer included in the vector (Invitrogen). Sequences were analyzed using the Basic Local Alignment Search Tool (BLAST) function of GenBank (Altschul *et al.*, 1990), and strains with the highest level of sequence identity were recorded as top BLAST matches regardless of whether or not they were associated with a formal publication. Top matches and cloned sequences belonging to the *Micromonosporaceae* (560 bp) and *Streptomycetaceae* (533 bp) were clustered into OTUs based on ≥ 99% 16S rRNA gene sequence identity using the java application Clusterer (http://www.bugaco.com/mioritic/clusterer_jlp.php). In addition, OTU representatives from cultured strains derived from all 11 samples used in a prior study (Prieto-Davó *et al.*, 2008) were included in the clustering analyses. Sequences were then aligned using ClustalX (Larkin *et al.*, 2007) and imported into MacClade for manual curation of the alignment (http://macclade.org).

Plasmid inserts derived from the Canary Basin PCR products were sequenced by Qiagen using the SP6 universal primer. Plasmid inserts from the SPG samples were sequenced using the 16S primers or vector primers (T7, SP6). Sequencing was performed on an Applied Biosystems 3130xl Genetic Analyser (Applied Biosystems) at the University of Rhode Island's Genomics and Sequencing Center (Kingston, RI). The resulting sequences were trimmed and contigs built using Sequencher™ (Gene Codes, Ann Arbor, MI).

Maximum-Likelihood rooted phylogenetic trees (HKY85 substitution model, 1000 bootstraps) were constructed for the *Streptomycetaceae* and *Micromonosporaceae* using PhyML (Guindon *et al.*, 2010) via http://www.phylogeny.fr/ and http://www.atgc-montpellier.fr/phyml/ (Dereeper *et al.*, 2008). Trees were edited using Inkscape (inkscape.org). For both phylogenetic trees, top BLAST matches, as well as previously cultivated OTU representatives (Prieto-Davó *et al.*, 2008), were included.

Diversity estimations

Estimates of the diversity present in the California clone libraries were performed using the statistical package EstimateS (http://purl.oclc.org/estimates) with 1000 runs for each library.

Results

Clone libraries targeting the order *Actinomycetales* (actinomycetes) and the families *Streptomycetaceae* and *Micromonosporaceae* were generated from five independent sediment cores collected off the coast of California (Table S1). The *Actinomycetales*-specific primers yielded largely nonactinobacterial sequences and *Actinobacteria* in the subclass *Acidomicrobidae*, which is outside of the *Actinomycetales*. In response, primers specific for the *Streptomycetaceae* and *Micromonosporaceae* were used because these were the dominant families cultured previously from these samples. Clone libraries were generated from two of the sediment cores using the *Streptomycetaceae* primers, two using the *Micromonosporaceae* primers, and

Table 1. Clustering of *Streptomycetaceae* and *Micromonosporaceae* clones into OTUs based on > 99% 16S rRNA gene sequence identity over 560 bp (*Streptomycetaceae*) and 533-bp (*Micromonosporaceae*)

Family	Cloned OTUs	Cultured OTUs*	GenBank cultured OTUs
Streptomycetaceae	37	5	29
Micromonosporaceae	4	3	4
Total	41	8	33

*Based on a prior analysis of the same sediment samples (Prieto-Davó *et al.*, 2008).

one using both primer sets (Table S4). All libraries were generated from the surface section of the cores, with the exception of sample SD06-13, from which libraries were generated from all five sections. Seventy-seven of the combined 79 clones obtained using the *Streptomycetaceae* primers belonged to this family confirming the specificity of that primer set. The cloning efficiency of the *Micromonosporaceae* primers was poor and yielded only 23 sequences, of which 22 belonged to the targeted family.

The 99 *Streptomycetaceae* and *Micromonosporaceae* clones were clustered based on $\geq$ 99% sequence identity (Table 1). This yielded a total of 41 OTUs, each of which contained 1–10 clones (average = 2). Of these, representatives of eight OTUs were cultured as part of a prior study (Prieto-Davó *et al.*, 2008; Table 1, Figs 1 and 2). When compared more broadly to GenBank, which includes the sequences from the prior study, 33 of the 41 OTUs from this study have cultured representatives, and thus, eight can be considered new in terms of publically available sequence data. The majority of the OTUs (37) and all of the eight new OTUs belong to the *Streptomycetaceae*. The OTUs in this family are scattered throughout the phylogenetic tree; however, two of the new OTUs (represented by clones SD06-09A-02 and SD06-13C-01) form a well-supported clade with the marine-derived actinomycete strain CNQ-085 (Fig. 1). Two more of the new OTUs, represented by SD06-07A-01 and SD06-13E-03, belong to a much larger and previously identified marine clade that includes the new species *Streptomyces marinus* (Prieto-Davó *et al.*, 2008) and the cloned OTU (SD06-13A-10). One additional OTU (SD06-13B-01) belongs to another cultivated marine clade (Prieto-Davó *et al.*, 2008), while the two remaining new OTUs (SD06-09A-06 and SD06-13A-02) are distantly related to previously observed sequences.

In an effort to estimate the total diversity in the sediments sampled, a rank abundance curve was generated from the combined *Streptomycetaceae* clone libraries. This curve shows little duplication and a long right-hand tail, as is characteristic of a highly diverse community (Fig. 3). This is supported by three diversity estimators (ACE, Chao 1, and Jackknife 1), which predict that, on average, 70 OTUs were present in the three samples analyzed (Fig. 4). The shape of the accumulation curve provides clear evidence that additional sequencing could reveal additional diversity. The *Micromonosporaceae* clones formed only four OTUs, and thus, a rank abundance curve was not generated. The poor cloning efficiency and low number of sequences analyzed suggest that additional studies of this family are warranted. Although none of the *Micromonosporaceae* OTUs were considered new, one cladded with *Catellatospora* spp. (clones SD06-11-02 and SD06-12-08) and a second, which included three cloned sequences, cladded with *Salinispora* spp. (clone SD06-12-01; Fig. 2). The other two OTUs cladded with *Micromonospora* spp. (clones SD06-12-05 and SD06-13-01). This is the first evidence that *Salinispora* spp. occur in temperate marine environments (Jensen & Mafnas, 2006; Freel *et al.*, 2012). Given that neither *Catellatospora* nor *Salinispora* spp. had previously been cultured from these samples, cultivation techniques specific for both genera were applied (Ara & Kudo, 2006; Freel *et al.*, 2012). However, only *Micromonospora* spp. were recovered.

Independent analyses of deep-sea marine sediment samples collected from the Canary Basin and the South Pacific Gyre (SPG) were also performed to test for the presence of *Salinispora* sequences. A clone library generated from a Canary Basin sediment sample collected at a depth of 3814 m revealed the presence of one *Salinispora* sequence (Table 2). Eleven deep-sea sediment cores obtained from the SPG were subdivided into 51 sections and analyzed by T-RFLP. PCR products were obtained using *Salinispora*-specific primers (Mincer *et al.*, 2005) from 25 of the 51 subsamples including samples from each of the 11 sites (Table S2). The second depth below the sediment surface (0.3–0.89 m) yielded the largest number of successful PCR amplifications. T-RFLP analyses were performed on the 25 PCR products, and all 25 yielded T-RFs of the expected size for *Salinispora* spp. (Supplemental Table S5). Clone libraries generated from three of the PCR-positive samples (SPG1, SPG2-1, and SPG2-3) yielded eight *Salinispora* sequences and confirmed the presence of the genus in sediments collected at a depth of 5699 m (Table 2). Additional sequencing would be required to confirm the presence of the genus in the remaining 22 samples. All of these sequences clade with *Salinispora* spp. (Fig. 2) and fall within a single 99% OTU. However, due to the lack of species-specific nucleotides in the region sequenced, it is not possible to assign species level identifications. Collectively, these results provide the first evidence that *Salinispora* spp. occur at depths > 1100 m (Mincer *et al.*, 2005) and outside of the geographical range from which the genus has been reported using culture-dependent methods (Freel *et al.*, 2012).

Fig. 1. Neighbor-joining phylogenetic tree of *Streptomycetaceae* 16S rRNA gene sequences. Environmental sequences from California begin with SD and are followed by number of clones (in parentheses) and accession number. Cultured strains were derived from a prior study (sequences beginning with CN). BLAST matches are included with species and strain name followed by accession number. Bold = new OTU (shares $\leq$ 98% sequence identity with any sequences deposited in Genbank), italics = marine OTU (all sequences in the OTU were derived from marine sources), underlined = OTU representative in culture. Boxed clade = marine clade (all sequences in the OTU plus top BLAST match and cultivars were derived from marine sources). Based on the analysis of 533-bp nucleotide positions and 1000 bootstraps.

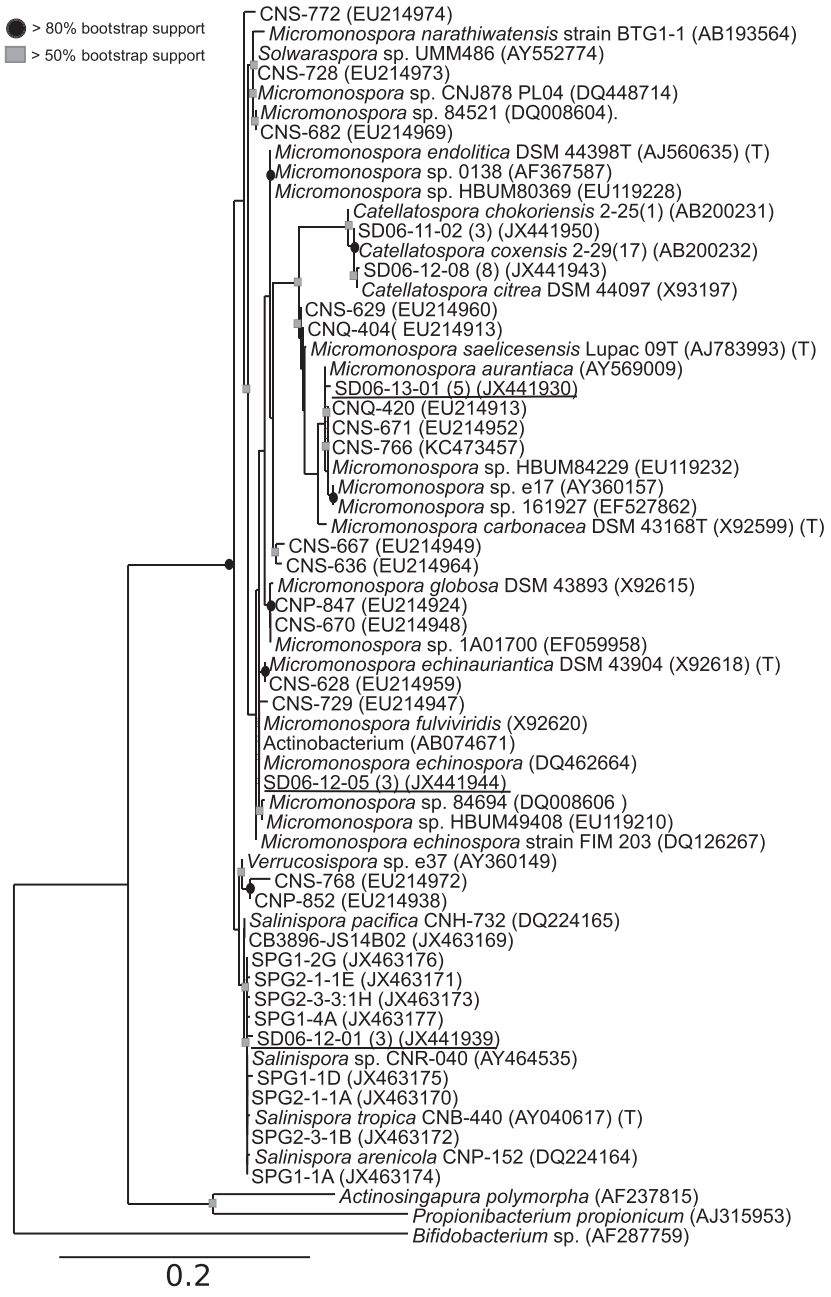

Fig. 2. Neighbor-joining phylogenetic tree of *Micromonosporaceae* 16S rRNA gene sequences. Environmental sequences from California begin with SD and are followed by number of clones (in parentheses) and accession number. Sequences from the Canary Basin start with CB and the South Pacific Gyre with SPG. Cultured strains were derived from a prior study (sequences beginning with CN). BLAST matches are included with species and strain name followed by accession number. Bold = new OTU (shares $\leq$ 98% sequence identity with any sequences deposited in Genbank), italics = marine OTU (all sequences in the OTU were derived from marine sources), underlined = OTU representative in culture. Based on the analysis of 560-bp nucleotide positions and 1000 bootstraps.

Discussion

Cultivation-independent methods are widely recognized as providing a more accurate estimation of bacterial diversity than culture-based approaches (Wagner *et al.*, 1993; Hugenholtz *et al.*, 1998). As a follow-up to a prior study of cultured marine actinomycete diversity in sediments collected off the coast of California (Prieto-Davó *et al.*, 2008), we performed culture-independent analyses on a subset of the samples. The results reveal eight new OTUs in the family *Streptomycetaceae* and numerous clones that expand the extant diversity associated with previously cultured marine actinobacterial lineages. Statistical analyses suggest that additional sequencing will uncover additional diversity in this family, a conclusion also reached by Stach & Bull (2005) in relation to the analysis of Canary Basin sediments. In addition, it is also clear that some of the cultured OTUs were not observed in the clone libraries (Fig. 1). This could be due to insufficient sequencing; however, it supports the concept that culture-dependent methods provide a method to access members of the rare biosphere that are frequently missed

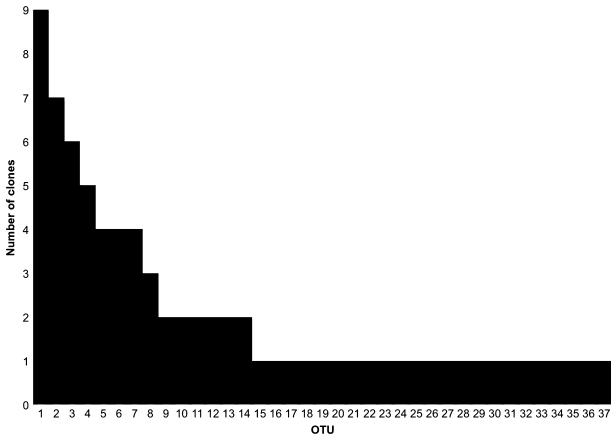

Fig. 3. Rank abundance curve for the San Diego *Streptomycetaceae* clone library. Seventy-seven clones generated from three independent libraries were combined and grouped into OTUs based on 99% 16S rRNA gene sequence identity.

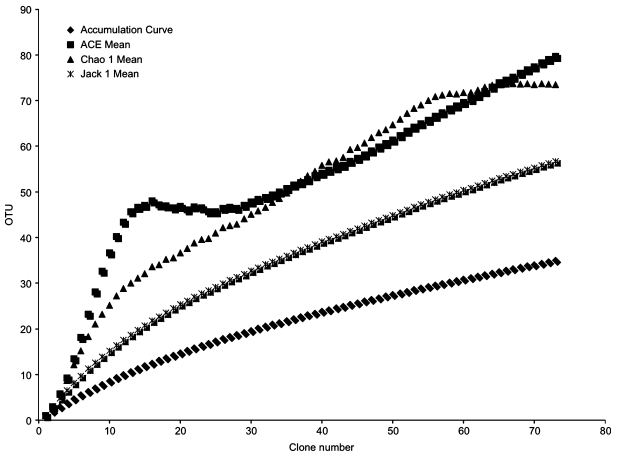

Fig. 4. Diversity estimators for the San Diego *Streptomycetaceae* clone library. Seventy-seven clones generated from three independent libraries were analyzed.

when culture-independent methods are applied (Shade *et al.*, 2012), even in cases such as this where taxon-specific primers are employed.

In the case of the *Micromonosporaceae*, the small size of the library made it difficult to predict the diversity in this family. Despite the small number of clones sequenced, seven (32%) were identified as *Salinispora* spp. This result was surprising considering that this genus has not previously been reported from temperate environments (Freel *et al.*, 2012) and that extensive cultivation efforts over many years have failed to recover this taxon from sediments collected off the coast of California. Additional cultivation efforts using the samples that yielded *Salinispora* clones also failed to produce these bacteria in culture suggesting that techniques other than those proven successful for tropical samples (Gontang *et al.*, 2007) are required.

The culture-independent results obtained for the sediments collected off the coast of California suggest that the biogeographical distributions of *Salinispora* spp. may be broader than those previously estimated based on culture-dependent approaches (Freel *et al.*, 2012). To further explore this possibility, deep-sea sediment samples collected from the Canary Basin (3814 m) and the South Pacific Gyre (5126 and 5699 m) were analyzed using culture-independent techniques. Both locations yielded *Salinispora* clones, providing a new depth record for the detection of this genus and additional evidence for its widespread occurrence in deep-ocean sediments. Samples collected from the SPG sites, which extend from American Samoa to New Zealand (Figure S1), consistently tested positive for *Salinispora* 16S rRNA gene sequences, providing evidence that the genus is broadly distributed in deep-ocean sediments. It remains unclear whether the DNA from which these sequences originated was derived from actively growing cells or spores. Conditions in the deep sea include high pressures and low temperatures, which may inhibit growth. Yet, extensive cultivation efforts applied to the California (Prieto-Davó *et al.*, 2008), Canary Basin (Stach *et al.*, 2003a, b), and SPG samples failed to yield *Salinispora* strains, suggesting they require cultivation conditions that differ from the strains that have been obtained from more tropical sites. While there was no phylogenetic evidence that the deep-sea *Salinispora* sequences are distinct (Fig. 2), less-conserved phylogenetic markers may need to be analyzed before any conclusions can be reached about their population structure.

Due to the specificity of the primers, it is likely that considerable actinomycete diversity was omitted from this study. However, the families targeted are among the most prolific in terms of secondary metabolite production and include a number of new marine taxa (Jensen *et al.*, 2005; Goodfellow *et al.*, 2012). The results presented here suggest that additional new diversity within the *Streptomycetaceae* remains to be cultured from marine sediments, thus supporting further studies of deep-sea actinomycetes as a resource for natural product discovery. In addition, there is intriguing new evidence that *Salinispora* spp. are broadly distributed in deep-sea marine sediments. It will be important to develop cultivation techniques appropriate for these bacteria and to determine whether they have developed adaptations to life in the deep sea that may include the production of new secondary metabolites.

Table 2. *Salinispora* clones obtained from deep-sea sediment samples

Clone	Location depth (m)	NCBI closest BLASTn match*	Accession numbers	Similarity (%)
SD06-12-01	San Diego 1133	*S. pacifica* CNH-732; *Salinispora* sp. CNR-040	DQ2244165.1; AY040617	99
SPG2-1-1A	Pacific Gyre 5126	*S. pacifica* CNT-138; *S. arenicola* CNT-850	HQ642853.1; HQ642848.1	100
SPG2-1-1E	Pacific Gyre 5126	*S. pacifica* CNT-138; *S. arenicola* CNT-851	HQ642853.1; HQ642848.2	99
SPG2-3-1B	Pacific Gyre 5126	*S. pacifica* CNT-138; *S. arenicola* CNT-852	HQ642853.1; HQ642848.3	100
SPG2-3-3:1H	Pacific Gyre 5126	*S. pacifica* CNT-138; *S. arenicola* CNT-853	HQ642853.1; HQ642848.4	99
SPG1-1A	Pacific Gyre 5699	*S. pacifica* CNT-138; *S. arenicola* CNT-854	HQ642853.1; HQ642848.5	100
SPG1-1D	Pacific Gyre 5699	*S. pacifica* CNT-138; *S. arenicola* CNT-855	HQ642853.1; HQ642848.6	99
SPG1-2G	Pacific Gyre 5699	*S. pacifica* CNT-138; *S. arenicola* CNT-856	HQ642853.1; HQ642848.7	100
SPG1-4A	Pacific Gyre 5699	*S. pacifica* CNT-148; *S. arenicola* NH13C	HQ642899.1; FJ232412.1	99
3896_JS14B02	Canary Basin 3814	*S. pacifica* CNT-148	HQ642899.1	99

*In cases where equivalent best matches were found to two species, both are reported.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Fig. S1. Sampling locations for South Pcific Gyre (SPG) sediments.
Table S1. Sample depth and location.
Table S2. South Pacific Gyre sampling sites (see Table S1 for locations) and depths (m) below the sediment surface (in parentheses) from which the cores were sub-sampled.
Table S3. PCR primers. *New to this study.
Table S4. Culture-independent analyses of sediments collected off the coast of California.
Table S5. T-RFLs in base pairs of cultured *Salinispora* species.