Detailed methodology for dataset "WS1209 strains"

from Patin,NV; Duncan,KR; Dorrestein,PC; Jensen,PR. (2012) Competitive strategies differentiate closely related species of marine actinobacteria. ISME J 10:478-490. doi:10.1038/ismej.2015.128

Sediment collection and processing
Sediment samples were collected via SCUBA at depths from 3 to 16 m in July 2012 during a research cruise aboard the R/V Walton Smith (U Miami). Individual sediment samples (5–10 g per sample) were collected from the sediment surface to depths of ca. −3 cm using sterile Whirl-Pak bags (Nasco, Ft. Atkinson, WI, USA). Locations included sites off Miami and the Dry Tortugas in the United States and Cancún, Cozumel, Akumal, and Banco Chinchorro in the Mexican Caribbean. All samples were processed immediately aboard ship using two methods: drying and stamping for selective actinomycete cultivation (Mincer *et al.*, 2002) and serial dilution and plating for the general cultivation of marine bacteria. For the latter, ca. 1 g of sediment was serially diluted 1:1, 1:10 and 1:100 in sterile seawater, vortex mixed, and 50 µl of the supernatant inoculated onto agar media and spread with a sterile glass rod. Three types of media were used for both methods: (1) 25% marine agar (9.3 g Marine Broth Difco 2216, 16-g agar, 750-ml 0.2-µm filtered seawater, 250-ml deionized water), (2) seawater-agar (16-g agar, 1-l 0.2-µm filtered seawater) and (3) 25% A1 (2.5-g starch, 1-g yeast extract, 0.5-g peptone, 16-g agar, 750-ml 0.2-µm filtered seawater, 250-ml deionized water).

Strain isolation
Salinispora strains were recognized based on colony morphology (Mincer *et al.*, 2002) and repeatedly transferred onto new agar media until pure cultures were obtained as evidenced by uniform colony morphology. The collection of sediment-derived bacteria used in the direct challenge assays were purified in a similar manner and selected to represent a diverse range of colony morphologies and pigmentation. All strains were maintained on medium A1 prepared with 75%artificial seawater (22 g/l Instant Ocean, United Pet Group, Cincinnati, OH, USA), grown with shaking in A1 without agar (hereafter 'A1') and cryopreserved at −80 °C with 10% glycerol.

DNA extraction, PCR and 16 S ribosomal RNA gene sequencing
All strains presumed to be actinomycetes based on morphology were grown in 7-ml A1 for 3–10 days while shaking at 160 rpm. DNA was extracted from 1 ml of the resulting culture according to the DNeasy protocol (Qiagen Inc., Valencia, CA, USA) with previously described changes (Gontang *et al.*, 2007). For all other strains, colony PCR was performed by suspending a single colony in 5-µl dimethyl sulfoxide and using 1 µl as the PCR template. 16 S ribosomal RNA PCR primers are described in Supplementary Table S4. Each PCR consisted of a 25 µl mixture containing 10 × PCR Buffer (Applied Biosciences, Foster City, CA, USA), 2.5 mM $MgCl_2$ (Applied Biosciences), 0.7% dimethyl sulphoxide, 10 mM dNTPs, 1.5 U AmpliTaq Gold DNA Polymerase (Applied Biosciences) and 10 µmol of each primer. PCR thermocycling conditions were as follows: 5 min of initial denaturation at 95 °C followed by 30 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 30 s and extension at 72 °C for 1 min. Sequencing was performed by SeqXcel, Inc. (San Diego, CA, USA). Sequences were submitted to the National Center for Biotechnology Information Basic Local Alignment Search Tool (BLASTN) and identified based on the taxonomic assignment of the closest Basic Local Alignment Search Tool match.

Direct challenge assays
Salinispora cultures were inoculated from frozen stocks into 25 ml A1. Cultures were shaken at 160 rpm for 6 days (*S. tropica*) or 11 days (*S. arenicola*) after which 60 µl was transferred by pipet to square 150 × 150 mm petri plates containing 100 ml of A1 agar media. The cultures were spread with a sterile loop down the center of the plate to create a narrow (ca. 3–5 mm) lawn of growth and allowed to grow for 7 days (*S. tropica*) or 10 days (*S. arenicola*), based on the time required to reach confluence. These incubation periods correspond to the entry of liquid

cultures into stationary phase (Supplementary Figure S1). Environmental isolates were then inoculated in triplicate from established plate colonies to within 1–2 mm of the *Salinispora* lawn (cross-streaking) with up to 50 perpendicular inoculations made per plate using sterile toothpicks (replicates inoculated onto different plates). Growth of the test strains was considered inhibited if a clearing zone ≥5 mm was observed in the area adjacent to the*Salinispora* lawn in at least two of three replicate assays. The pH within the zones of inhibition was tested in comparison with a medium control using pH test strips (Micro Essential Laboratories Inc., New York, NY). Given that antibiotic production can be time-dependent (Bibb, 1996), a second series of assays was performed in which strains from both *Salinispora* spp. were allowed to grow on agar plates for 23 days prior to adding the bacterial strains. Only strains that showed no evidence of inhibition in the initial assays were tested at this second time point.

Interference vs exploitation competition assays

Follow-up assays were performed to distinguish between interference competition (the production of diffusible growth inhibitors) and exploitation competition (nutrient depletion) as the source of the growth inhibition detected in the direct challenge assays. Agar diffusion assays were performed in triplicate using two *S. arenicola* (CNY-679 and CNY-685) and two *S. tropica* (CNY-678 and CNY-681) strains. These strains were grown in the same manner used in the direct challenge assays, after which 1 cm^2 agar blocks were cut from the area immediately adjacent to the culture and placed over a freshly inoculated lawn of an environmental isolate that previously tested positive for growth inhibition. The lawns were periodically checked (1–10 days) for zones of inhibition surrounding the agar block and scored as positive when a clear zone (≥5 mm) was observed. The blocks were visually assessed throughout the tests to be free of *Salinispora*colonies, which are easily recognized based on morphology, so that any observed inhibition could be linked to the diffusion of compounds that were transferred with the agar blocks. All strains that were not inhibited in the agar diffusion assay were further analyzed to determine whether the cause of the activity observed in the direct challenge assay was due to iron depletion. In this case, the direct challenge assays were repeated using standard A1 and A1 supplemented with $FeSO_4$ (10 μg ml^{-1} final concentration). If growth was inhibited on A1 but not on iron-replete A1, the inhibition was attributed to iron depletion.

Growth rates

Salinispora growth rates were determined by changes in liquid culture dry weight biomass over time. *Salinispora* cultures were inoculated from frozen bacterial stocks (1.8 ml) into 50 ml A1. Following 7 days of growth (25 °C, 160 rpm), 1-ml of this culture was inoculated into each of 24 glass tubes containing 10 ml A1 and shaken at 160 rpm (25 °C). On days 0, 3, 6, 9, 12, 15 and 18, triplicate tubes were filtered onto pre-weighed 47-mm glass fiber filters (Pall Corporation, Ann Arbor, MI, USA), dried overnight (32 °C) and weighed. Cell mass was calculated as mg dry weight per ml and growth curves generated by plotting the log of cell mass vs time. Growth rates were calculated as the change in biomass over time during the exponential phase of growth.

Chemical extractions and disc-diffusion assays

S. arenicola CNY-679 and *S. tropica* CNY-678 were grown on A1 agar plates for 10 and 23 days, respectively. Cell-free agar adjacent to *Salinispora* growth was removed and cut into small pieces using a sterile scalpel and extracted using methanol (500 ml, 160 rpm, 2 h). The volume of agar extracted was measured by solvent displacement. The extract was filtered (0.45 μm Whatman), dried *in vacuo*, dissolved in ca. 10 ml of water and extracted with an equal volume of ethyl acetate. The ethyl acetate layer was separated, filtered (0.45 μm Whatman), dried under N_2, and weighed. A1 agar media control extracts were similarly prepared. Extracts were dissolved in methanol at 1 ×, 10 × and 100 × volumetrically equivalent concentrations with 1 × equal to the extract being dissolved in a volume of solvent equivalent to the volume of agar extracted. Extracts were tested for antibiotic activity against environmental isolates using standard disc-diffusion assays. For these assays, 15-μl of *Salinispora* extract, media extract or solvent controls (MeOH) were added to paper discs, allowed to dry and placed onto A1 agar plates, along with an antibiotic control disc (5 μg ciprofloxacin; BD, Sparks, MD, USA), onto which a bacterial strain

had been inoculated as per the agar diffusion assays. Zones of inhibition were recorded as the diameter of clear halos surrounding the discs. Thirteen of the strains showing sensitivity to *S. arenicola* CNY-679 culture extracts were similarly tested for sensitivity to commercially available rifamycin SV (Sigma-Aldrich, St Louis, MI, USA) at concentrations of 0.01, 0.1, and 1 mg ml^{-1}.

Liquid chromatography-tandem mass spectrometry and MALDI-TOF imaging mass spectrometry

High-resolution liquid chromatography-tandem mass spectrometry was performed using an Agilent 6530 Accurate Mass Q-TOF coupled to an Agilent 1260 LC system (Santa Clara, CA, USA) as described in the Supporting Information. Imaging mass spectrometry was performed on agar plates prepared as per the direct challenge assays using *S. arenicola* CNY-679 and *Kytococcus* sp. CUA-766. The latter was chosen based on the consistently large zones of inhibition observed in the direct challenge assays with all four *S. arenicola* strains. The interaction zone, along with monocultures of both strains and a medium control, were processed for MALDI-based imaging mass spectrometry in positive mode using a Microflex Bruker Daltonics mass spectrometer as described in the Supporting Information.

Statistical analyses

A non-parametric PERMANOVA analysis was used to test for correlations between growth inhibition and the taxonomy of the strains used in the direct challenge assays (Anderson, 2001). This test was performed using the 'adonis' function provided by the vegan package (Oksanen *et al.*, 2014) and run in the statistical program R (R Core Team). Welch's two-sample *t*-tests (Welch, 1947) were performed in R to test for significant differences between growth rates (n=4 for each species), average zones of inhibition (n=4 for each species) and average percentage of strains inhibited (n=12 for *S. arenicola*, n=13 for *S. tropica*).

Genome sequencing and analysis

All genome sequences were generated as previously described (Ziemert *et al.*, 2014) according to the guidelines of the Department of Energy Joint Genome Institute. Twenty-four genomes (Supplementary Table S3) were downloaded from the Joint Genome Institute website and submitted to antiSMASH for identification of genes associated with secondary metabolism (Medema *et al.*, 2011). Gene clusters linked to rifamycin biosynthesis were submitted to NaPDoS (Ziemert *et al.*, 2012) to confirm their identity based on a phylogenetic analysis of the associated ketosynthase domains. To assess the potential for siderophore biosynthesis, previously identified siderophore gene clusters (Supplementary Table S5) were extracted from the closed genomes of *S. tropica* CNB-440 and *S. arenicola* CNS-205 (Penn *et al.*, 2009) using Geneious Pro 5.5.9 (created by Biomatters, available at http://www.geneious.com). These gene clusters were used as queries against a database created from 24 *Salinispora* genomes (12 *S. arenicola* and 12 *S. tropica*) using MultiGeneBlast 1.1.13 (Medema *et al.*, 2013). Genome sequences were considered to contain a gene cluster if the sequence coverage and identity values were >50% to the query sequences.