Supporting Information

High-Resolution Liquid Chromatography-Mass Spectrometry (LC-MS)

Samples were run under the following LC conditions: 1–5 min: 10% MeCN (0.1% TFA) in H_2O (0.1% TFA), 5–26 min: 10–100% MeCN (0.1 % TFA), 26–30 min: 100 % MeCN (0.1 % TFA) for, 0.7 mL/min). Q-TOF MS settings during the LC gradient were as follows: Acquisition: positive ion mode, mass range m/z 300–1700, MS scan rate 1/s, MS/MS scan rate 5/s, fixed collision energy 20eV; Source: gas temperature 300°C, gas flow 11 L/min, Nebulizer: 45 psig, Scan source parameters: VCap 3000, Fragmentor 100, Skimmer1 65, OctopoleRFPeak 750. Samples were prepared by dissolving dry extract in methanol at a concentration of 1 mg /ml, and 15 μg was injected onto a Phenomenex (Torrance, CA) Kinetex C18 reversed-phase HPLC column. Standard Rifamycin S (Sigma) compound was prepared by dissolving dry compound in methanol at a concentration of 0.1 mg/ml and injecting 1.5 μg. LC data were analyzed using ChemStation software (Agilent) and MS data were analyzed using MassHunter software (Agilent).

Matrix-Assisted Laser Desorption Ionization Time of Flight Imaging Mass Spectrometry (MALDI-TOF IMS)

Briefly, the section of agar encompassing the *S. arenicola* colony, zone of inhibition, and cross-streaked CUA-766 colony was cut from the plate using a sterile scalpel and placed on a Bruker MSP 96 anchor plate. The sample was covered with a matrix consisting of a 1:1 mixture of α-cyano-4-hydroxycinnamic acid and 2,5-dihydroxybenzoic acid using a 20 μm sieve. Once the sample was completely covered with matrix, it was dried in a 37 °C oven for 14 hours, at which point it was subjected to IMS.

The plate containing the sample was photographed and then run on a Microflex Bruker Daltonics mass spectrometer outfitted with FlexImaging 2.0, FlexControl 3.0, and FlexAnalysis 3.0 software for run setup and data analysis. The sample photo was loaded onto the FlexImaging command window and three teach points were selected in order to align the background image with the sample target plate. The sample was run in positive reflectron mode, with 400-600 μm laser intervals in XY, 40% laser power, and a mass range of 200-2000 Da. Detailed instrument parameters for collecting MS data are described in [S1]. The data were analyzed using FlexImaging 2.0. The resulting mass spectrum was filtered manually in 0.5–3.0 Da increments with individual colors assigned to the specific masses associated with the peaks.

Supporting references

1. Yang, Y.L., et al., *Translating metabolic exchange with imaging mass spectrometry*. Nat Chem Biol, 2009. **5**(12): p. 885-7.
2. Mincer, T.J., W. Fenical, and P.R. Jensen, *Culture-dependent and culture-independent diversity within the obligate marine actinomycete genus Salinispora*. Applied and Environmental Microbiology, 2005. **71**(11): p. 7019-7028.

3.   Muyzer, G., E.C. de Waal, and A.G. Uitterlinden, *Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA.* Appl Environ Microbiol, 1993. **59**(3): p. 695-700.
4.   Patin, N.V., et al., *Effects of OTU clustering and PCR artifacts on microbial diversity estimates.* Microb Ecol, 2013. **65**(3): p. 709-19.
5.   Wilmotte, A., G. Van der Auwera, and R. De Wachter, *Structure of the 16 S ribosomal RNA of the thermophilic cyanobacterium Chlorogloeopsis HTF ('Mastigocladus laminosus HTF') strain PCC7518, and phylogenetic analysis.* FEBS Lett, 1993. **317**(1-2): p. 96-100.

Supporting figure legends

Figure S1: Average size of the zone of inhibition (ZOI) produced by each strain over both time points. Only non-zero values were included.

Figure S2: Number of environmental isolates inhibited by 0, 1, 2, 3, or 4 *Salinispora* strains for each species.

Figure S3: Number of strains inhibited by each *Salinispora* species. The red section of each bar represents the results for the first time point (*S. tropica* and *S. arenicola* grown for seven and 10 days, respectively, prior to the addition of the challenge strain). The blue section of each bar represents the number of strains inhibited at the second time point (23 days of growth for both *Salinispora* species) prior to the addition of the challenge strain. Only strains that were not active at the first time point were tested at the second time point.

Figure S4: Growth curves for four strains each of *S. arenicola* (a) and *S. tropica* (b) on a semi-log scale. Error bars represent the standard deviations for three biological replicates.

Figure S5: Identification of rifamycin S from culture extracts of *Salinispora arenicola*. (A) MS-MS spectrum of a peak (*m/z* 696) detected in the extract of *S. arenicola* CNY-679 monoculture. (B) Extract generated from a zone of inhibition between *S. arenicola* CNY-679 and *Bacillus* sp. CUA-897. (C) MS spectrum of rifamycin S standard. (D) UV chromatograph of the *m/z* 696 peak matches the rifamycin S spectrum, along with the chemical structure of rifamycin S. Arrows indicate the parent ion in each spectrum.