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A. Experimental

1. Materials and Methods

All reagents were obtained from the Sigma-Aldrich Chemical company unless otherwise specified. *Streptomyces coelicolor A3(2)* was obtained from the John Innes Center, *Tsukamurella pulmonis* from the American Type Culture Collection *(ATCC 700081)*, and *Rhodococcus wratislaviensis* was obtained via dilution plating from hypogean sediments.

2. Eliciting antibiotic resistance and fermentations. To generate antibiotic resistant mutants, the spore inoculum of *S. coelicolor* was uniformly spread on GYM (glucose 0.4 %, yeast extract 0.4 %, malt extract 1 %, peptone 0.1 %, sodium chloride 0.2 %, agar 2 %) agar plates containing streptomycin at one of two concentrations (100 μg/mL, 300 μg/mL) or rifampicin at either 200 μg/mL or 400 μg/mL (concentrations of antibiotics were chosen so they exceed the minimum inhibitory concentration for *S. coelicolor* on GYM medium). After two weeks of incubation at 30 °C, the agar plates were inspected for the presence of resistant colonies, which were then aseptically transferred to antibiotic-free ISP2 (glucose 0.4 %, yeast extract 0.4 %, malt extract 1 %, agar 2 %) plates. Each *S. coelicolor* mutant was then inoculated to 20 mL ISP2 liquid seed culture, incubated for 7 days, and from seed culture to 50 mL liquid ISP2 fermentation culture for 7 days of incubation at 30 °C. Progenitor *S. coelicolor* was incubated under the same conditions to generate the control culture.

3. Rare earth element fermentations. For rare element additives, the spore suspension of *S. coelicolor* was inoculated on ISP2 agar plates for incubation at 30 °C for 7 days, then inoculated from plates into 20 mL liquid seed culture and from seed culture to 50 mL liquid ISP2 production cultures containing various concentrations of scandium chloride (20 μM, 50 μM, 100 μM, 200 μM, 500 μM) or lanthanum chloride (1500 μM, 1700 μM, 1900 μM, 2100 μM, 2500 μM) for 7 day incubations at 30°C. To generate a control, *S. coelicolor* was incubated in 50 mL additives-free ISP2 medium under the same conditions.

4. Extraction of liquid fermentations. Total culture metabolite extracts from liquid cultures were generated by adding 50 mL of methanol to each fermentation flask and shaking the flasks on a rotary shaker for 1 h. Mycelia were then separated on a centrifuge, and supernatants were dried *in vacuo* to yield crude extracts.

5. Co-culture generation. Co-culture plates were prepared by addition of 40 mL of sterile ISP2 medium to a one well OmniTray plate. Cryogenic spore suspensions of *S. ceolicolor* were cultivated on agar plates (100 x 15 mm) containing 30 mL of ISP2 medium and incubated at 30°C until the production of spores occurred. The spores were removed from the surface of the plate using a sterile loop and suspended in 25 mL of ISP2 liquid medium at a concentration of approximately 10^8 spores/mL as determined via hemocytometer. This suspension was homogenized and decanted into a one well plate as a reservoir. The pins of a 96 well replicator were submerged into the spore solution and applied to the surface of the solid support within the previously prepared one well OmniTray plate without puncturing the surface (**Figure S1**). The plates were incubated for 24 hours at 30°C. Cryogenically stored *M. luteus* was inoculated into 5 mL of sterile ISP2 medium 8 h prior to application to the co-culture plate. *Rhodococcus wratislaviensis* stock was inoculated into 5 mL of sterile ISP2 medium 24 h prior to application to the co-culture plate. Cryogenically stored *T. pulmonis* stock was inoculated into 5 mL of sterile heart infusion medium 24 h prior to application to the co-culture plate. For all competing organisms, once an OD_{600} of ~1 was achieved, the 5 mL sample was diluted into 30 mL of medium in separate one well plate reservoirs. The pins of a 96 well replicator were submerged into the solution and applied to the surface of the solid support within the one well OmniTray plate without puncturing the surface in an offset manner relative to the previously inoculated actinomycete. After 7 days (**Figure S2**), co-culture plates were cut into 1 x 1 cm segments and extracted with equal volumes of methanol by shaking for 3 h at 170 rpm and 30 °C.

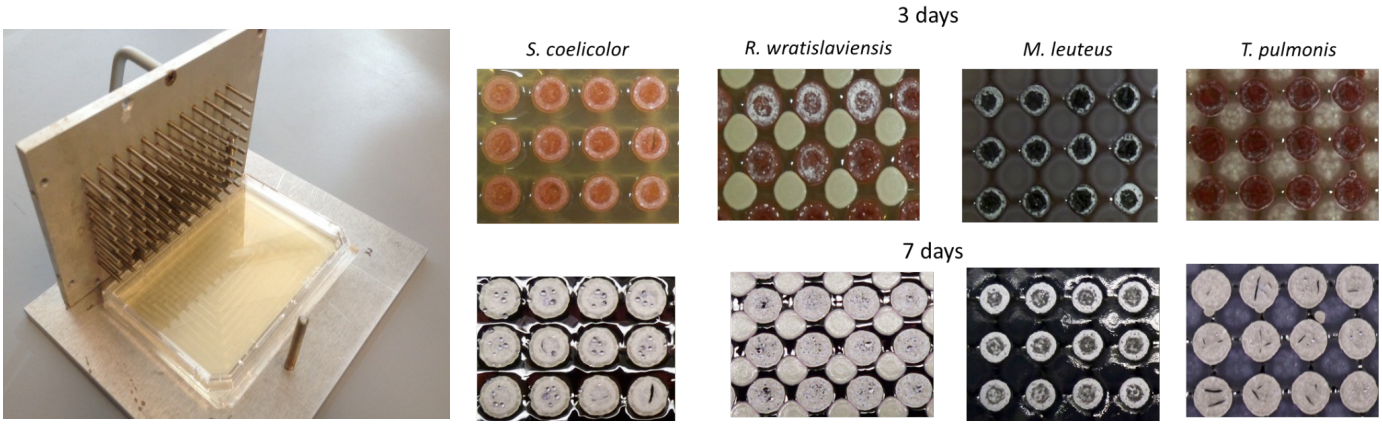


Figure S1. Left, apparatus used for co-culture on agar plates generating heat maps for co-culture panel in **Figure 3**. A guide rail permits the 'checkerboard' application of two organisms. Right, *Streptomyces coelicolor A3(2)*, grown in monoculture on the left, was co-cultured with *Rhodococcus wratislaviensis*, *Micrococcus leuteus*, and *Tsukamurella pulmonis*. Upper set shows growth and phenotype after incubation for 3 days, below, incubation for 7 days.

6. UPLC-IM-MS Data Acquisition and Processing. Extract samples were resuspended in methanol at a concentration of 200 mg/mL. UPLC-IM-MS^E data acquisition was performed analysis on a Synapt G2 HDMS platform (Waters Corporation, Milford, MA) with a 25 min gradient. Mobile phase A consisted of H_2O with 0.1 % formic acid, and mobile phase B consisted of acetonitrile with 0.1 % formic acid. A 1x100 mm 1.7 µm particle BEH-T3 C18 column (Waters Corporation, Milford, MA) was used for chromatographic separations with a flow rate of 75 µL/min and a column temperature of 40 °C. An autosampler with a loop size of 5 µL held at 4 °C was used for sample injection. The initial solvent composition was 100 % A, which was held for 1 min and ramped to 0% A over the next 11 min, held at 0 % A for 2 min, and returned to 100 % A over a 0.1 min period. The gradient was held at 100 % A for the next 10.9 min for equilibration. Prior to analysis of the sample queue, ten sequential column-load injections were performed with 5 µL of the quality control. This protocol increases retention time stability and is critical to reproducible analyses. Quality control injections were then performed after every 10 sample injections to ensure instrument stability. Quality controls were comprised of pooled equal aliquots of all samples analyzed.

IM-MS^E spectra were acquired at a rate of 2 Hz from 50-2000 Da in positive ion mode for the duration of each sample. The instrument was calibrated to less than 1 ppm mass accuracy using sodium formate clusters prior to analysis. A two-point internal standard of leucine enkephalin was infused in parallel to the sample at a flow rate of 7 µL/min, and data were acquired every 10 s. This was used for post-processing determination of accurate mass for prioritized ions. The source capillary was held at 110 °C and 3.0 kV, with a desolvation gas flow of 400 L/h and a temperature of 150 °C. The sampling cone was held at a setting of 35.0, with the extraction cone at a setting of 5.0. In the MS^E configuration, low and high energy spectra were acquired for each scan. High energy data provided a collision energy profile from 10-30 eV in the transfer region, providing post-mobility fragmentation. Ion mobility separations were performed with a wave velocity of 550 m/s, a wave height of 40.0 V, and a nitrogen gas flow of 90 mL/min, with the helium cell flow rate at 180 mL/min. Internal calibrant correction was performed in real time.

Data were converted to mzXML format using the msconvert tool from the ProteoWizard package.[1] Peak picking and alignment were performed using XCMS in R.[2] See **Figure S3** for details and package locations. The resulting data matrix contained 2154 detected features and was formatted for analysis using both GEDI and Umetrics. Formatting for GEDI is outlined below, formatting for Umetrics was performed by extracting and transposing the sample-feature intensity matrix generated from XCMS, and importing it into Umetrics software. Prior to GEDI and MVSA, analytical triplicates were averaged. For GEDI analysis, a grid of 25 x 26 was generated. Software specific parameters include: 100 first phase training iterations with an initial training radius of 10.0, a learning factor of 0.5, a neighborhood block size of 20, and a conscience of 5.0, and 160 second phase training iterations with a neighborhood radius of 1.0, learning factor of 0.05, neighborhood block size of 2, and conscience of 2.0. A random seed of 10 with a Pearson's correlation distance metric and random selection initialization was used.

Metabolite identifications were performed using accurate mass measurements and fragmentation spectra extracted from IM-MS^E data. Utilizing drift time correlations, product ions were correlated appropriately to precursors for extraction of high energy spectra.

7. Mass spectrometry data processing workflow

(1) To begin, data are converted from vendor-specific formats to open source mzXML formats. For Waters data, msconvert was used for conversion purposes through the command prompt:

> msconvert [input file] --mzXML –filter "sortByScanTime" –o [output location]

(2) The msconvert utility is included in the ProteoWizard package[1], which can be download at:

http://proteowizard.sourceforge.net/.

To perform feature detection, xcms was used within R. The download and a fantastic tutorial are available on the METLIN website:

(http://metlin.scripps.edu/xcms/)

(2) The resulting output from xcms analysis (report.tsv) was then used for further analysis. Technical replicates were averaged within any available spreadsheet software, and formatted for GEDI analysis. Formatting and download can be found at:

(http://apps.childrenshospital.org/clinical/research/ingber/GEDI/GEDI_Help.htm)

(4) Regions of interest were first generated through differential analysis within GEDI.
These regions were then given defined boundaries. This was done manually, though boundary recognition algorithms are being investigated.

(5) Within GEDI, metabolite locations were exported through

>File>Export Results>Save Gene Assignments List

This generated a list of the SOM locations of all metabolites, which were then compiled into lists corresponding to ROIs as seen in **Tables S2-S7** in this ESI material. Using the original data matrix, intensities for all features within an ROI were summed, and the percent contribution of each determined for prioritization purposes.

(6) Metabolite identification then began, with the only notable caveat being the extraction of fragmentation spectra using post-mobility fragmentation, which allowed untargeted fragmentation with relatively contaminant-free spectra. Retention time and drift time occurrences were also used to determine if certain features were in-source fragments. Retention times were similar, and the product ion appeared both at a drift time unique to the in-source fragment within the intact spectrum and as a product ion with the same drift time as the precursor within the fragmentation spectrum.

(7) Monoisotopic peaks were used for database searching, with candidate molecules compared to fragmentation data and retention time occurrence.

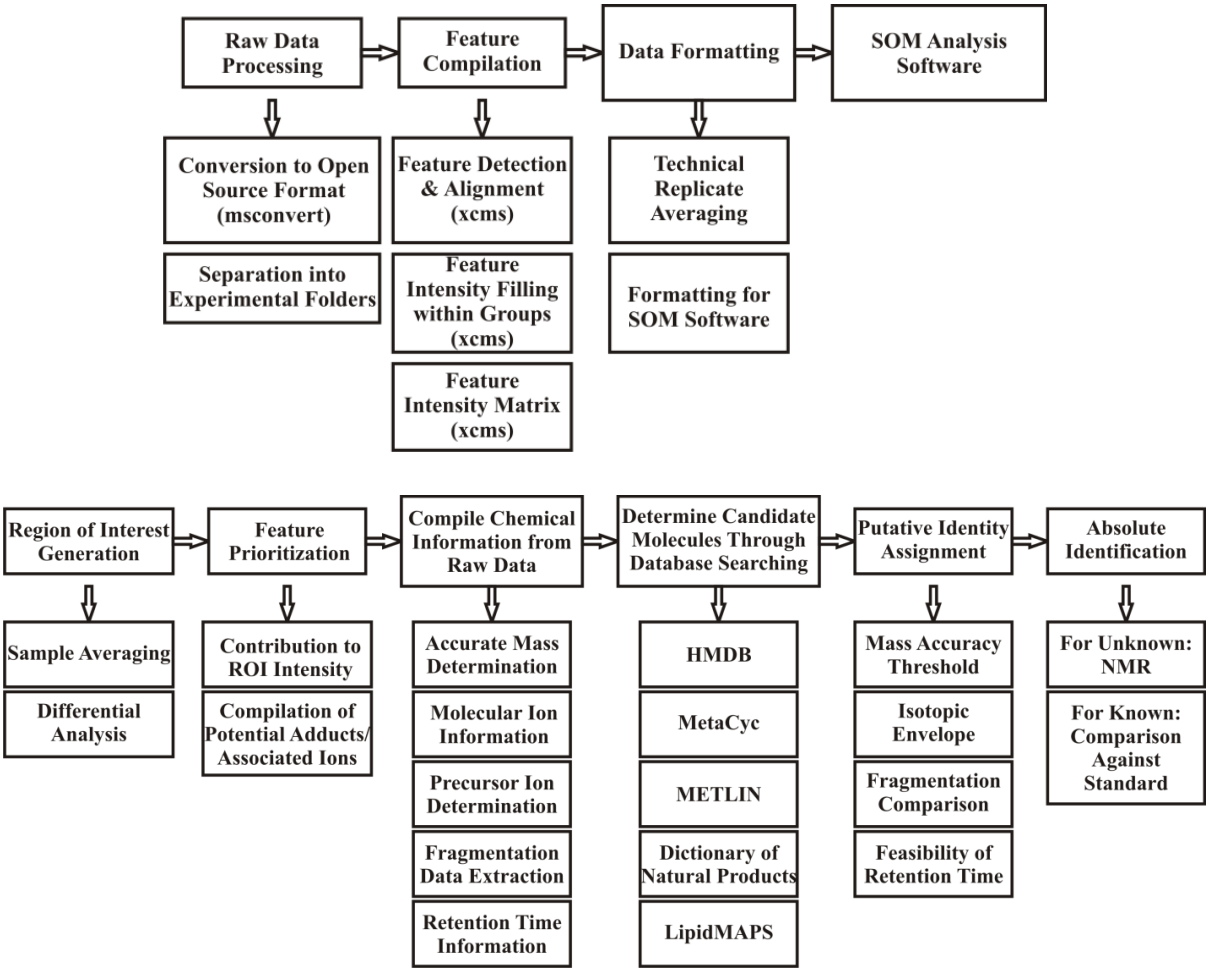


Figure S2. Above, work flow spanning data acquisition to data analysis using SOM software used to generate **Figure 3**. Below, depicted is a workflow spanning region of interest (ROI) generation to the assignment of molecular identity to features of interest. This method is specific to the UPLC-IM-MS^E platform utilized in this work.

B. Supplemental Data

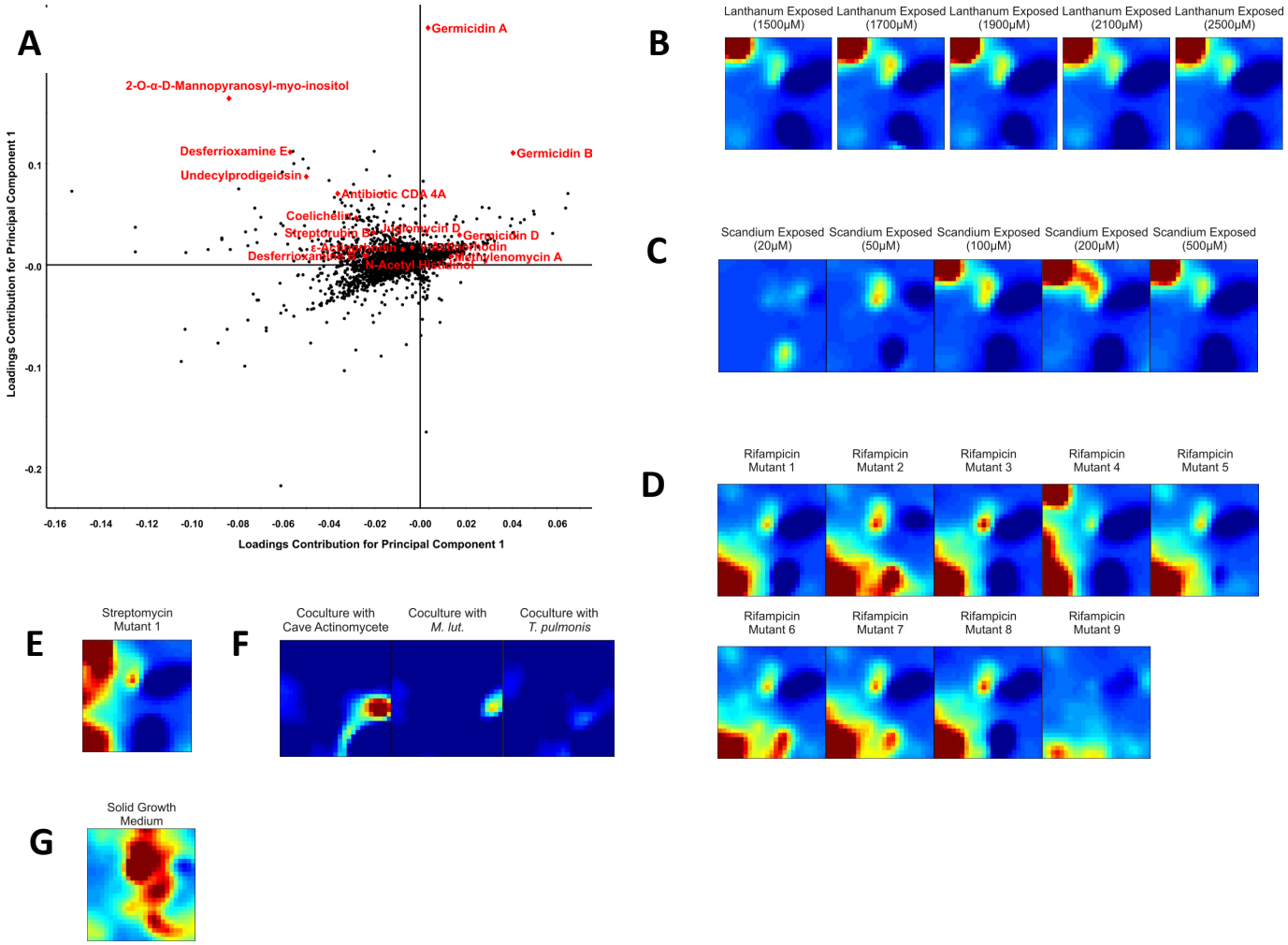
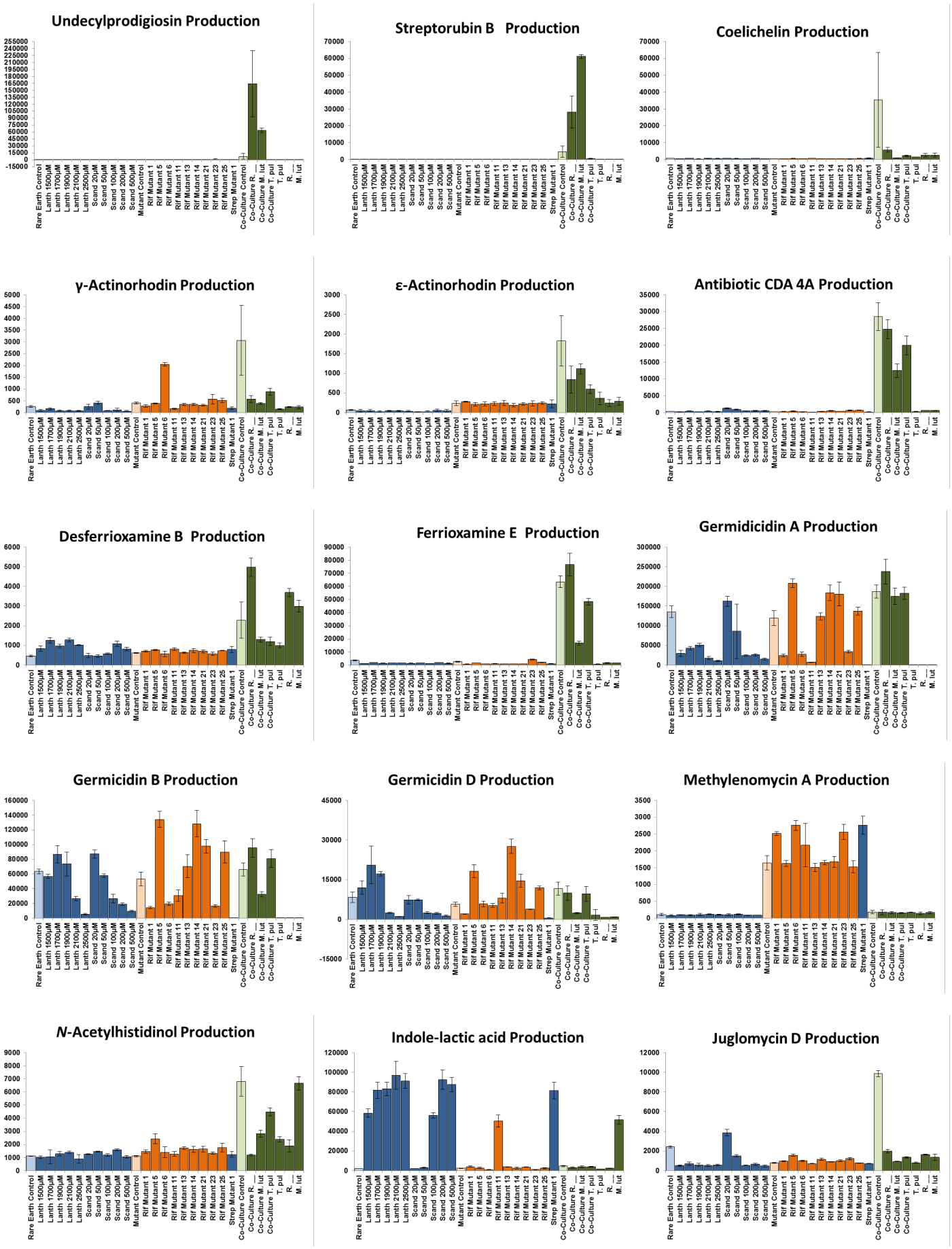


Figure S3. (A) Loadings plot for **Figure 2A.** The locations of identified secondary metabolites are annotate in **Figure 3**. Differential self-organizing map depictions of **(B)** lanthanum, **(C)** scandium, **(D)** rifampicin-resistant, **(E)** streptomycin-resistant, **(F)** mixed fermentation, and **(G)** *Streptomyces coelicolor A3* grown on ISP2 agar.

Table S1. Gene clusters correlated to secondary metabolites production (shown in **Figure 3**) in *Streptomyces coelicolor (A3)*.

Detected Metabolite(s)	Correlated Gene Cluster
Desferrioxamine	SCO2782-2785
Coelichelin	SCO0489-0499
Actinorhodin	SCO5071-5092
Prodiginine	SCO5877-5898
Calcium dependent antibiotic	SCO3210-3249
Germicidin	SCO7221
Ectoine	SCO1861-1871
Methylenomycin	SCP1


Undecylprodigiosin Production
Streptorubin B Production
Coelichelin Production
γ-Actinorhodin Production
ε-Actinorhodin Production
Antibiotic CDA 4A Production
Desferrioxamine B Production
Ferrioxamine E Production
Germidicidin A Production
Germicidin B Production
Germicidin D Production
Methylenomycin A Production
N-Acetylhistidinol Production
Indole-lactic acid Production
Juglomycin D Production

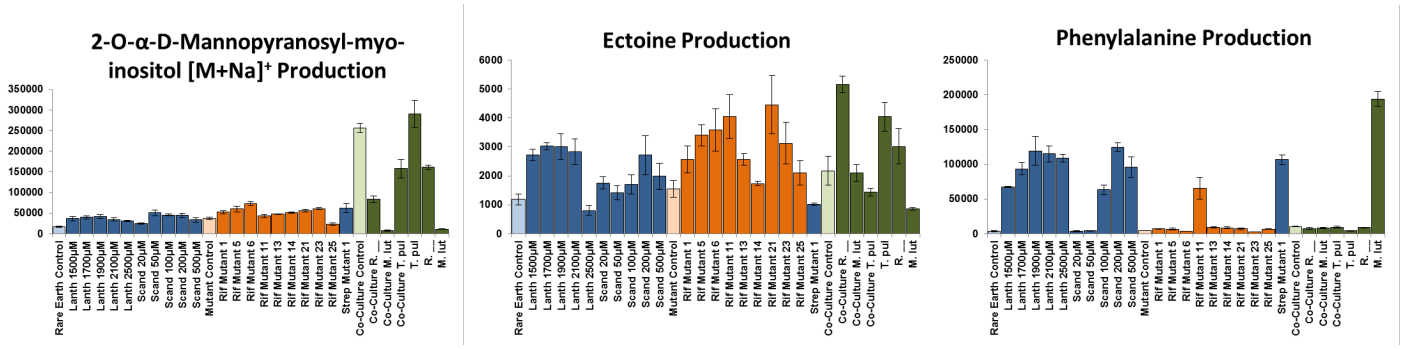


Figure S4. Production profile of selected metabolites from **Figure 4** across culture conditions (continued from previous page).

REFERENCES

1. Darren Kessner, Matt Chambers, Robert Burke et al., *Bioinformatics* 24 (21), 2534 (2008).
2. C. A. Smith, J. Elizabeth, G. O'Maille et al., *Analytical Chemistry* 78 (3), 779 (2006).