Synthetic ecology for quantitative prediction of anti-comtamination strategies in biofuel-producing cultures of *Synechocystis* PCC 6803

- We previously engineered the cyanobacterium *Synechocystis* PCC 6803 to secrete laurate, a J8 jet fuel precursor.
- Reduced laurate yield correlated with contamination of *Synechocystis* cultures with laurate-consuming heterotrophic bacteria ('scavengers').
- We hypothesized that adding salt, antibiotic and/or alkalinity would selectively inhibit scavengers while allowing *Synechocystis* to flourish, increasing laurate yield.
- We used statistical modeling to generate and test quantitative predictions of heterotroph growth in response to levels of added salinity, alkalinity, and the antibiotic kanamycin in rich media (LB broths) and also in *Synechocystis* cultures.
- We hypothesized that Defined Consortia made from diverse scavenger isolates can be used to simulate the PBR contaminome under controlled reproducible laboratory conditions.

Methods and Results I: Factorial analysis to generate and test model with diverse Consortia

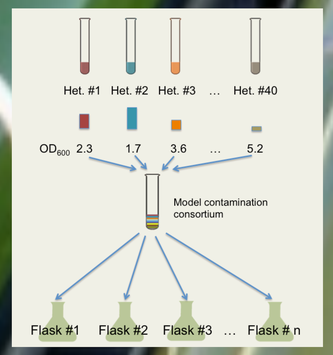
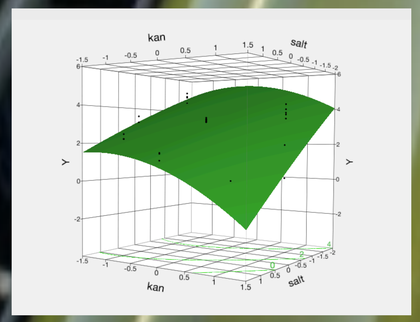


Fig. 1: Defined Consortia.
Left: *Synechocystis* mutants secrete fatty acids which accumulate in supernatant (Liu et al, PNAS 2011).

Middle: Heterotroph isolates with diverse colony phenotypes were selected to generate Defined Consortium 1 and 2, representing diverse phyla across the naturally occurring PBR contaminome.

Right: Defined Consortia were reproduced by combining equal OD_{600} of known isolates for each experiment. Each flask was inoculated with a total Consortium starting OD_{600} of 0.001 and incubated with shaking at 30° C.

Coded Levels	Uncoded Levels		
Factors	Salt, Molar	Kan, ug/mL	pH
-√2	0.0	0	7.5
-1	0.09	29	7.8
0	0.3	100	8.5
1	0.51	171	9.2
√2	0.6	200	9.5



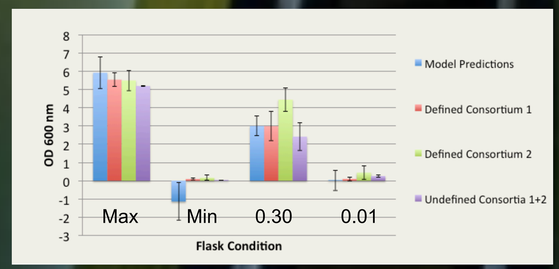
Regression (coded units):
Y = het OD = 3.28 – 1.36(salt) – 0.75(kan) – *0.16(pH)* – 0.44(kan²) + 0.31(pH²) –0.22(salt*kan)

Fig. 2: Design and Regression.
Left: Central Composite Design was chosen to generate surface response model. Levels of additives were determined empirically to encompass the entire sample space biocompatible with our *Synechocystis* mutant. A total of 17 conditions were generated from this design: 8 conditions from full factorial (2^3), 3 center points, and 6 axial points.

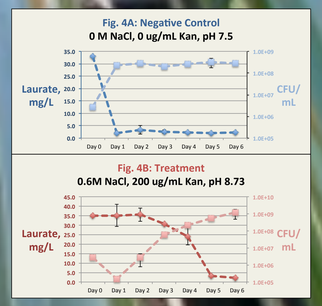
Right: Regression and one surface response model generated from Defined Consortium 1 in rich media. Regression p-value < 0.0001; R-squared pred. of 83% for raw data (not normalized or transformed).

The main effect for pH was not statistically significant (p-value = 0.072).

Fig. 3: Model Accurately Predicts Growth of Diverse Consortia in Rich Media.
Four model predictions of Consortium 1 growth at 24 hours (blue bars;Maximum OD_{600}, Minimum OD_{600}, OD_{600} = 3.0 and OD_{600} = 0.01) were generated with 95% confidence intervals. These predictions were tested using Defined Consortium 1 (red bars) and a second, novel Defined Consortium 2 (yellow bars), comprised of additional unique 40 heterotroph isolates. Finally, two Undefined Consortia were tested (purple bars). These Undefined Consortia were generated by sampling 1.5 liters of Tempe Canal Water (in January and August) which was then propagated in LB plain broth to create the inoculum.



Results II: Additives protect laurate for 72+ hours in deliberately contaminated cultures



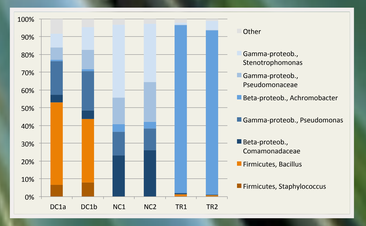
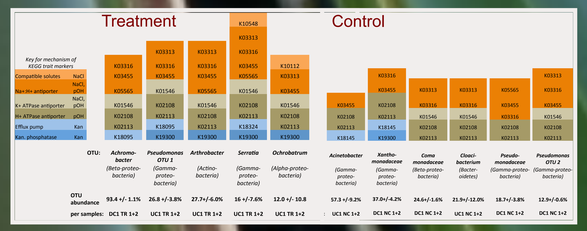


Fig 4: Laurate time-course assay in *Synechocystis* supernatants.
Left: Axenic *Synechocystis* supernatants (100 mL; log-phase) were spiked with sodium laurate to 100 mg/L (100 ppm), and inoculated with Defined Consortium 1 (left) or Undefined Consortium 1 (Figure 5, below) at a starting OD_{600} of 0.001, incubated at 30° C. with shaking. 10 mL aliquots of culture were sampled every 24 hours to quantify laurate via FID-GC. Cell densities were measured by plating (left) or flow cytometry (not shown).
Right: 16s Illumina sequence data are shown for the Defined Consortium 1 inocula replicates (DC1a and DC1b), and replicates of negative control (NC1, NC2) and Treatment (TR1, TR2) at the Day 6 time point.

Methods and Results III: Metagenomic analysis shows 'minimum core' of trait markers



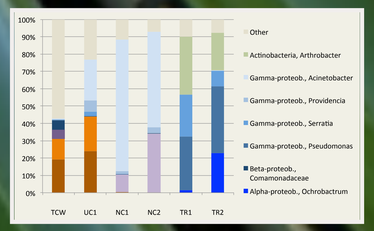


Figure 5: 16s Illumina sequence and PICRUSt metagenomic prediction of KEGG trait markers
To predict function of Consortia, a comprehensive list of 17 KEGG ortholog trait markers (not shown) was curated via literature review and PICRUSt library search. Markers were chosen for relevance to additives: for example, markers for tolerance to NaCl include NhaA (K03313), a sodium:proton antiport protein. Markers conferring tolerance to NaCl are shown in shades of orange according to molecular mechanism; alkalinity tolerance in tan, and antibiotic tolerance in blue (above left). For each of the abundant phyla shown in the 16s bar charts (above right and Fig 4), PICRUSt data for marker genes is reported (above left). We found that all Treatment phyla contained at least 7 markers covering all three additives (the 'minimum core'), whereas negative controls had no more than 6 markers and lacked markers for 1 or more additive. Therefore, phyla are sorted both by type and abundance of markers in treatment v control conditions, independent of 16s OTU identity.

Conclusions: Research platform for synthetic ecology of PBR contaminome is established

- Presence of salt, alkalinity and kanamycin protects laurate by suppressing contaminant growth in *Synechocystis* supernatants.
- Metagenomic function and community structure consistent with hypothesis that PBR contaminome comprised of guild of unrelated bacteria with a minimum core of adaptive traits.
- Future testing by adaptive trait marker gene expression measurements and additional diverse Consortia replicates.
- This finding informs a genetic-based strategy for choosing better combinations of additives by avoiding those with overlapping resistance mechanisms (for example, a single anti-porter marker can confer tolerance to both NaCl and alkalinity, which is sub-optimal for maximum contaminant mitigation).
- Proof of concept established for new research platform of quantitative synthetic ecology of PBR contaminome. Future improvement by adding pH stat and automated OD data logger for kinetics; and additional additives and contaminant Consortia categories (eukaryotes, phage).

Authors: Rey C. Allen[1], Alex Zevin[2], Bruce E. Rittmann[1], Roy Curtiss III[3], and Rosa Krajmalnik-Brown[1]
[1]Swette Center for Environmental Biotechnology, Arizona State University, Tempe, AZ, 85281
[2]Department of Pharmaceutics, University of Washington, Seattle, WA, 98195
[3]Department of Infectious Disease and Pathology, University of Florida. Gainesville, FL 32611

ASU BIODESIGN INSTITUTE
ARIZONA STATE UNIVERSITY