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Sandia National Laboratories

Microbial management: Monitoring

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Advancements in Algal Biomass Yield (ABY) DOE-FOA-0000811-1547
Kick Off Meeting, September 9 – 10, 2014
Kailua-Kona, Hawaii

Technical goals

Identification and development, for each algal strain, a "probiotic" microbial community that consistently promotes the growth/lipid production of the algae,

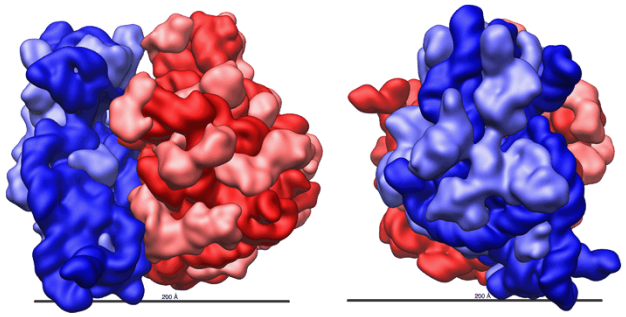
Track the development and progression of microbial communities in outdoor runs of targeted algal strains, simultaneously using culture-based methods and deep sequencing of small-subunit ribosomal RNA genes.

Develop qPCR-based strategies for tracking key microbial species, once these have been identified, through algal production runs.

Develop qPCR probes for tracking, and facilitating the isolation of key species.

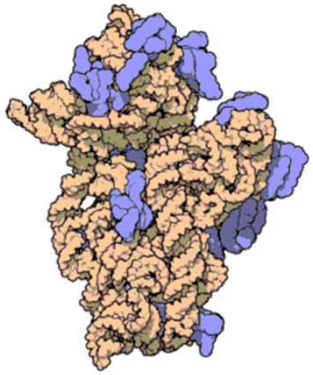
Small subunit ribosomal RNA (SSU rRNA) gene is used for phylogenetic analysis

Ribosome structure



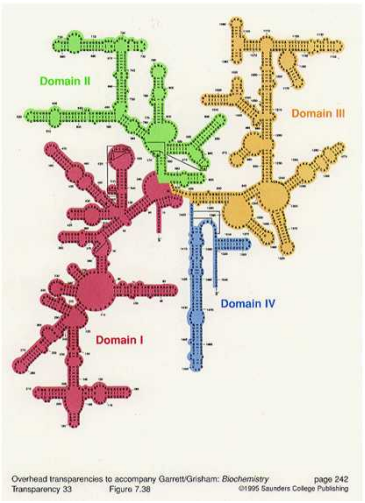
Large Subunit, Small Subunit

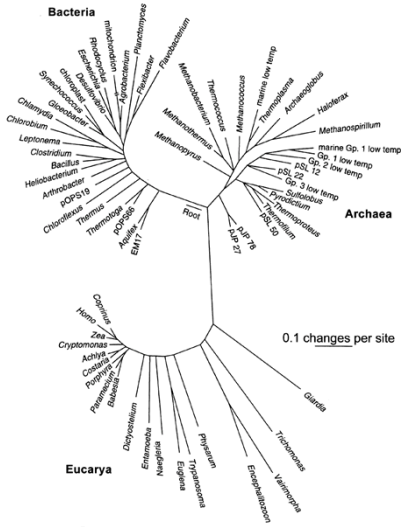
Small subunit structure



RNA, Protein

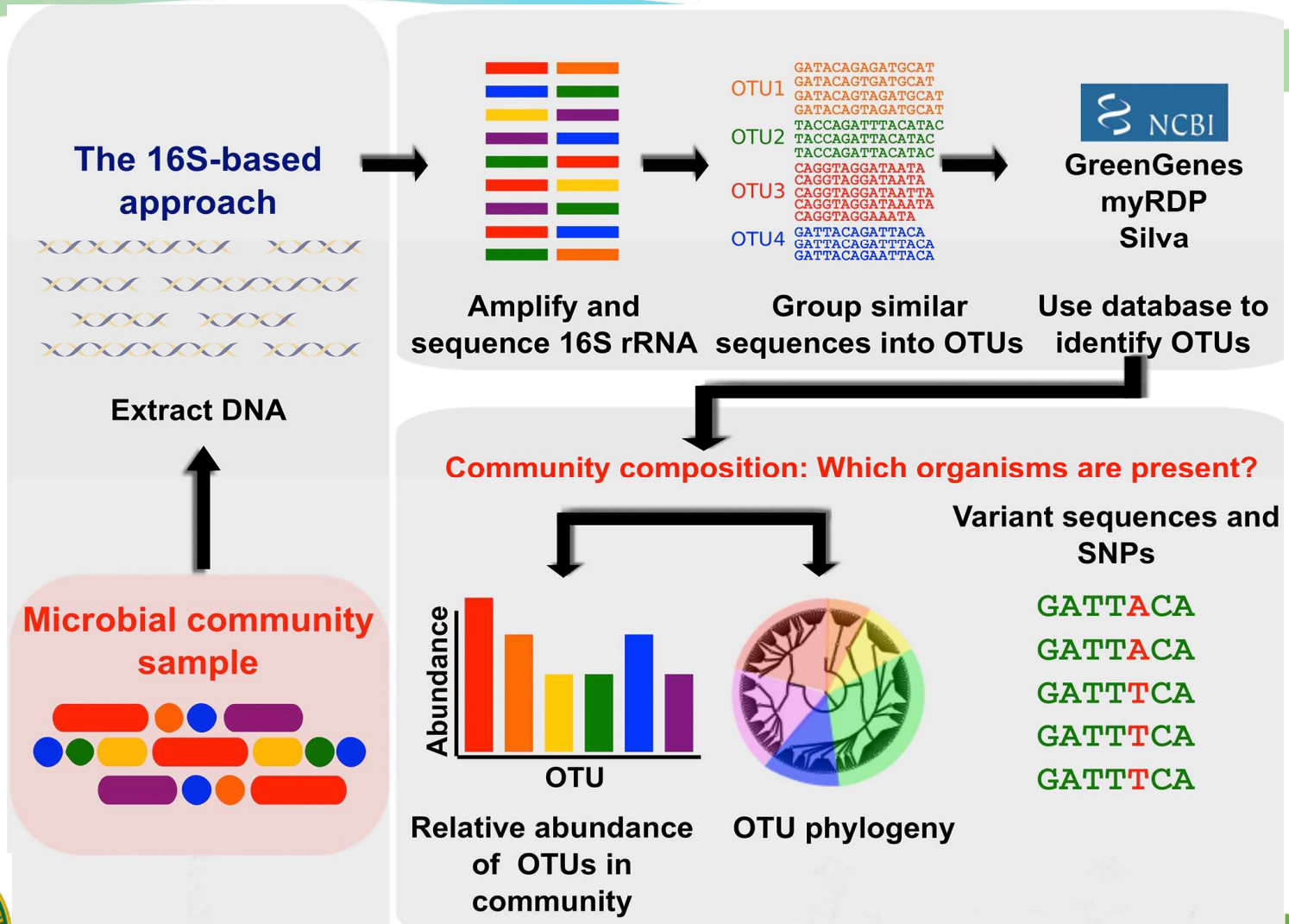
SSU rRNA





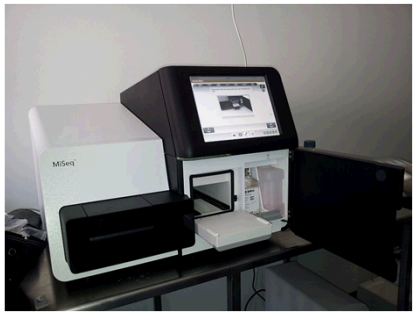
Phylogenetic "Tree of Life" based on SSU rRNA sequences

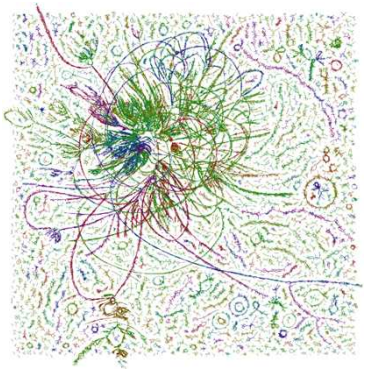
Microbial community analysis by deep sequencing of SSU rRNA genes is a multistep process



Morgan XC, Huttenhower C (2012) PLoS Comput Biol 8(12): e1002808.

Microbial community analysis will leverage work carried out in other BETO funded projects.





V Iverson/U of Washington

Pond

↓

Sequencer

↓

Microbiome

From "Pond Crash Forensics" and ATP^3, we have developed methods for all the steps in the process

- Sample acquisition
  - Stabilization for shipment
- Nucleic acid extraction
  - Extraction bias
- Sequencing library prep
  - Plastid exclusion
  - Amplification bias
- Data analysis
- qPCR primer design

Sample Acquisition: leveraging the ATP^3 protocol

Harvesting by centrifugation: 50 mL samples

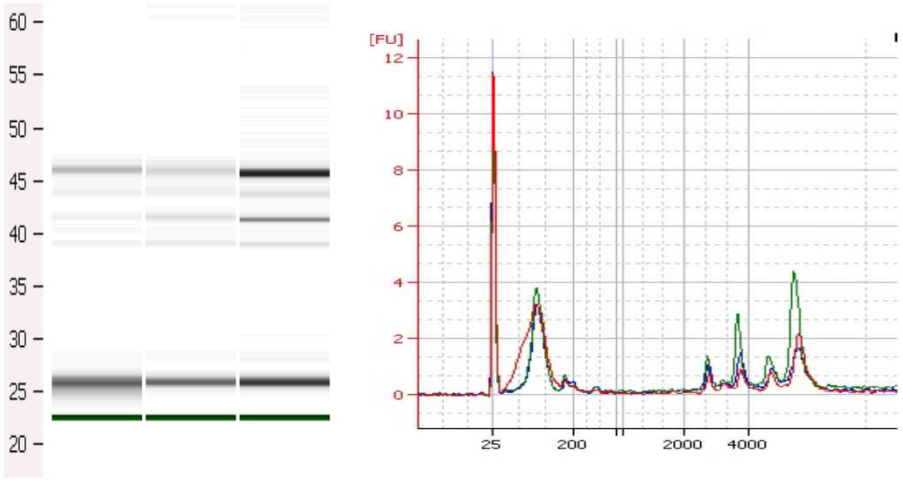
Resuspension in RNALater based on AFDW

Storage at 4° overnight followed by -20°

Shipment on wet ice.

50 mL culture at AFDW (g/L) of	Milliliters of RNALater
0.13	0.625
0.25	1.25
0.38	1.88
0.5	2.5
0.75	3.75
1	5
1.25	6.25
1.5	7.5

RNA from *N. salina* (Zymo Bact/Fungal Kit)



1. Cells in ESAW at 4C (3 weeks); Red
2. Cells in RNA Later, 4C (3 weeks); Blue
3. Cells in RNA Later, -20C (3 weeks); Green

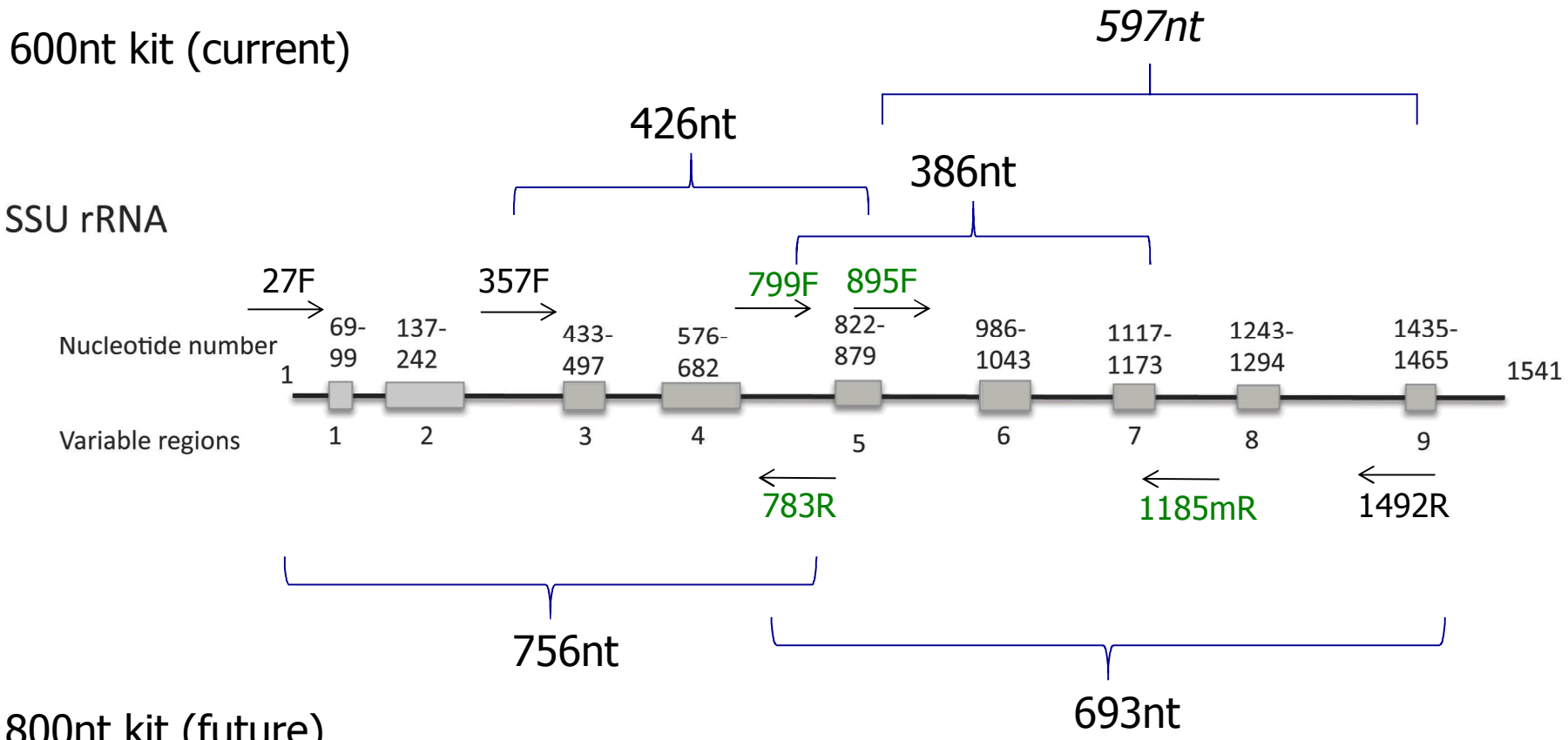
Microbiome analysis versus pond crash diagnostics requires more stringent nucleic acid extraction

2-3 separate extraction protocols to partly compensate for differential extraction efficiencies.

Zymo bacterial
Qiagen Plant Kit
Zymo Soil Kit
} 96 well format

Mock microbial communities as control for extraction
Genomic DNA from mock microbial communities as control for amplification.

SSU rRNA gene sequencing strategy (bacterial)

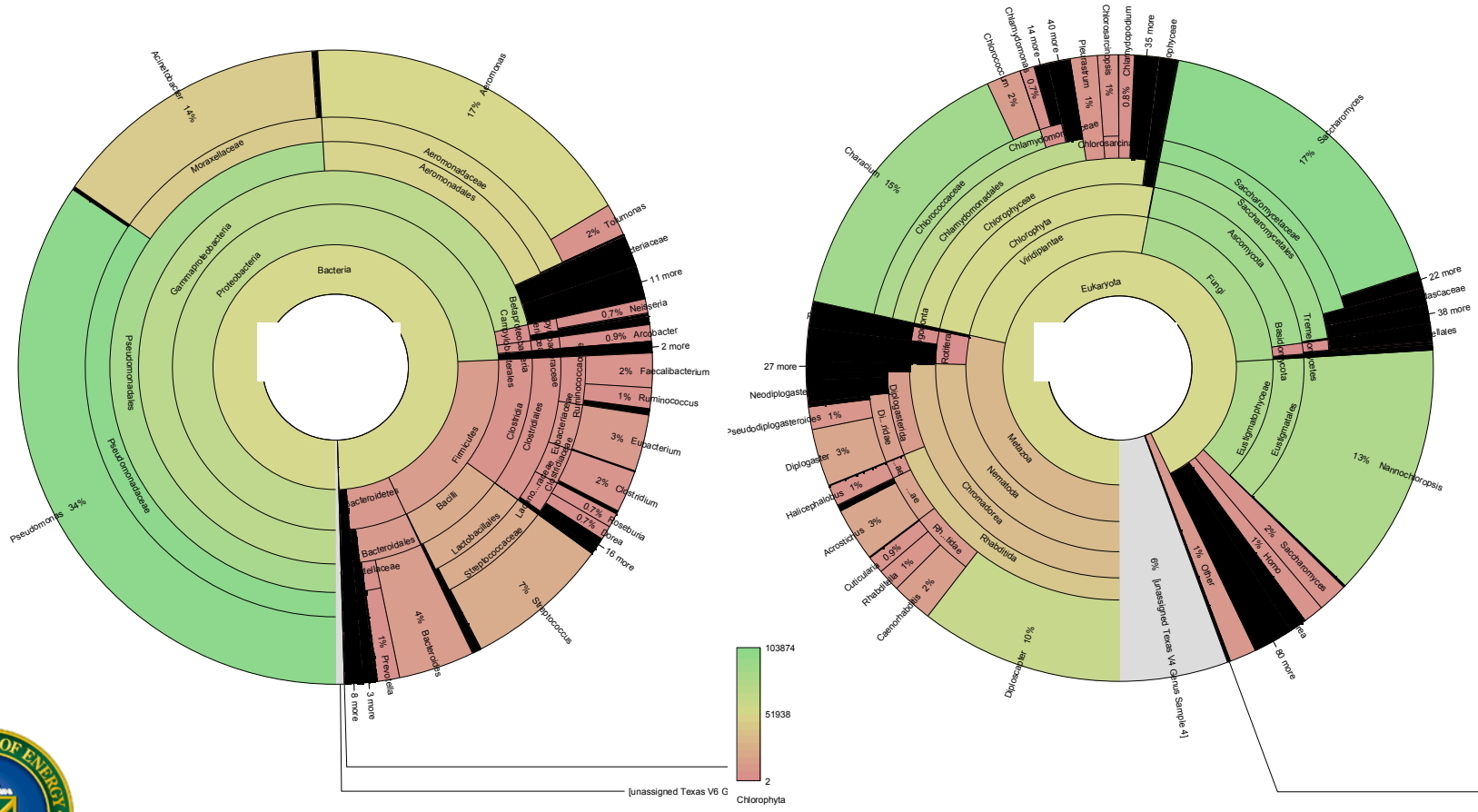


Data Visualization from single ponds

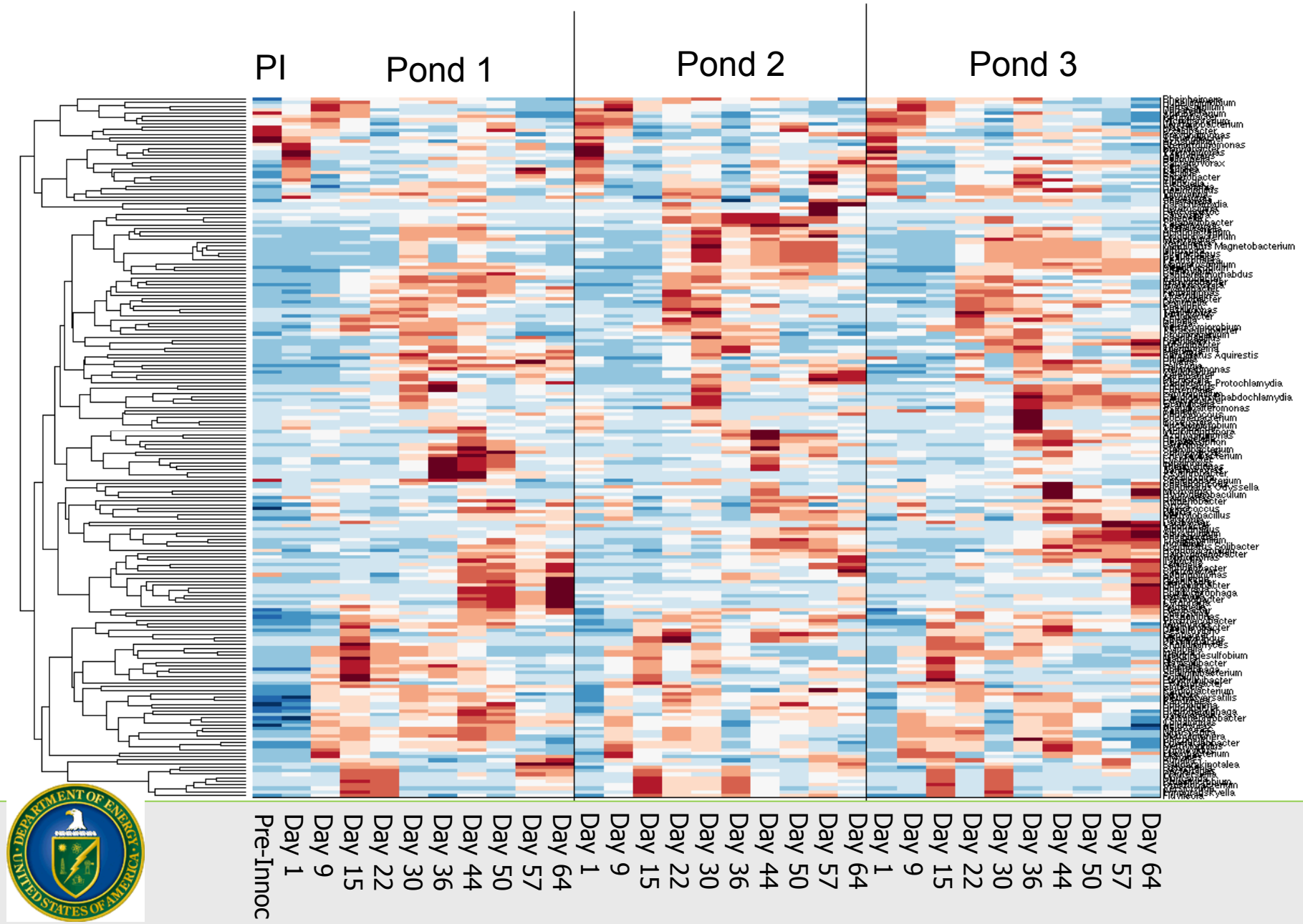
Krona (snapshot) - Texas V6 Genus Sample 1

Krona (snapshot) - Texas V4 Genus Sample 4

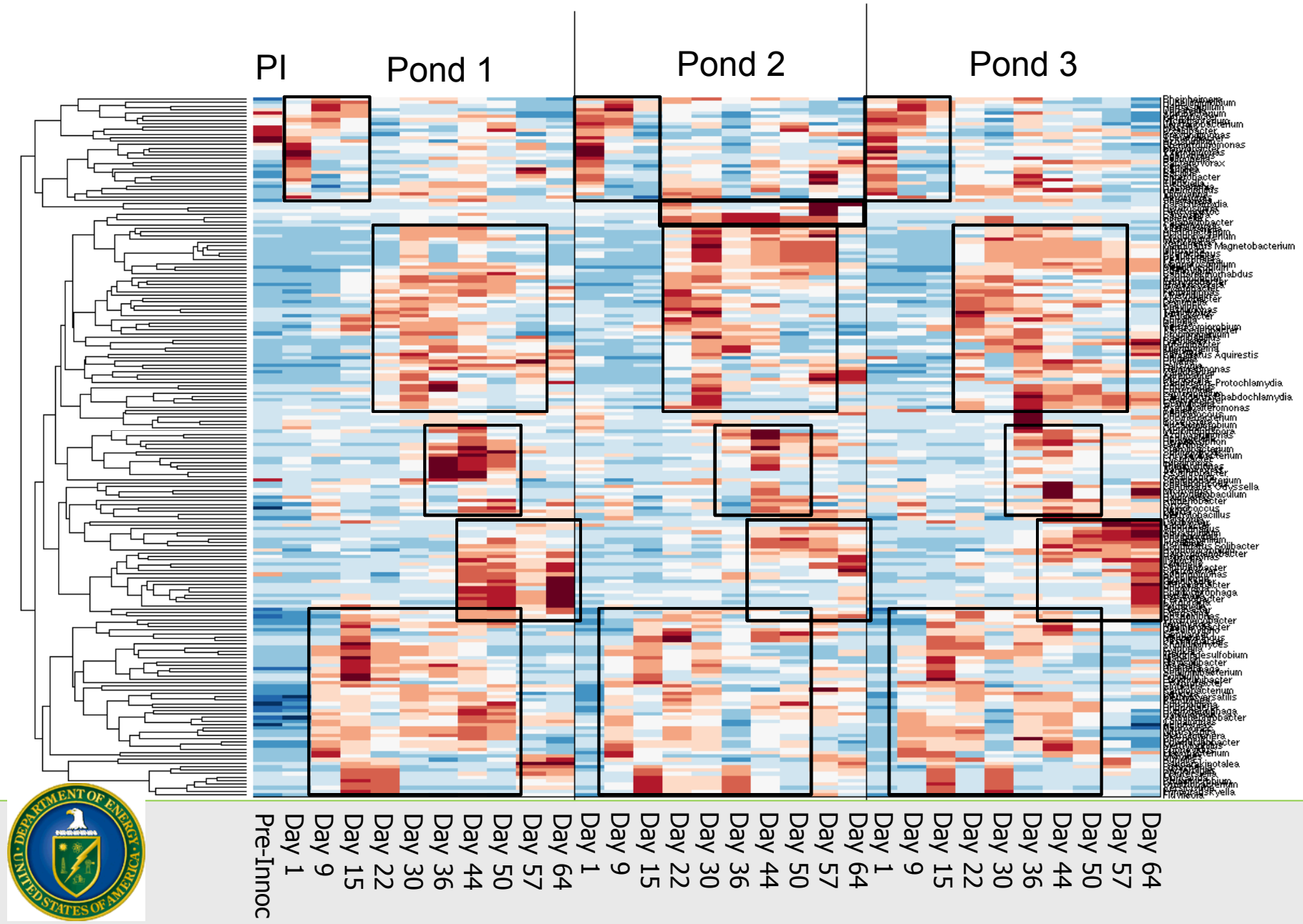
8/7/14 2:22 PM



Tracking of Microbial Consortia in Algal Ponds

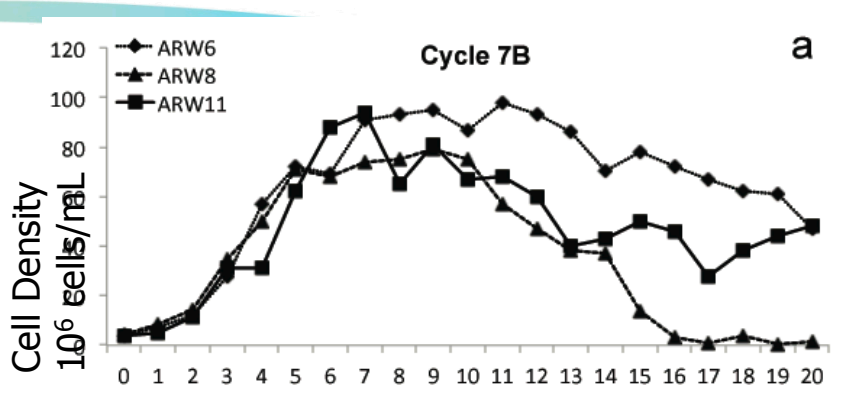


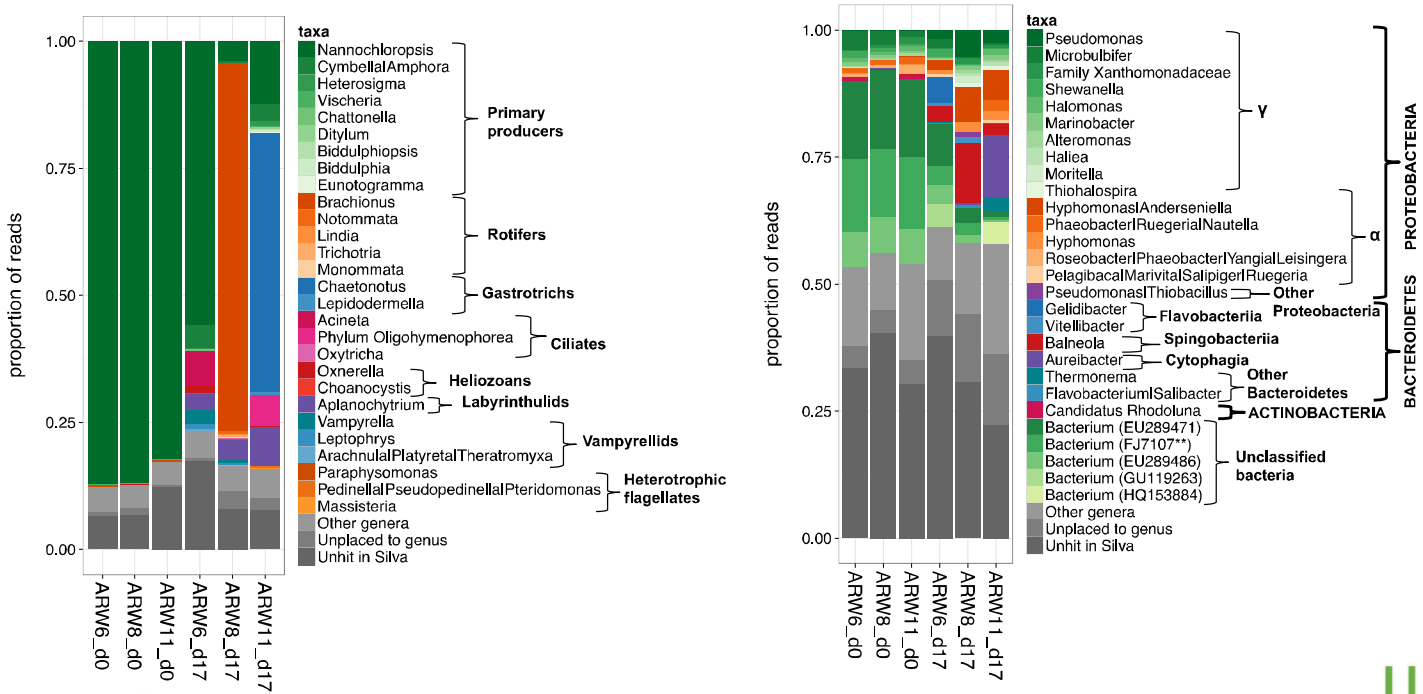
ecific clusters of species arise concurrently in parallel ponds



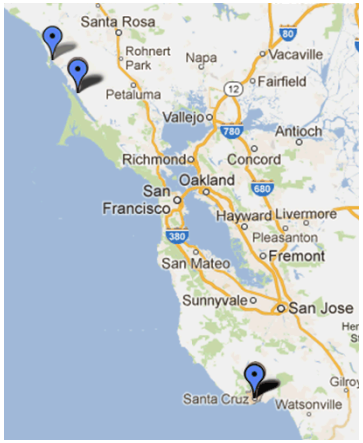
In cases where ponds crash for unknown reasons we can carry out forensic analysis.

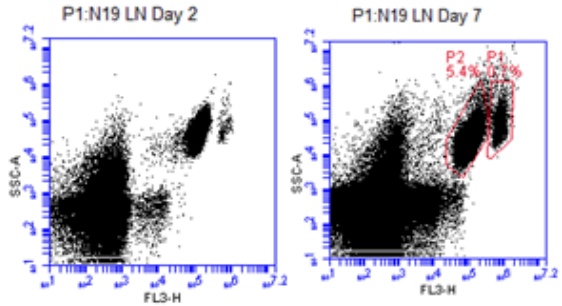
Carney *et al* 2014

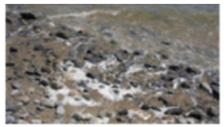




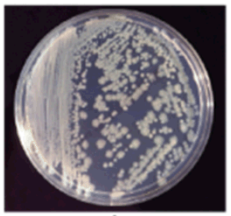
Probiotics: identification of microbes that improve yield



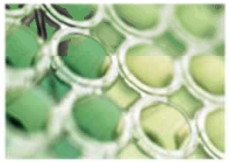




Environmental sampling



Isolation

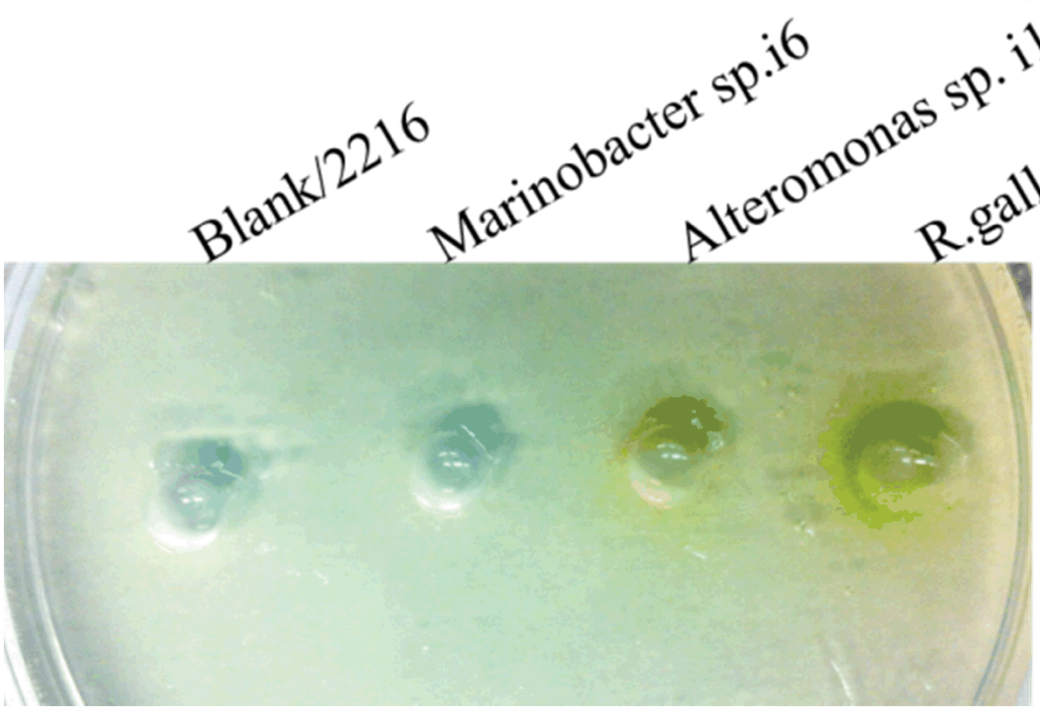


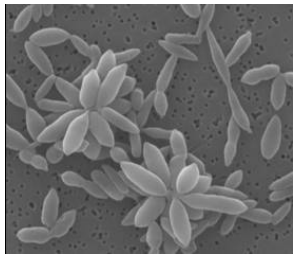
co-culture screen 200 µl

co-culture confirmation 8 ml

co-culture confirmation 60 ml

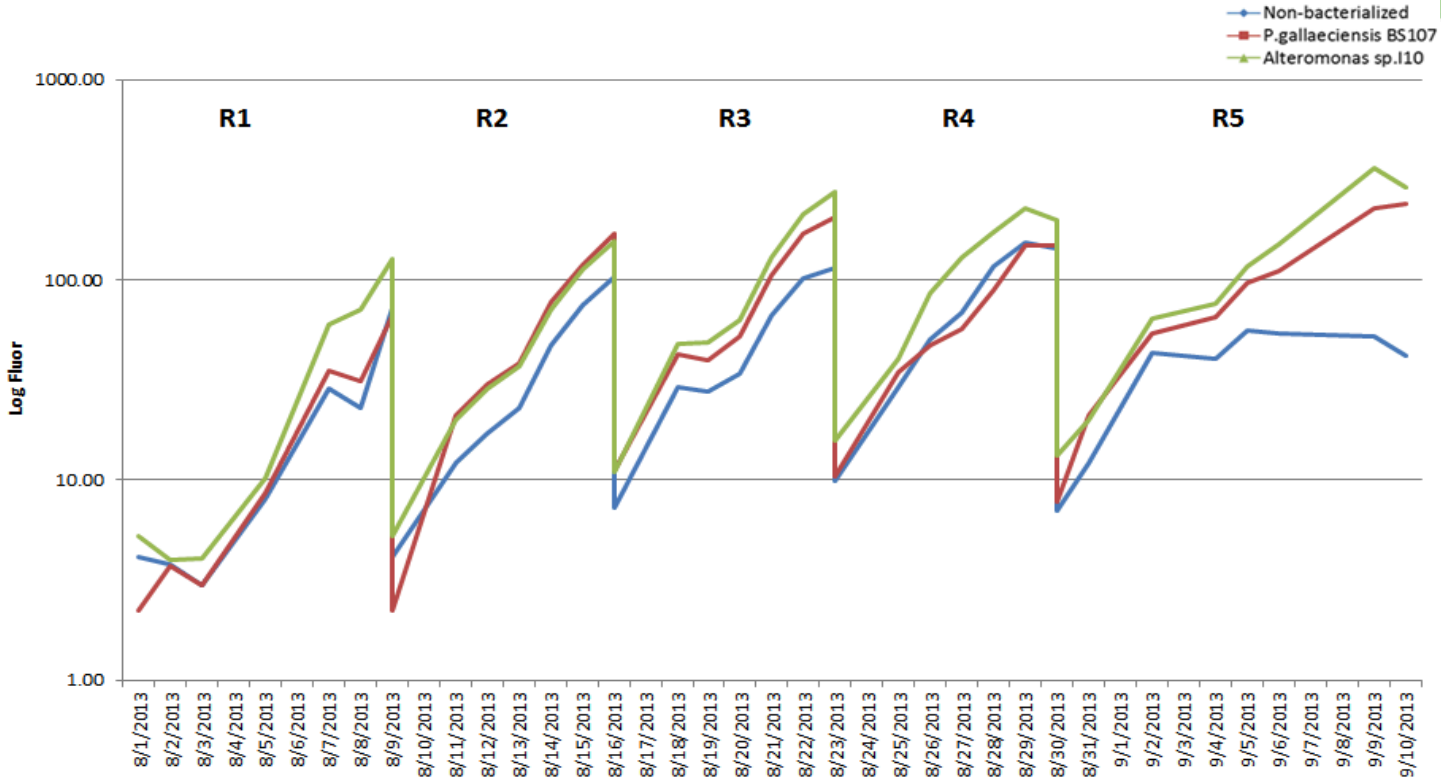
Co-culture of N. salina with isolated strains improves growth on plates





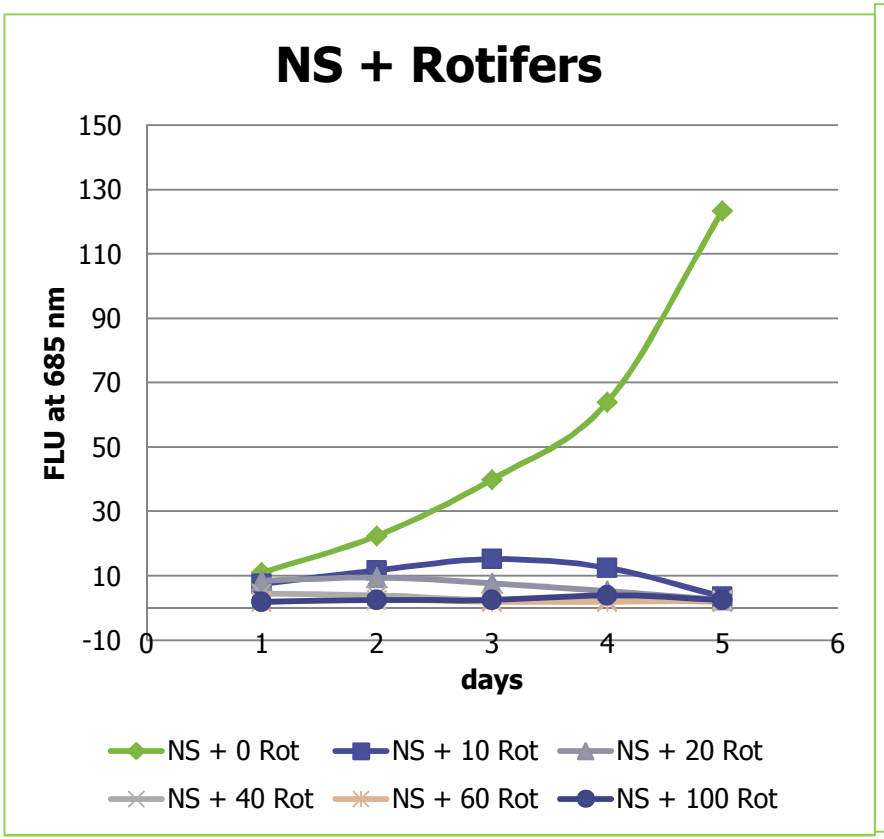
R. gallaenciensis

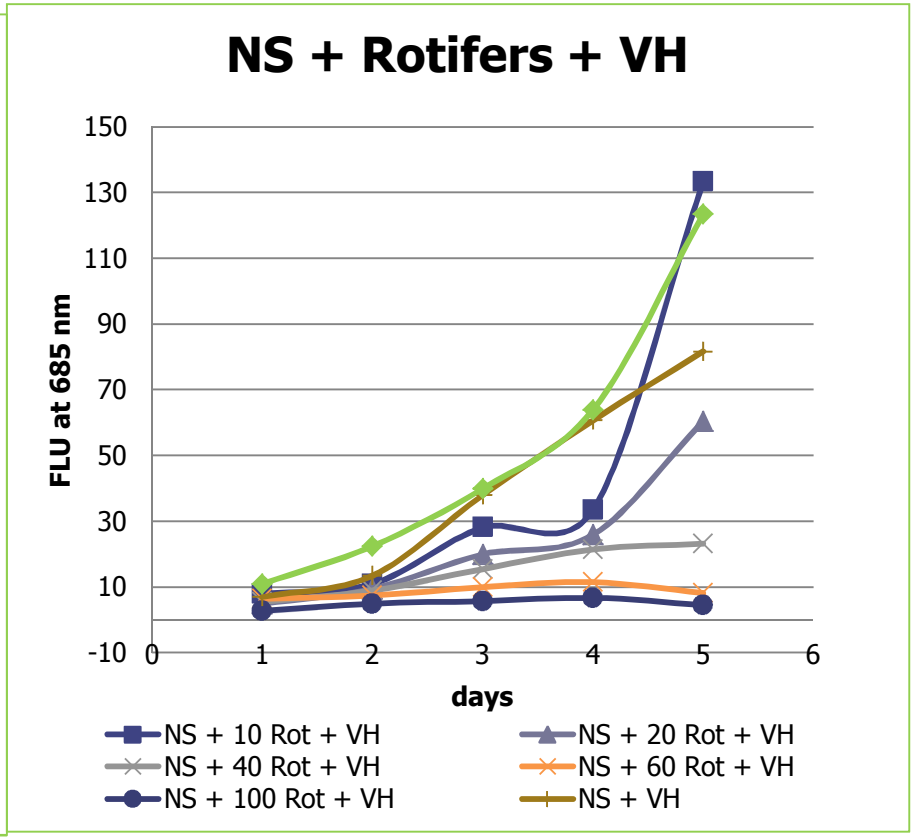
Probiotics + *N. salina* co-culture in outdoor mesocosms

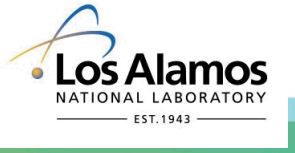


8-week outdoor cultivation period (20L mesocosms)

Probiotic strain Vh mitigates Rotifer grazing







Acknowledgments