Continuous Biological Protection & Control of Algal Pond Productivity (TABB)

&

Protective Bacteria in Algal Ponds: Inducible Protection to Maximize Response (AOP)

DOE BIOENERGY TECHNOLOGIES OFFICE

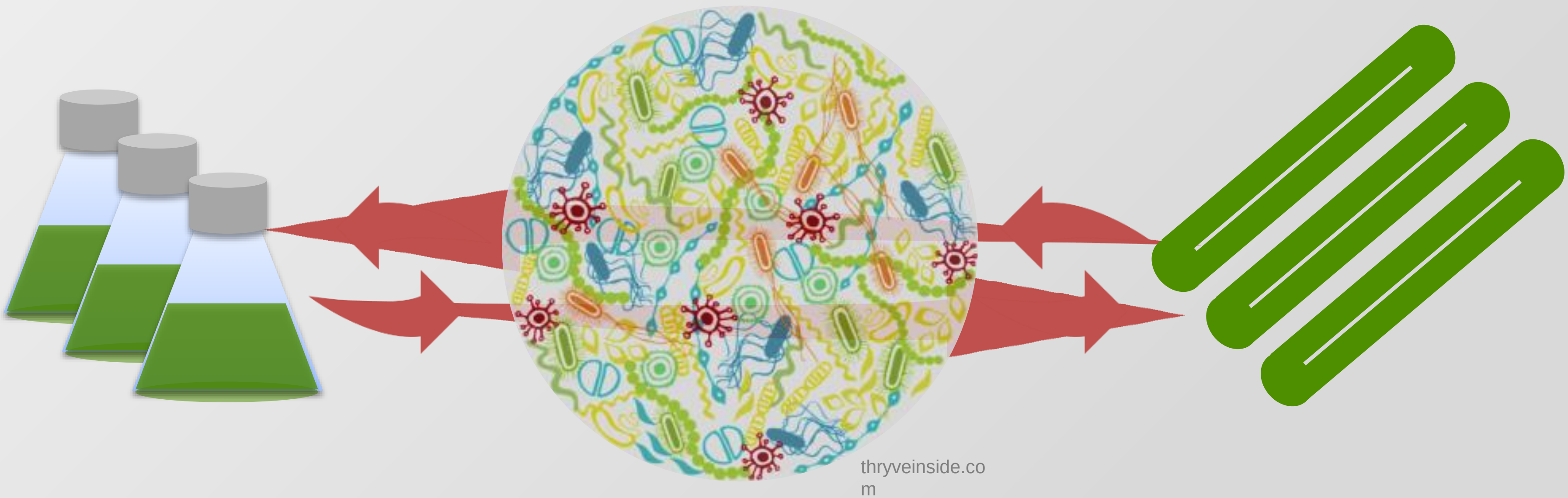
2019 PROJECT PEER REVIEW

March 7, 2019

ADVANCED ALGAL SYSTEMS

PIs: Rhona Stuart & Michael Thelen, LLNL

This work was performed under the auspices of the U.S. Department of Energy by Lawrence Livermore National Laboratory under Contract DE-AC52-07NA27344.



LAB CULTURE

ALGAL POND MICROBIOME

RACEWAY

Goal Statements

TABB: Find probiotic bacteria that protect algal crops against predators and pathogens

- Uncover candidate bacteria and establish protective probiotic effects that improve the resilience of algal crops to predators and pathogens & increase annual algae biomass yields above the 2015 State Of Technology baseline.

AOP: Improve protective effect of probiotic bacteria uncovered through TABB

- Demonstrate probiotic application regimes that significantly increase the magnitude and duration of the probiotic protective effect by 25% each, over current baseline, and significantly decrease in situ algal carbon loss as compared to untreated, ultimately contributing to improved algal cultivation yields

GOALS directly address barriers to sustainable algae production through algal cultivation research and development, and translating LABORATORY SUCCESS to SCALABLE OUTDOOR CULTIVATION STRATEGIES.

TABB Quad Chart Overview

- Project start: 10/1/15
- Project end: 12/31/18*
- 95% complete

	FY 17 Costs	FY 18 Costs	Total Funding (FY 16-project end date)
DOE Funded	$371k	$272k	$1M
Project Cost Share (Comp.)	--	$110k	$388k

(*no cost extension through 6/30/19 to finish publications)

- **Barriers addressed:**
  - "High costs of producing algal biomass and low yields of target biofuel and bioproduct feedstocks produced from algae"
  - "Translating laboratory success to demonstrated, scalable, outdoor cultivation environments"

Partners	%
LLNL	34
Sandia	30
Heliae, LLC	28
UC Davis	8

AOP Quad Chart Overview

- Project start: 10/1/18
- Project end: 9/30/21
- ~10% complete

	FY 17 Costs	FY 18 Costs	Total Planned Funding (FY 19-project end date)
DOE Funded	NA	NA	$1.25M

<u>Partners (FY19)</u> <u>%</u>

- LLNL 85
- LBNL 15

Barriers addressed:

- Aft-B, Sustainable Algae Production
- Aft-A, Biomass Availability and Cost

Project Objectives

1. Identify genes/toxins involved in bacterial protective mechanisms
2. Test and identify induction conditions for increased protection
3. Apply promising induction conditions at increasing scales
4. Determine effects of protection on in situ pond failure frequency

End of project goal:

Probiotic application regimes that:

- Increase probiotic effect over baseline
  - Magnitude ↑ 25%
  - Duration ↑ 25%
- Decrease in situ algal carbon loss

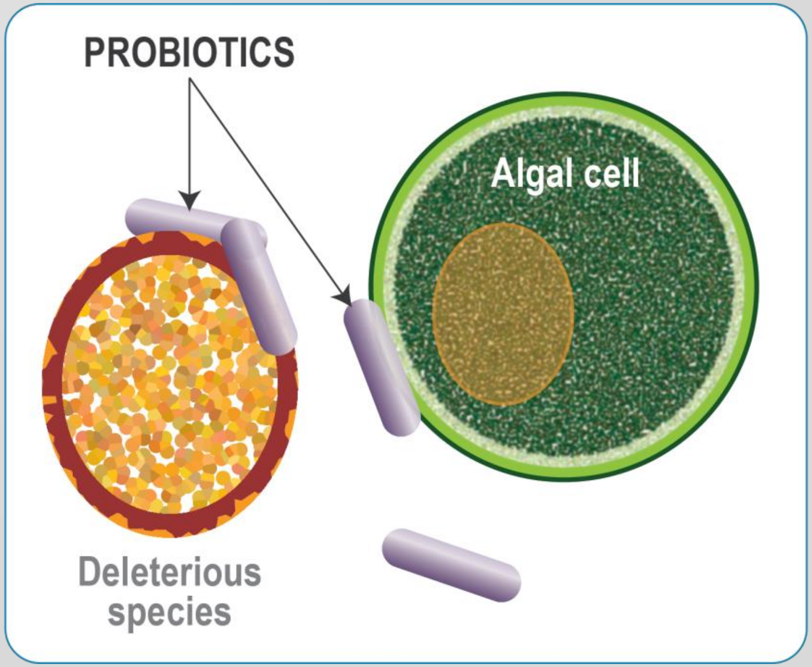
Outline

- TABB: Summary of completed project
  - Team and management
  - Approach
  - Accomplishments
  - Relevance
- AOP: summary of new project
  - Overview
  - Approach
  - Accomplishments/Plans
- Summary

Overview

- **Problem:** Current strategies to improve algal cultivation efficiency and stability are ineffective or have side effects
  - Crop losses from pests are treated with chemicals or extreme environments
  - Evolved resistance is a risk with these treatments

- **TABB project** aimed to:
  - Identify protective bacteria "probiotics" to prevent crop loss due to pests
  - Characterize their probiotic application at increasing scales



Unique team

We used the combined expertise from **multiple institutions** to tackle this multidisciplinary challenge

- **LLNL:** Complex microbial community analyses
- **Sandia:** Monitoring and mitigating algal pond contaminants
- **Heliae:** Industrial algal culturing
- **UC Davis:** Engineering and modeling

Lawrence Livermore National Laboratory

Sandia National Laboratories

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Management structure reflects task structure

Michael Thelen (PI), LLNL
Tracking and reporting

Rhona Stuart (Project Lead), LLNL
Protective assays
Microbial diversity analyses

Todd Lane, Sandia
Pathogen assays
1000 L raceway trials

Laura Carney, Heliae
Industrial-scale algal culturing

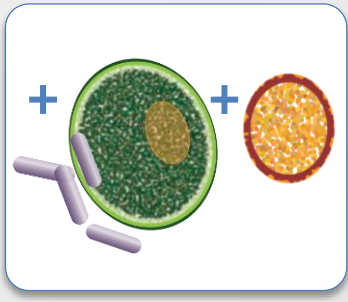
Jean VanderGhenyst, UCD
TEA and modeling

- PI Responsibilities
  - Tracks milestones & data
  - Generates quarterly reports
  - Synthesizes results into publications and solutions
- Meetings
  - Monthly teleconferences
  - Annual meetings
  - Other meetings as needed

- Decision making through consensus
- Team leads responsible for achieving task milestones
- PI retains ultimate decision-making authority

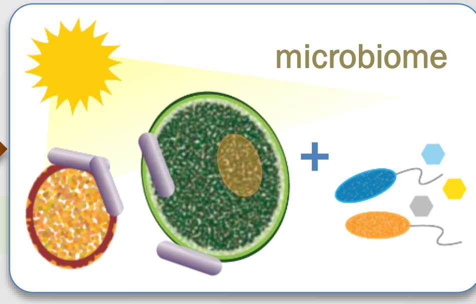
TABB: Technical Approach

BENCH SCALE



G/NG 1

OPEN SYSTEM



G/NG 2

PROCESS DEVELOPMENT SCALE AT SANDIA

INDUSTRIAL SCALE AT HELIAE

GOAL: Probiotics that increase algal resistance in co-cultures under relevant conditions

Increased algal resistance in an outdoor open mesocosm

Validate increased algal resistance at 1000 L scale

Test probiotic application

TRL-3

TRL-2

Stepwise scale-up in complexity to mitigate risks

TECHNOECONOMIC ANALYSIS

Bench & mesocosm scale data → *Kinetic Model* → *TEA model* → *Systems Comparison*

Accomplishments

1. Developed a model probiotic system
2. Selection-based generation of protective consortia
3. Microbial applications at the industrial scale
4. Technoeconomic modeling of protective bacteria

Accomplishments

1 Model probiotic system: *J. lividum* and *M. salina*

1. *Microchloropsis salina:*
   – A common algal strain
2. *Brachionus plicatilis:*
   – A rotifer that consumes *M. salina* algal cells
   – Common pest in mass algal cultures
3. *Janthinobacter lividum:*
   – We identified this promising candidate bacterium
   – Hypothesis: will protect against rotifer predation

Rotifers. (Photo credit: Microscopy UK)

Goals:

– Use this simple 3-member system—*M. salina, B. plicatilis, & J. lividum*—to establish a repeatable and consistent protection assay

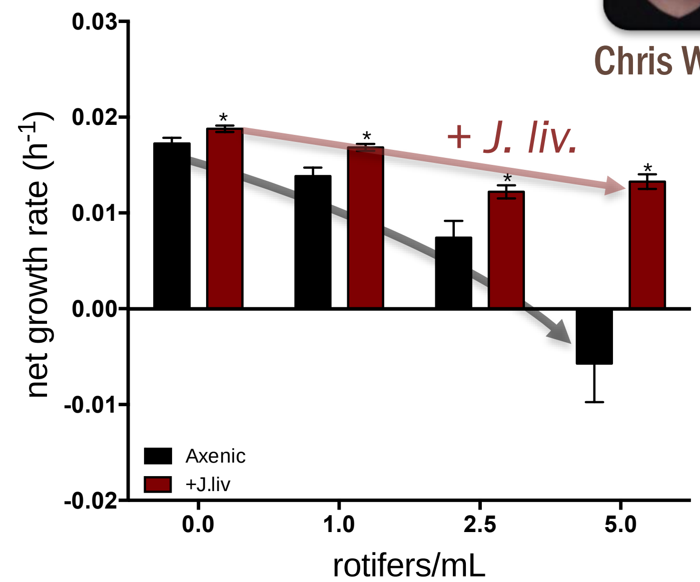
– Test scale-up of the assay to complex outdoor systems

Accomplishments

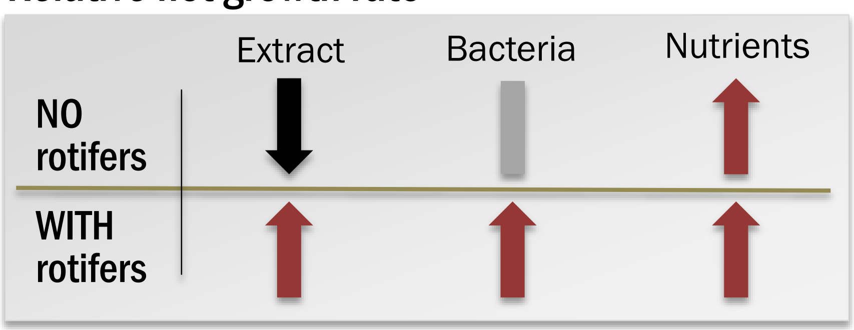
1 Laboratory Scale: *J. lividum* and *M. salina*

- Using our standardized grazing assay, we determined that *Janthinobacter* protects algae from rotifer predation
- *Janthinobacter* extracts also protect, even though algal growth is hindered
- *Janthinobacter* protects in presence of unrelated bacteria and with decreasing nutrients

Chris Ward



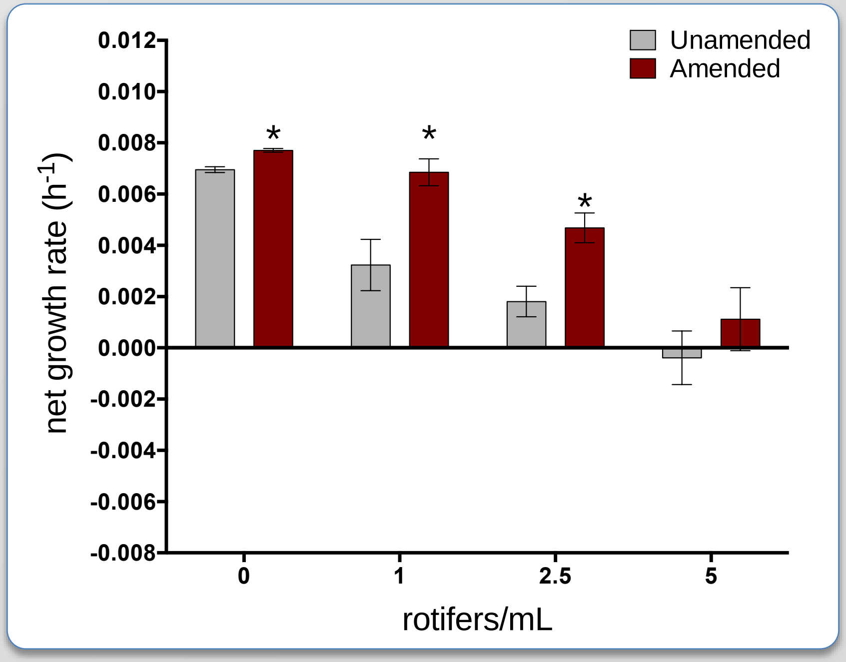
Relative net growth rate



Janthinobacter **protection is robust at laboratory scale**

Accomplishments

1 Protection with Increasing Complexity: *J. lividum* and *M. salina*



- Grazing assays conducted in mesocosm trials by dilution—assayed in laboratory conditions
  - Provides a conservative, likely underestimate, of protection
- Protection was most significant on day 1
- Detectable up to 6 days
- *J. lividum* persisted throughout 10-day culture

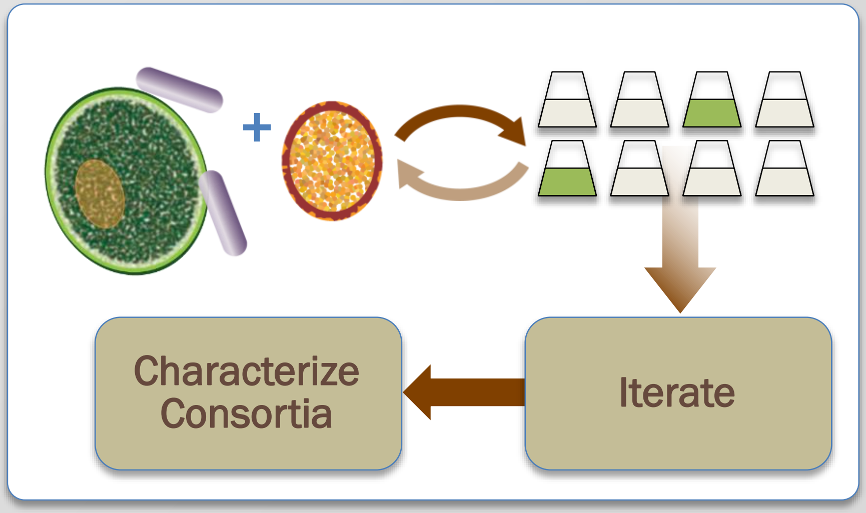
Janthinobacter protects in the presence of complex outdoor microbial communities

Accomplishments

2 Generation of novel protective consortia

Carolyn Fisher

- Developed a method to identify novel microbial consortia that protect *M. salina* from rotifer predation:
  1. Pond or environmental sample inoculated with algae (*M. salina*)
  2. Challenge with rotifers (*B. plicatilis*)
  3. Reiterate and demonstrate rotifer die-off is communicable



Goals:

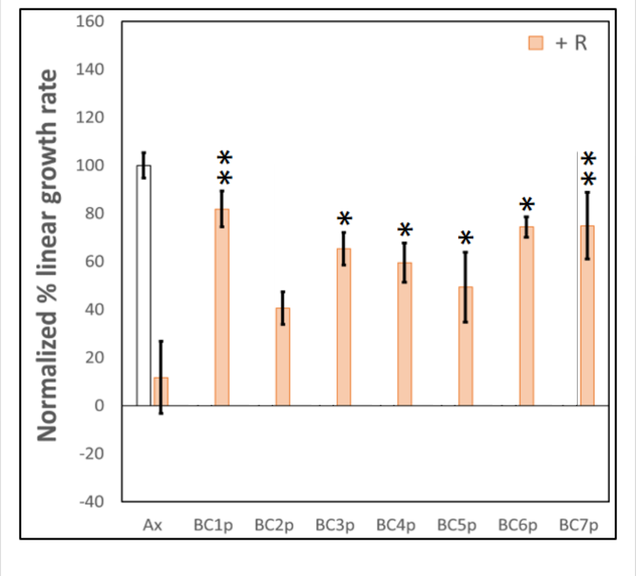
- Identify novel protective consortia
- Test effectiveness of protective consortia with increasing complexity

Sandia National Laboratories

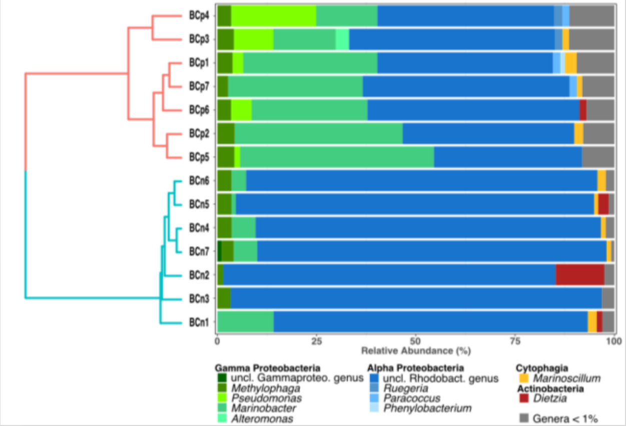
Accomplishments

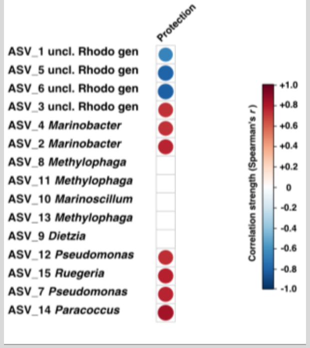
2 Selection-based generation of protective consortia

- Protective bacterial consortia (BC1p-BC7p) increase *M. salina* growth in presence of rotifers



- Determined which strains correlate with protection
  – Compared strain composition of protective and non-protective consortia



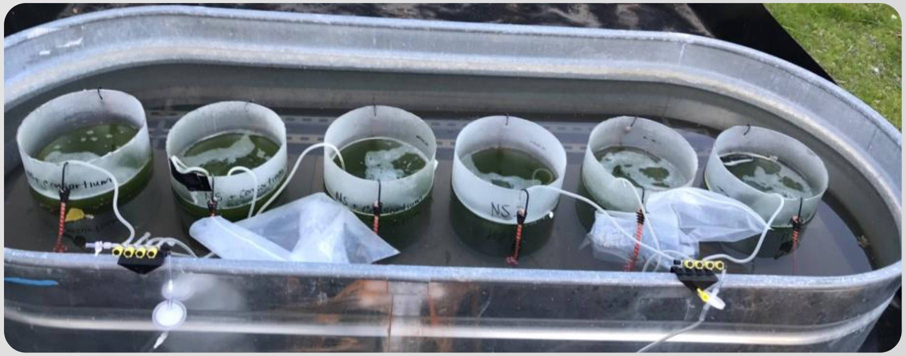


Fisher & Ward *et al*, in review

Accomplishments

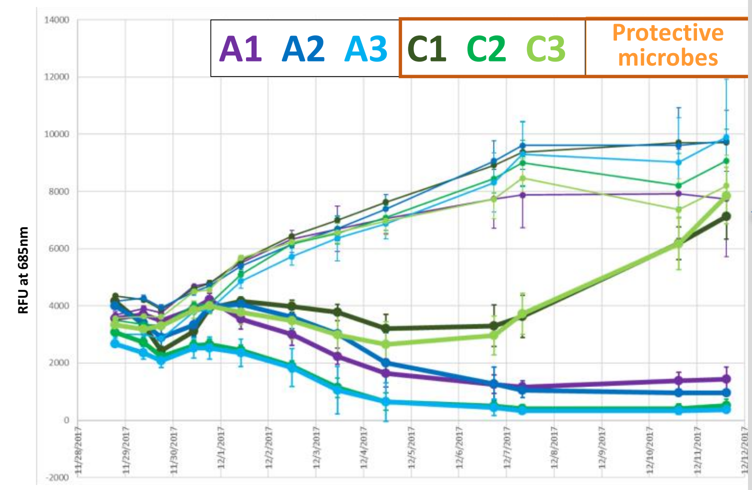
2 Applying protective consortia at increasing scales

- Applied our consortia to outdoor mesocosms
  - Tracked over several harvests and reseedings



6 X 16 L open outdoor cultivation system

- Detected protection in laboratory assays from the mesocosms after 2 harvests and reseeds



Laboratory challenge of samples from outdoor rotifer protection assays

Accomplishments

3 Microbial applications at industrial scale

- Collaboration with Heliae, LLC
  - Examined phototrophic algal ponds
- Distinct system from other aims:
  - Heliae algae production strain
  - Impacted by a different set of pests, particularly chytrids

Goals:

- Characterize microbiome factors in a relevant industrial system
- Determine important parameters for feasible probiotic additions at this scale

Chris Ward

Laura Carney

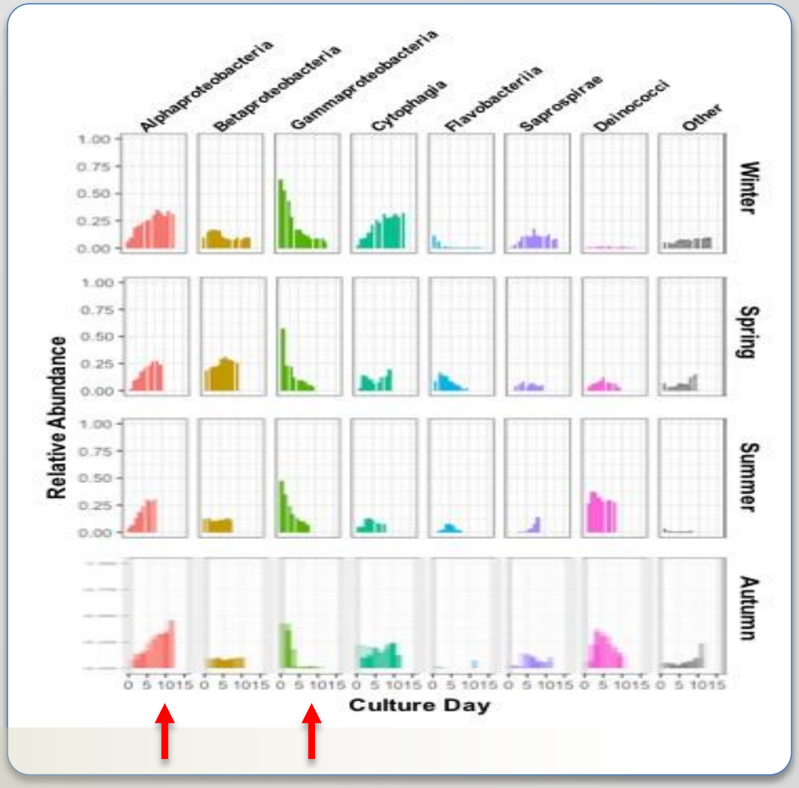
heliae®

Accomplishments

3 Microbial applications at industrial scale

A year in the life of the industrial scale microbiome

- Characterized the microbiome community composition
  - Sampled replicate 10,000 L reactors
  - From scale up to harvest, over one year (624 samples total)
- Found that distinct patterns emerged across seasons
  - Particular taxa dominant early in the reactor development and others arising later



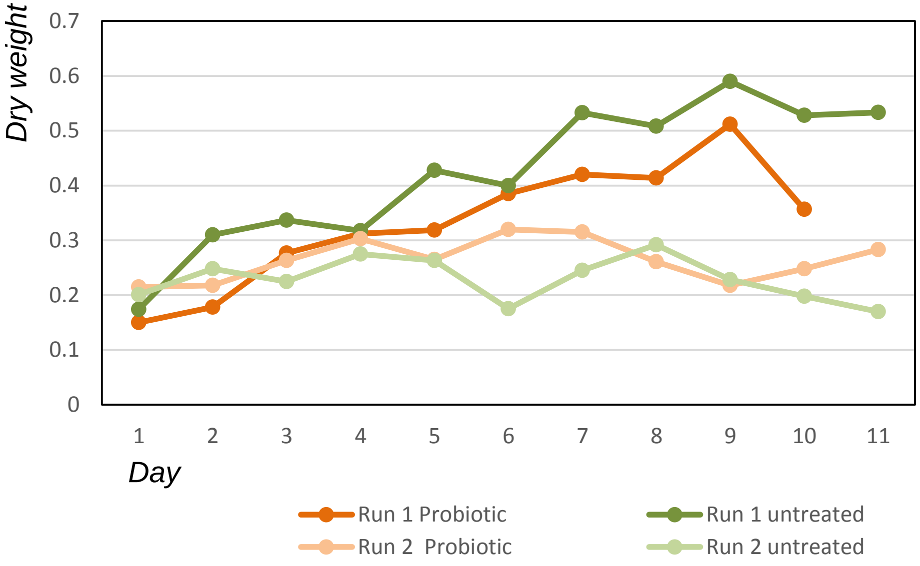
Surprisingly, microbiomes follow successional patterns across seasons, opening the door for manipulation

Accomplishments

3 Microbial applications at industrial scale

Probiotic application trial in 10,000 L reactors

Scale-up production and addition of probiotic conducted onsite by Heliae



Probiotic additions at this scale were feasible for industrial production

Accomplishments

4 Technoeconomic modeling of protective bacteria

Lauren Jabusch

- Developed a kinetic growth model
  - Incorporates our laboratory and mesocosm scale data of algae, rotifers, & bacterial growth
- Incorporated this growth model into a techno-economic assessment of protective probiotic additions

Goals:

- Model costs and benefits of protective bacteria application at industrial scale
- Determine areas for improvements to costs to increase economic feasibility

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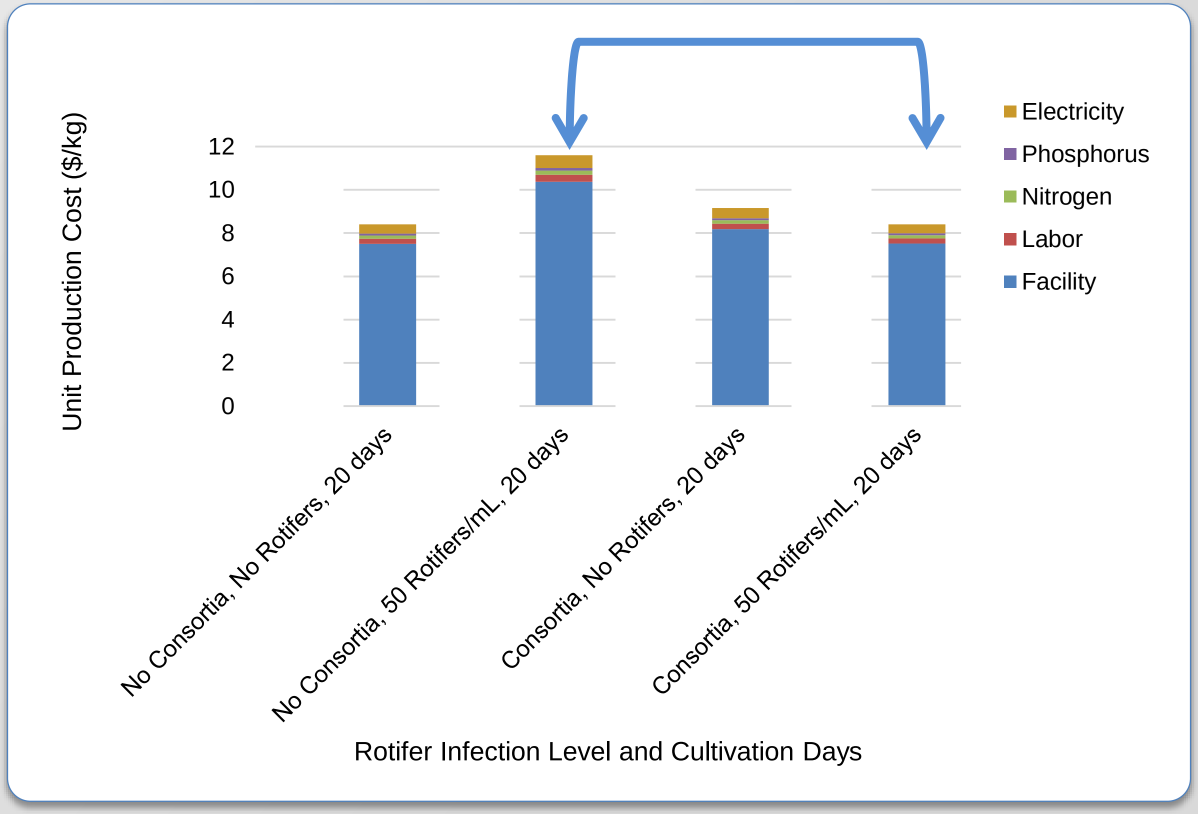
Accomplishments

4 Technoeconomic modeling of protective bacteria

- Developed a kinetic model that matched our experimental data
- Incorporated this model into a techno-economic analysis of algae production with probiotic and with grazer predation



Application of protective consortia reduces costs in the presence of high rotifers over longer time periods

TABB Relevance: Prevent Algal Crop Loss by Generating Biological Controls

GOAL: Improve resilience of algal crops to predators and pathogens using probiotic bacteria to increase annual algae biomass yields by 5–30%

- Addressed Targeted Algal Biofuels and Bioproducts (TABB) FOA barriers
  - Our probiotic approach directly addressed:
    - 1) “Low yields of target biofuel and bioproduct feedstocks” and 2) “Biological contamination presents one of the greatest challenges in cultivating robust, reliable algal cultures”
    - Accomplished by addressing 3) “novel, safe, and effective strategies need to be developed to control culture contamination events”
  - Our pipeline strategy addressed:
    - “Translating laboratory success to demonstrated, scalable, outdoor cultivation environments that capture all of the variables not present in laboratory systems”

This project advanced the state of technology and positively impacted the commercial viability of algal biofuels:

- Demonstrated how leveraging the microbiome can increase algal resistance to predators and pathogens
- Demonstrated the utility of a rapid translation from laboratory to outdoor testing and the importance of iteration between these experiments
- Demonstrated a feasible industrial scale (10,000 L) probiotic addition
- Incorporated laboratory and outdoor experimental data into a relevant TEA to assess sensitivities and future opportunities

Outline

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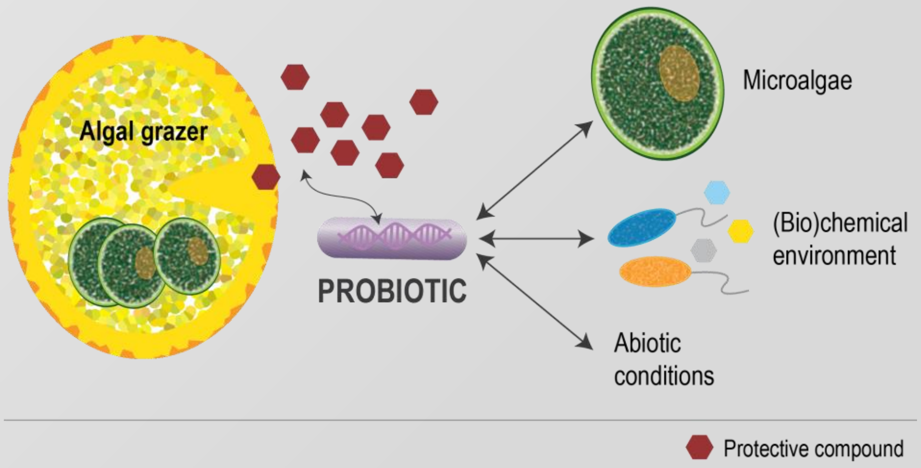
AOP Overview: Protective Bacteria in Algal Ponds
Inducible Protection to Maximize Response

NEED:

- *J. lividum* is a **promising probiotic**
  - Protected algae from predation at laboratory and outdoor scales
- ***BUT** protection was inconsistent in magnitude and duration at outdoor scale*



AOP GOAL: Probiotic application regimes that:

- Increase probiotic protective effect
  - Magnitude ↑ 25%
  - Duration ↑ 25%
- Decrease in situ algal carbon loss

Back to basics: By identifying the biological mechanism and regulation of protection, we can induce and increase the protective response

AOP: Team and Management

- LLNL: Expertise in complex microbial community analyses and algal ecophysiology
- LBNL: Our new partners provide expertise on our probiotic of choice
- As with management of the previous project:
  - Decision making through consensus
  - Team leads responsible for achieving task milestones
  - PI retains ultimate decision-making authority
  - Monthly telecon with team members

Rhona Stuart (PI), LLNL
Protective assays
Microbial diversity analyses

Romy Chakraborty, LBNL
Probiotic induction trials
Related probiotic strains

LLNL Team:
Michael Thelen
Chris Ward
Kristina Rolison
Grazing protection assessment at bench and outdoor scales

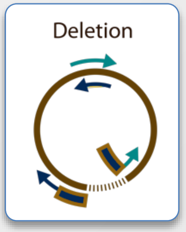
Jean VanderGhenyst,
U.Mass Dartmouth
TEA adaptation (year 2.5-3)

Texas A&M
Year 3 only
industrial scale trial

Lawrence Livermore National Laboratory

BERKELEY LAB

Technical Approach



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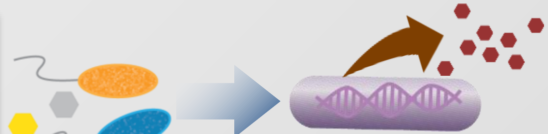
Mutagenesis

Grazing assays

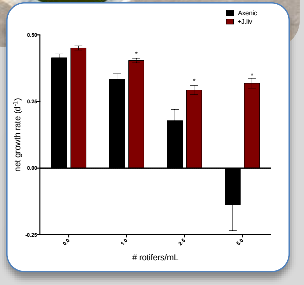


Modify conditions and measure expression

G/NG

Screening at bench scale

Open system testing



AIM 1: Identify protective genes

AIM 2: Test induction conditions with scale

AIM 3: Assess improvements with increasing complexity

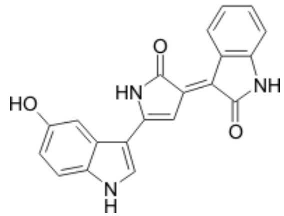
AIM 4: Methods Development:
Effect of protection on algal carbon loss and in situ pond failure frequency

Stepwise scale up and early testing outdoors to mitigate risks

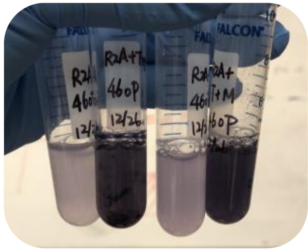
YEAR 1 Identify the mechanism of protection

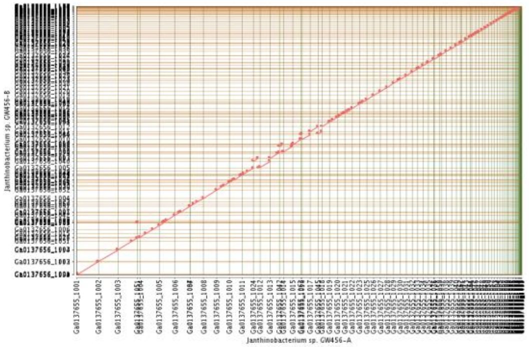
Xioaqin 'Sherry' Wu

- *J. lividum* produces a purple pigment—violacein
  - We ***hypothesize*** that this is the **protective compound**
- Our LBNL collaborators have a collection of novel *Janthinobacter* isolates that are:
  - Nearly identical at the genome leve
  - ***BUT** produce violacein under distinct conditions*



violacein





Year 1 milestones include:

- Identify target gene lists
- Demonstrate gene knockout in *Janthinobacter*
- Characterize novel isolates' protective capabilities
- Identify a genetic mechanism of protection

BERKELEY LAB

Potential Challenges

- **Risk:** None of the target genes are responsible for protection
  - **Mitigate:** We are widening our list of target genes based on a transposon mutagenesis library screen
    - List will provide fitness of all genes in probiotic genome under 1000s of conditions
- **Risk:** Identified induction treatment is not feasible at scale
  - **Mitigate:** Use our current TEA to estimate feasibility and reach out to industry consultants to examine alternative applications of the treatment to test for effectiveness

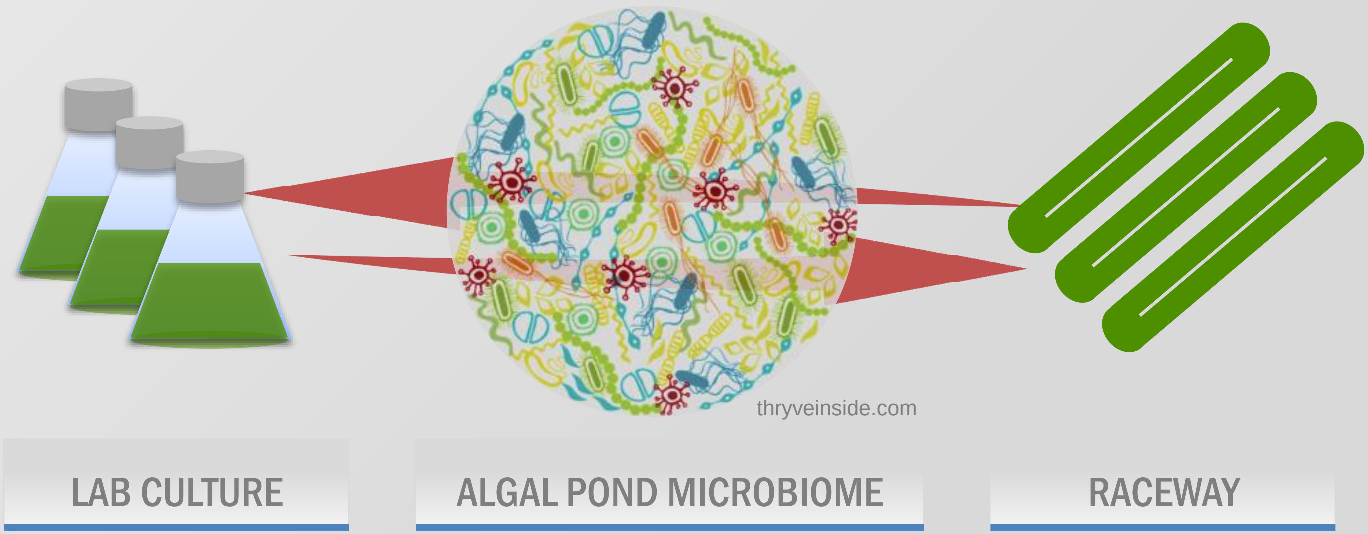
Potential Challenges, *continued*

- **Technical**
  - Systems with protective bacteria must have increased algal biomass yield compared to unprotected systems in the presence of deleterious species pressures
  - Protective bacteria cannot decrease algal productivity in the absence of deleterious species
- **Market/Business**
  - Cost of probiotics application must be cost-effective compared to:
    - Costs of alternative management using chemical treatments
    - Risks of pond crash when treatments fail
  - Application of probiotics must be realistic in the timeline of pond development

Summary

1. Leveraging the microbiome can increase algal resistance to predators and pathogens
2. Utility of a rapid translation from laboratory to outdoor testing & importance of iteration
3. Feasible industrial scale probiotic addition
4. Incorporated laboratory and outdoor experimental data into a relevant TEA to assess sensitivities and future opportunities

- Our new project will apply what we have learned, addressing the TEA sensitivities to make our probiotic application more economically feasible



Credits

U.S. DEPARTMENT OF ENERGY | Energy Efficiency & Renewable Energy

BIOENERGY TECHNOLOGIES OFFICE

LLNL

- Chris Ward*
- Xavier Mayali
- Max Li
- Kristina Rolison

SNL

- Todd Lane
- Pamela Lane
- Carolyn Fisher*

LBNL

- Romy Chakraborty
- Xiaoqin "Sherry" Wu

Heliae, Inc

- Laura Carney
- Braden Bennett
- Rachel Montoya

UC Davis

- Jean VanderGheynst
- Lauren Jabusch*

BETO project management support

Devinn Lambert, Dan Fishman, and Evan Mueller
(and Amanda Barry previously)

*postdocs and graduate student