SAND2020-9880C

μBIO

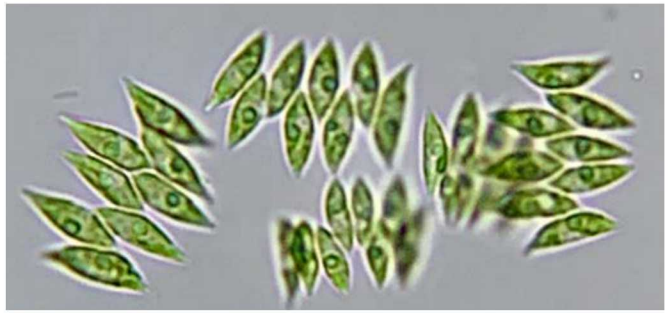
MICROBIO ENGINEERING

Algae Biomass Organization Summit
September 22, 2020

Sandia National Laboratories is a multimission laboratory managed and operated by National Technology & Engineering Solutions of Sandia, LLC, a wholly owned subsidiary of Honeywell International Inc., for the U.S. Department of Energy's National Nuclear Security Administration under contract DE-NA0003525.

Improved Strains for Combined Phycoremediation and Biofuels Production

Aubrey Davis, Ph.D. [1,2] *Presenting*



[1] MicroBio Engineering Inc., San Luis Obispo, CA
[2] California Polytechnic State University (Cal Poly), San Luis Obispo, CA

Notice: This work was supported by the U.S Department of Energy, Bioenergy Technologies Office (US DOE BETO) under Agreement DE-EE0007691

Algae Biomass Yield 2 (ABY2) DOE BETO Project Objective: Goal is to increase algal 'biofuel intermediates' (BFI) yields

- Identify promising strains for outdoor cultivation

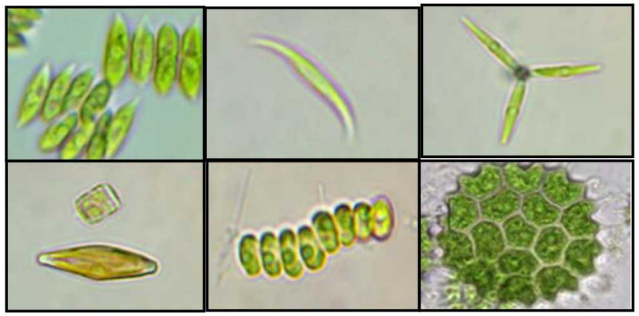
  Strains screened for productivity and biochemical content, promising culture collection strains & local isolates identified.

- Apply non-GMO approach for strain improvement

  24% and 30% productivity increase with a stable, non-GMO improved cultigen and strain, respectively.

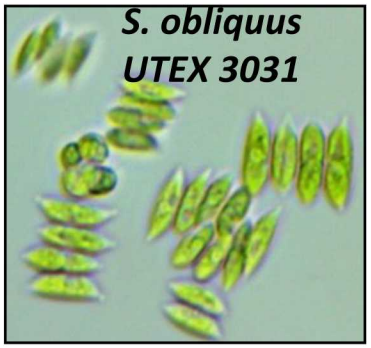
- Demonstrate long-term baseline productivity and composition in outdoor raceway ponds relative to native polyculture.

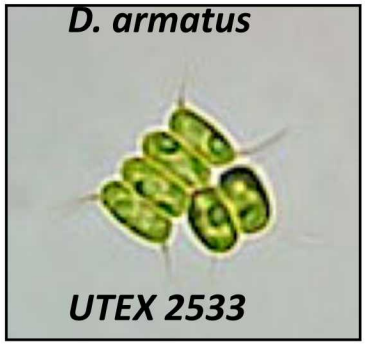
  Annual productivity and biochemical content determined for polycultures and strains.



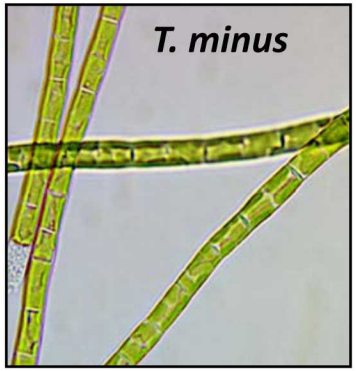
μBIO MicroBio Engineering

Novel and Promising Strains





Wastewater Isolates



Genomic Sequencing*

Species	Method	Status
D. armatus (UTEX 2533)	PacBio & Illumina	Assembled
C. zebra (wastewater isolate)	PacBio & Illumina	Sequencing in progress
T. minus (wastewater isolate)	PacBio & Illumina	Assembled

*Conducted by Krissy Mahan and Todd Lane at Sandia National Labs

Tribonema minus:

- Isolated from polyculture
- Stable and productive
- Filamentous, can be harvested with screens
- Produces EPA and palmitoleic acid

μBIO MicroBio Engineering

Sandia National Laboratories

Tribonema minus Draft Genome Assembly Statistics

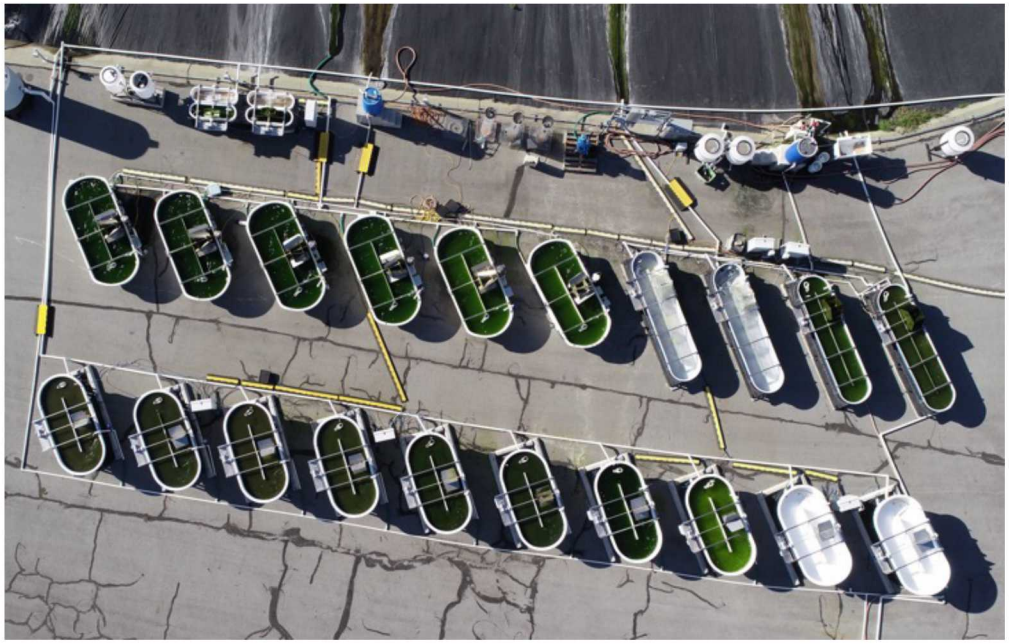
Sequencing effort and variant analysis conducted by Dr. Krissy Mahan (kmmahan@sandia.gov) and Dr. Todd Lane (twlane@sandia.gov) at Sandia National Laboratories

Assembly size	# of contigs > 5 kb	Largest contig	Contig N50	GC (%)
188.5 Mb	631	2.8 Mb	748252	57.5

Sandia National Laboratories

µBIO MicroBio Engineering

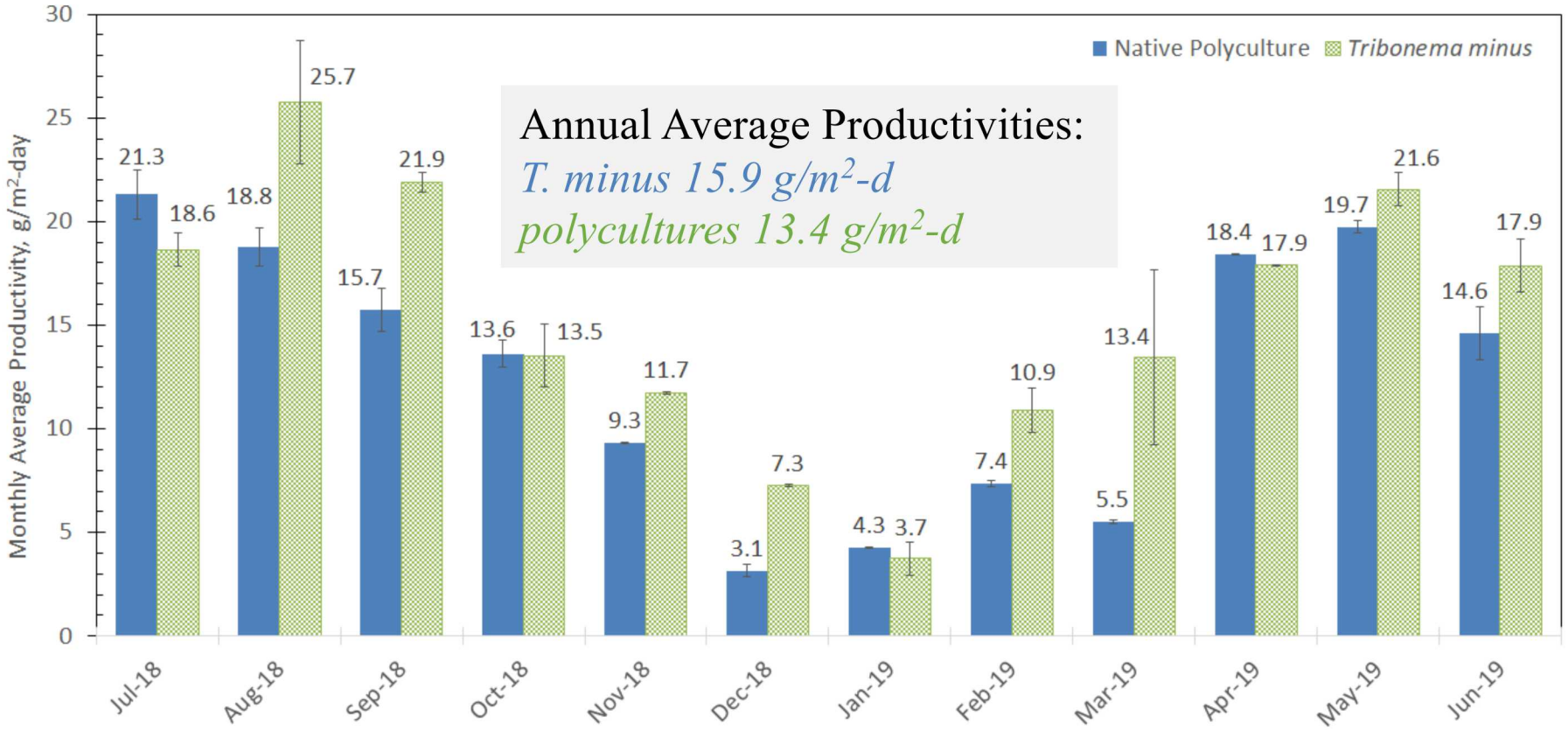
Outdoor Cultivation performed at the Algae Field Station operated by Cal Poly



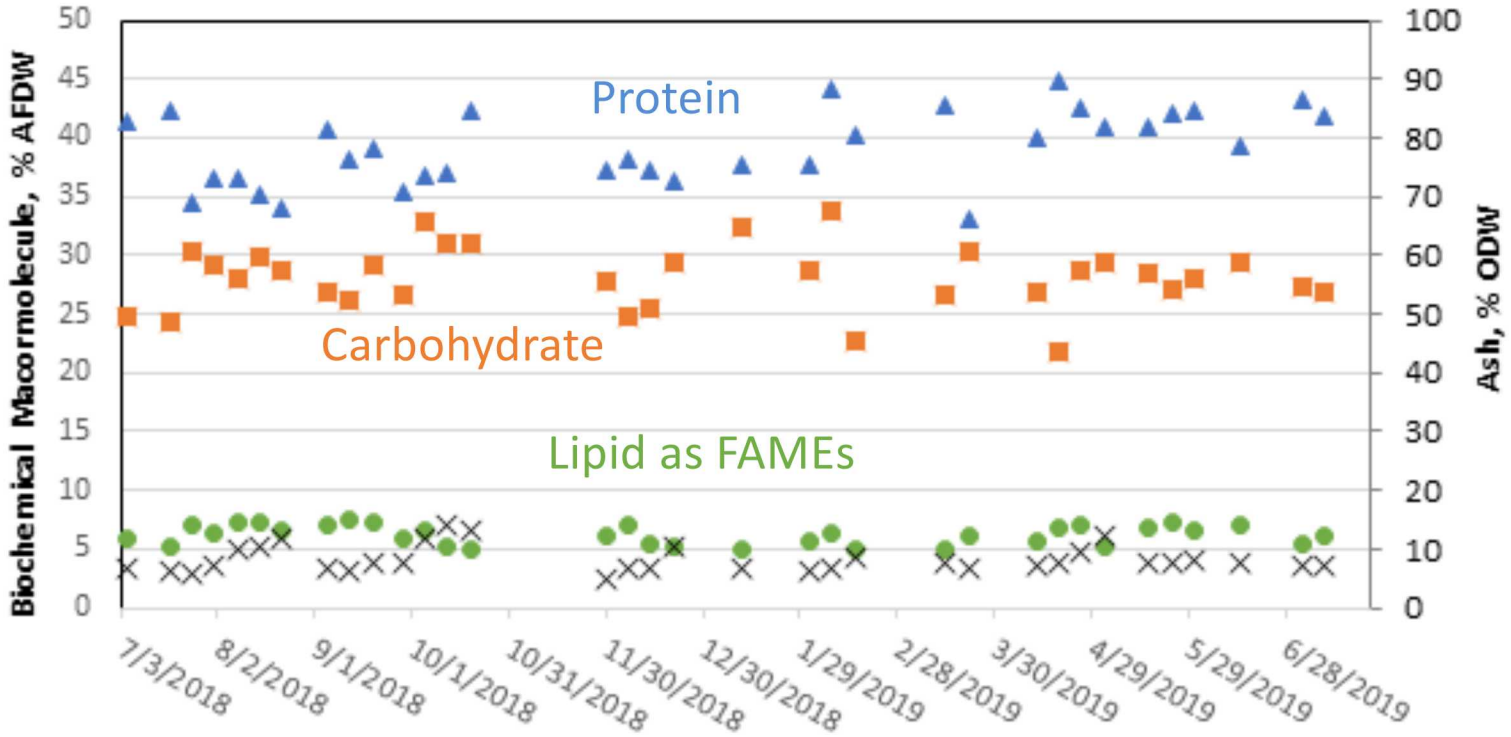
CAL POLY
SAN LUIS OBISPO

μBIO
MICROBIO ENGINEERING

Tribonema minus is More Productive Than Polyculture



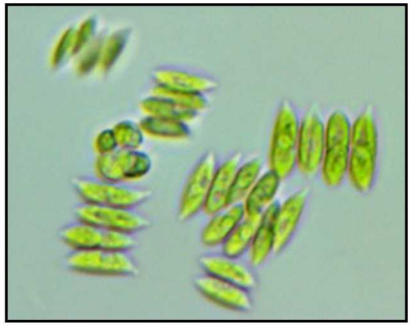
*T. minus B*iochemical Profile Stable in Ponds Over One Year in San Luis Obispo, CA

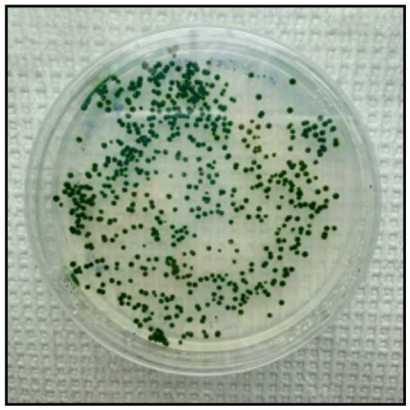


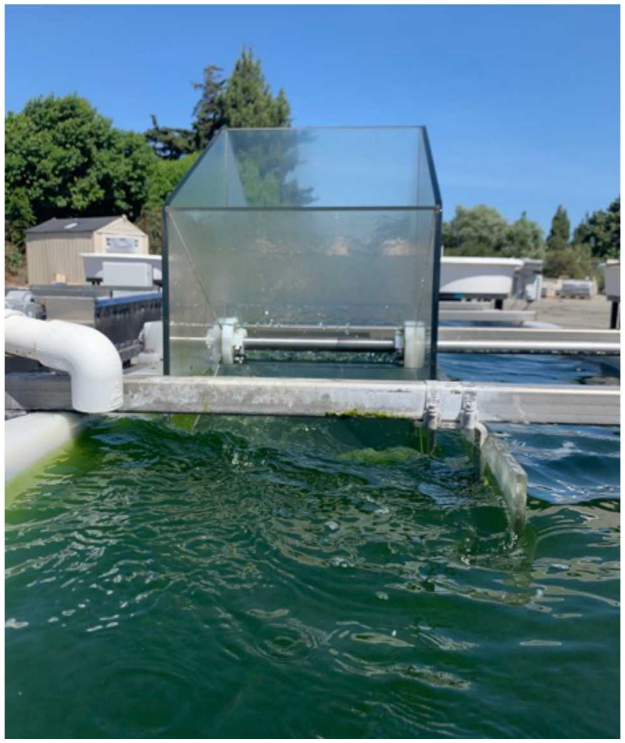
*EPA up to 4% of AFDW

S. obliquus (DOE0152z) Chosen for Improvement Through Adaptive Laboratory Evolution (ALE)

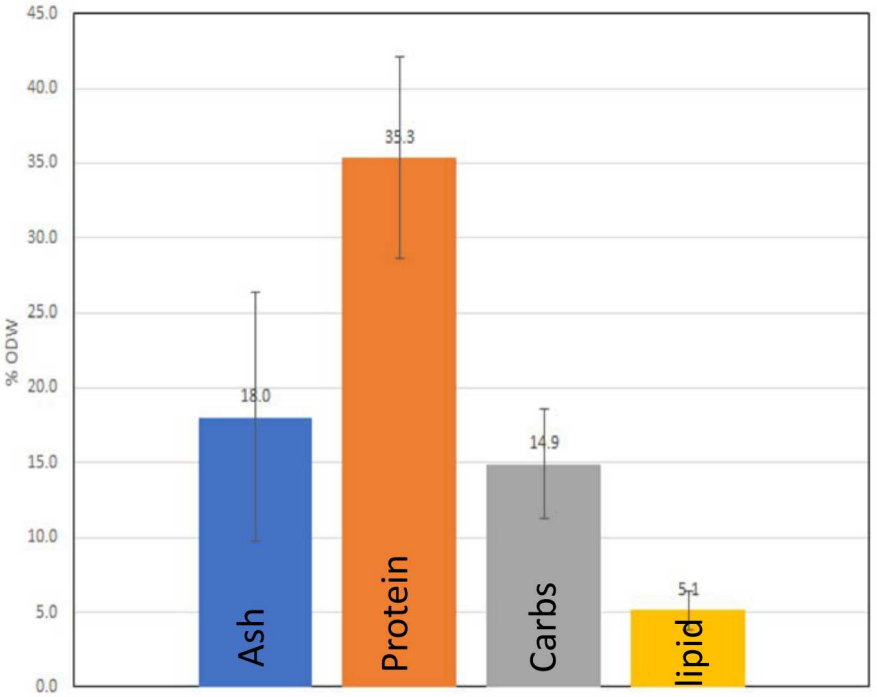
Annual productivity average (2017 – 2018): **10 g AFDW/m^2-day**





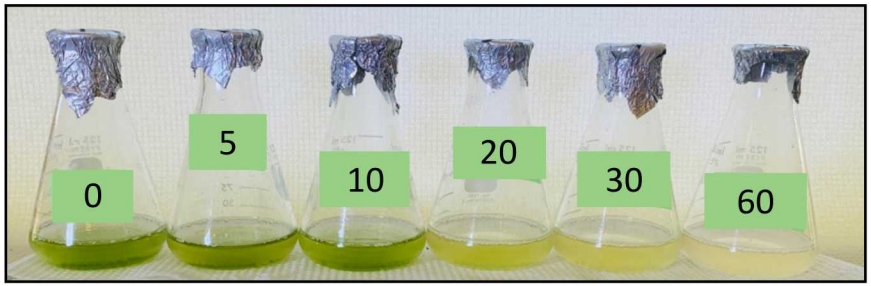


Annual Average Biochemical Profile



Strain Improvement Through ALE: Random Mutagenesis Followed by Selective Enrichment

Random mutagenesis



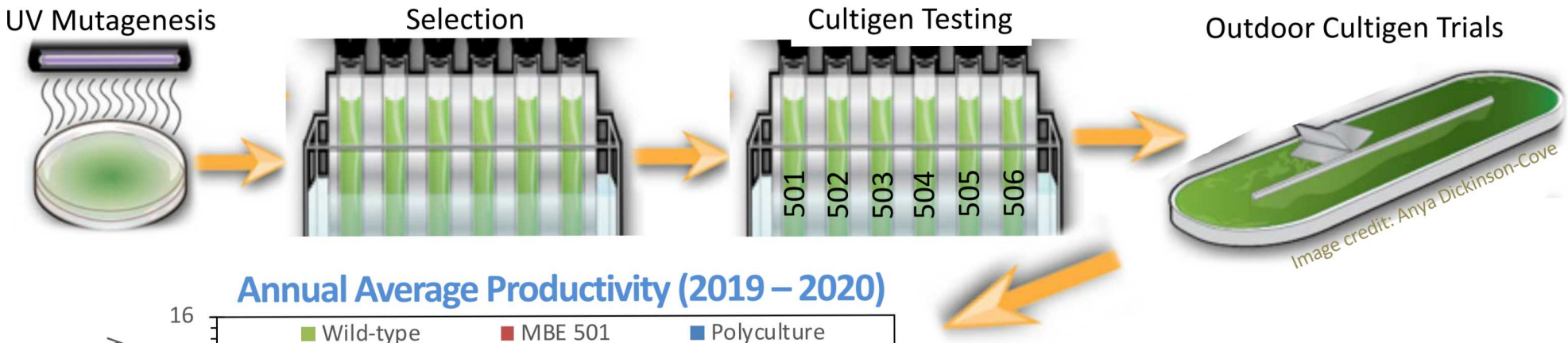
Exposure (s)	Lethality (%)
5	15
10	67
20	98
30	100
60	100

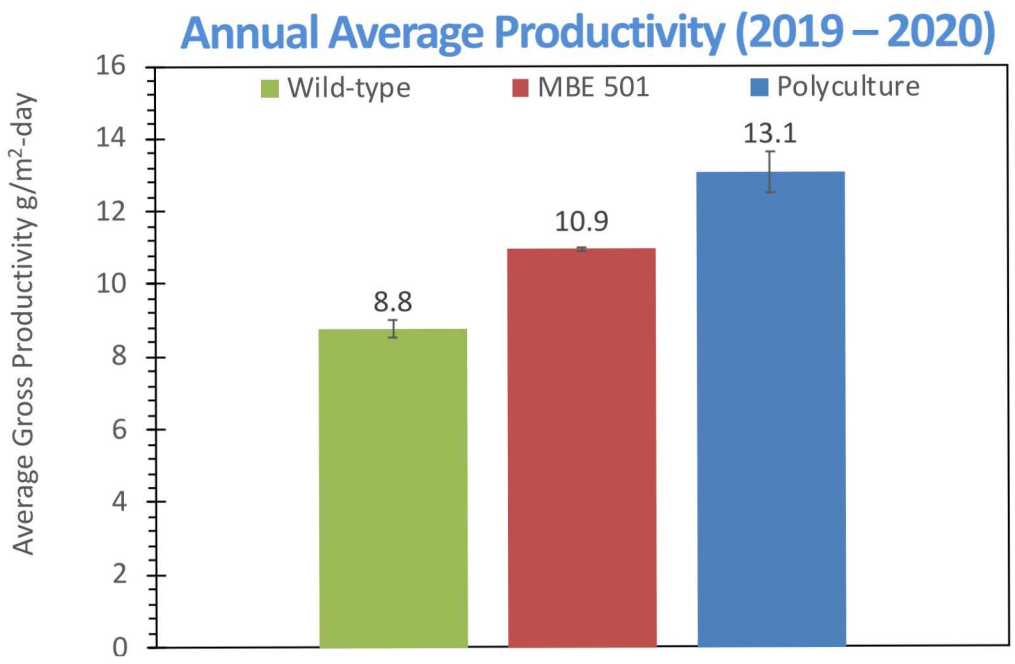
Applied a 5-sec UV exposure to encourage genetic and phenotypic heterogeneity in the population while minimizing deleterious effects. Mutagenesis rate is being assessed by collaborators at SNL.

Selection for growth improvements through routine dilutions in bubble column reactors

μBIO MicroBio Engineering

ALE-Generated Improved Cultigen (selected population)

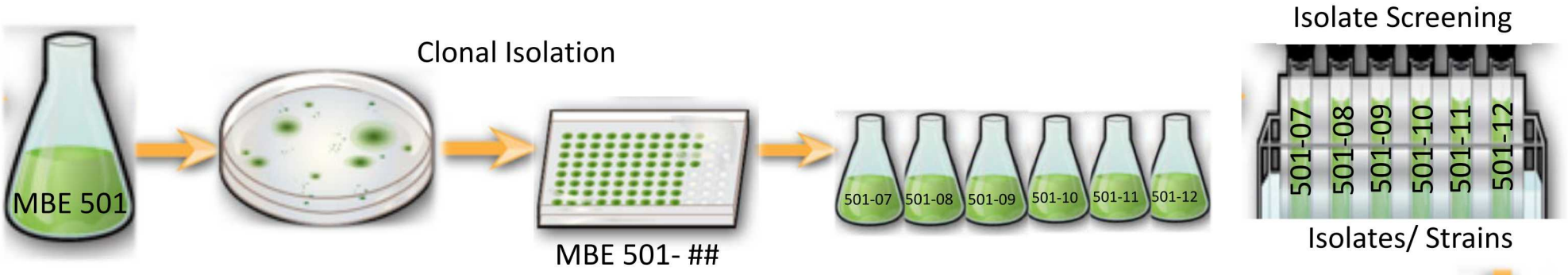




- Cultigen = heterogeneous, selected population
- Populations were under selection for 200 – 400 generations
- **Cultigen MBE 501 is more productive than wild type in outdoor, raceway trials**

μBIO MicroBio Engineering

ALE-Generated Improved Strain



- Laboratory trials demonstrated MBE 501-12 productivity improvements of at least 20% relative to the wild type.
- Outdoor trials of MBE 501-12 are underway. Preliminary data supports indoor results with productivity improvements of ~30%.

Outdoor trials for individual isolates

μBIO MicroBio Engineering

Summary

During this ABY2 project we:

- Isolated and characterized promising native species from wastewater, including strains that outperform polycultures.
- Sequenced and assembled novel genomes.
- Developed an *S. obliquus* strain with improved productivity in outdoor ponds using a non-GM approach.
- Advanced the state of technology towards meeting the goals of DOE-BETO for algae biofuel production.

MicroBio Engineering

Acknowledgments

CAL POLY
SAN LUIS OBISPO

Tryg Lundquist, Ph.D.

Staff/Grad Students
Shelley Blackwell
Sara Leader
Carly Lesne
Ryan Anderson
Nahel Ali
Claire Greco
Paul Camarena
Allison Shoemaker
Emma Hennig
Mike Scott
Anya Dickinson-Cove
Lucy Houghton

U.G. Students
Tim Beillevaire
Amelia Skeate
Maria Delfino
Tristan Jang
Megan Bell
Megan Bolling
Catherine Field
Laly Rouch
Jonathan Provost
Pascal Ung
Gabe Santos
Reid Dawley
Maddie Rons
Lacey Sikes
Nolan Thompson
Isalyne Gatheron
Arnaud Bizouarne

μBIO
MicroBio Engineering

John Benemann, Ph.D.
Ruth Spierling
Ian Woertz
Braden Crowe
Neal Adler
John Coyne
Cooper Gibson
Whitney Taylor
Bobbi Oberti

Consultant:
Juergen Polle, Ph.D.

US DOE BETO
DE-EE0007691