

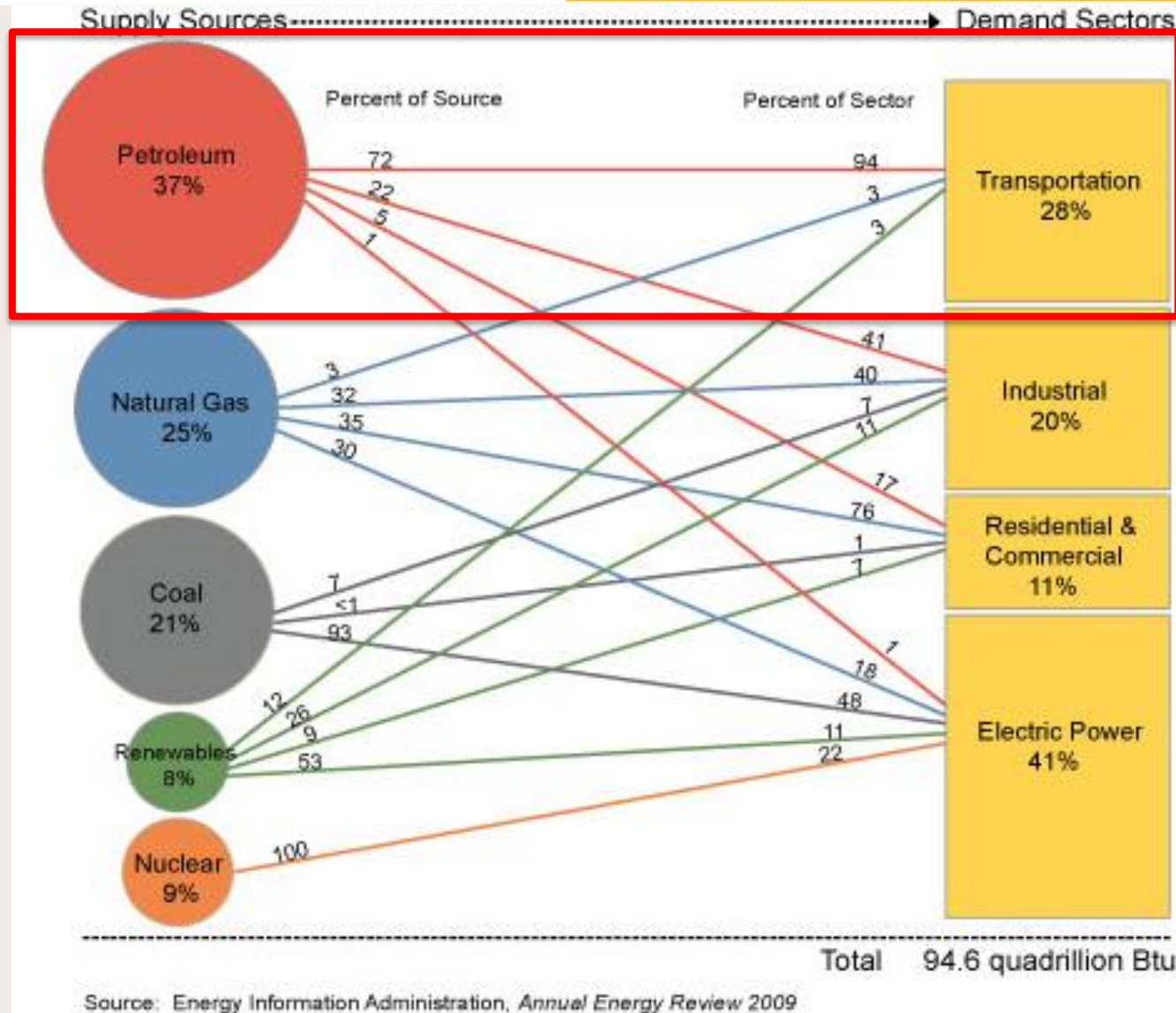
improving human health and the health of our planet

Microbial Ecology in a Photobioreactor

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Biotechnology



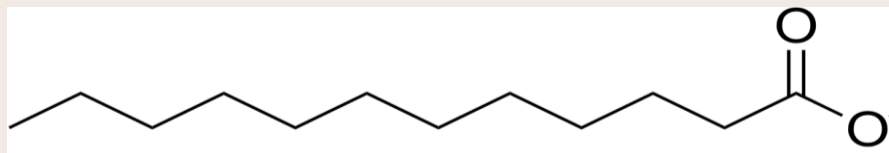


Most transportation is fueled by a finite resource!!



The Photobioreactor (PBR)

- **Purpose: Carbon neutral production of jet fuel**
 - Mutant *Synechocystis* sp. PCC 6803 secretes laurate into media

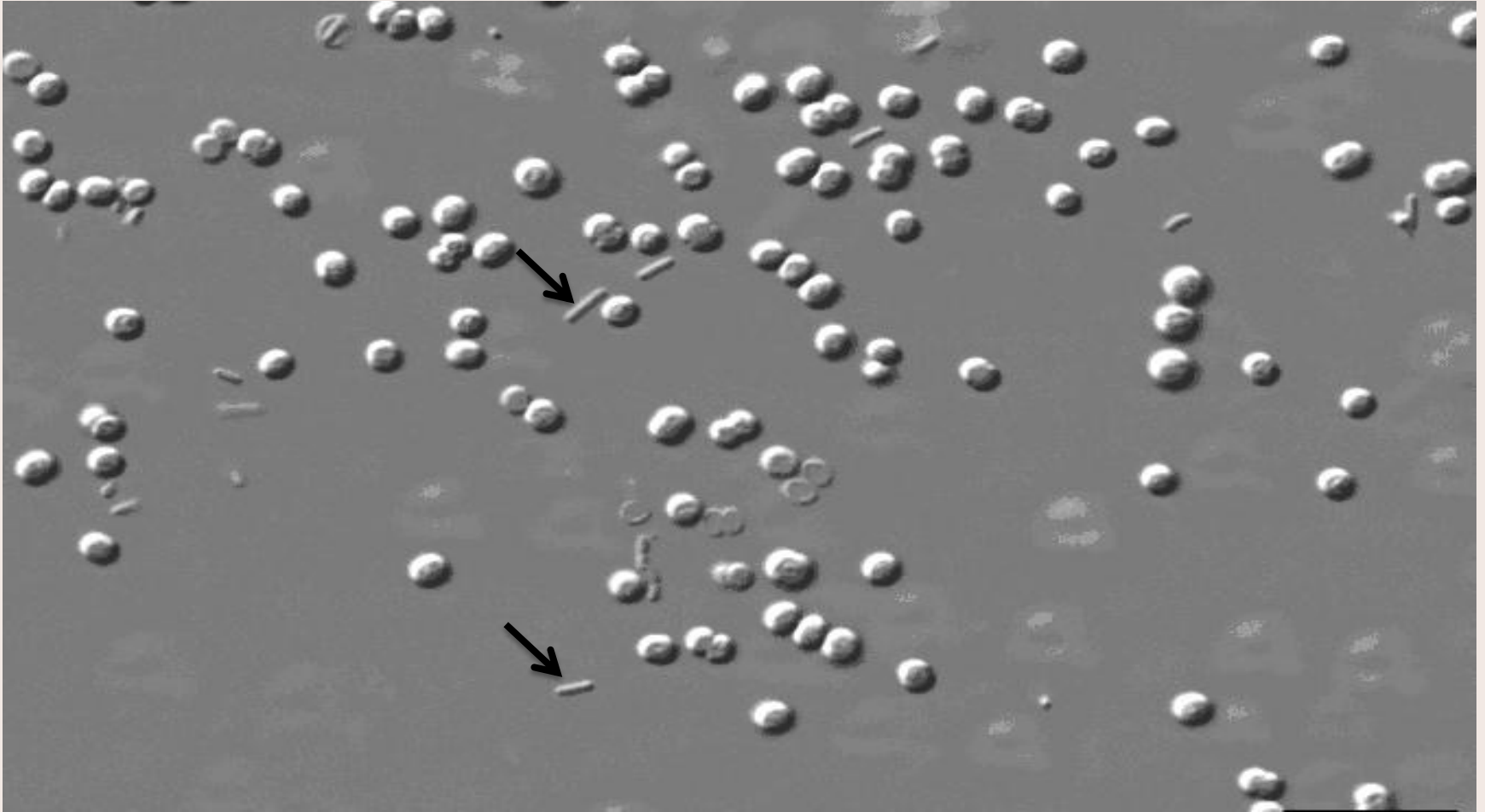


- Laurate is harvested and catalytically converted into undecane (jet fuel)
 - Carbon neutral because each C burnt as fuel was fixed by *Synechocystis* as CO₂





Microbial Ecology

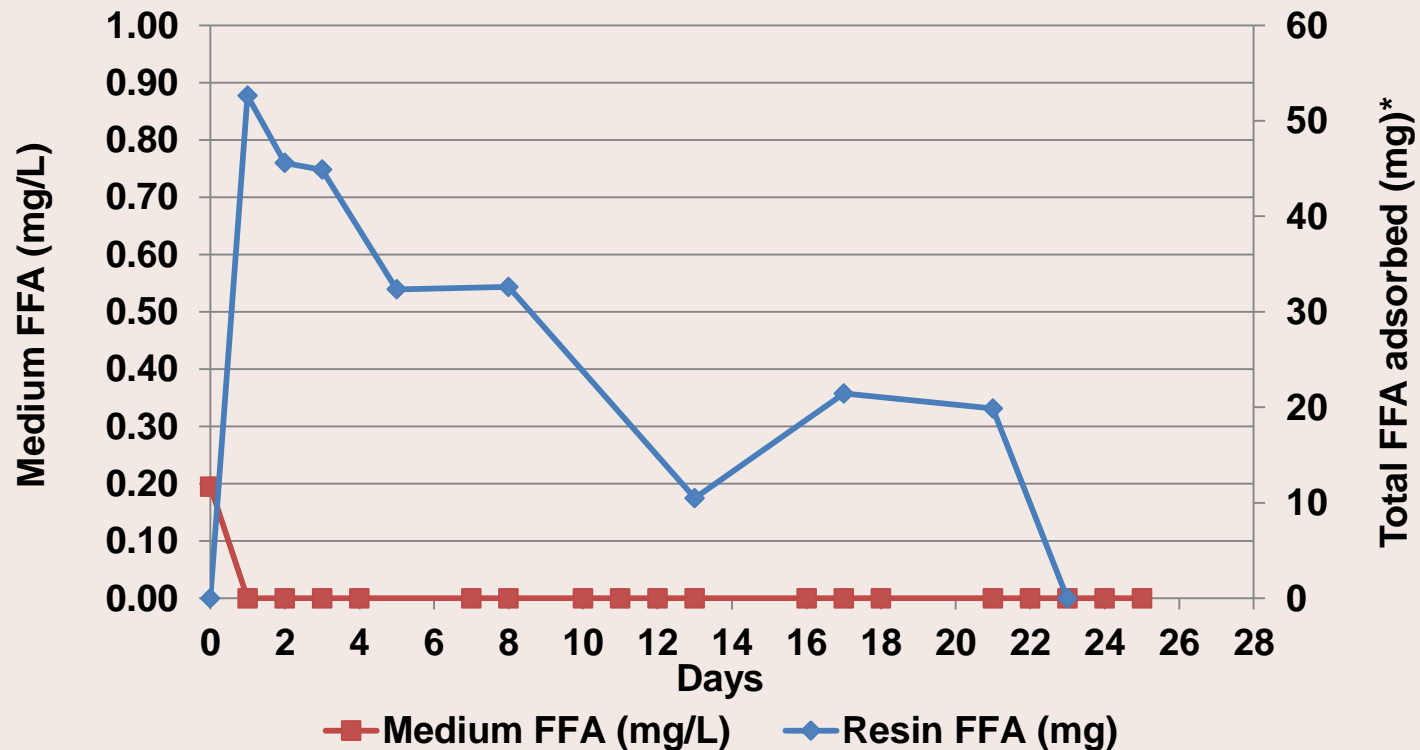


Contaminants invade the PBR culture!



Microbial Ecology

- Rising contamination levels are correlated to decreasing laurate production!





Studying the Microbial Community

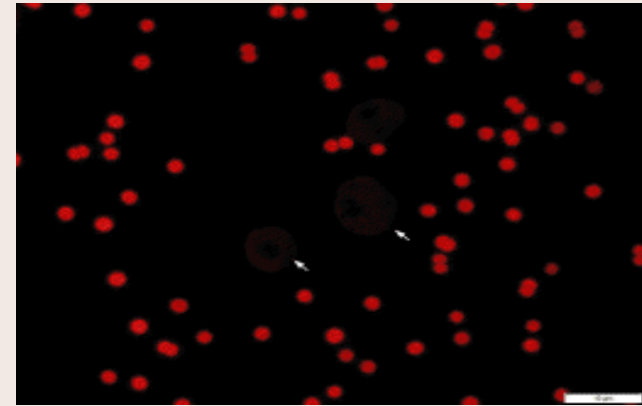
- **Goals:**

- Determine the structure of the microbial community in the PBR when *Synechocystis* is:
 - » Healthy and growing
 - » Unhealthy and dying
- Determine how heterotrophic members of the community interact with *Synechocystis*
 - » Are there good/bad heterotrophs?



Studying the Microbial Community

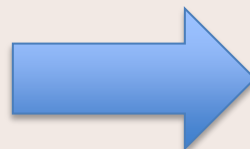
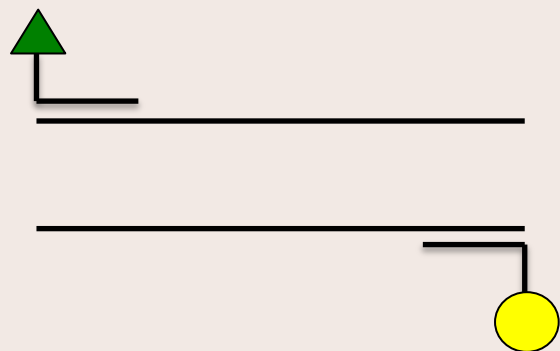
- Tools:
 - Microscopy
 - Light and fluorescent
 - Microbiology
 - Isolate species that can grow on laurate, sequence 16S rDNA



Exp	Closest BLAST match
4.6	<i>Novosphingobium subterraneum</i> strain B1AT14
	<i>Aquabacterium fontiphilum</i> strain CS-6
3.6	<i>Uncultured bacterium</i> clone ESM-3
	<i>Bacillus cereus</i> strain 9D
	<i>Staphylococcus</i> sp. CIFRI PTSB-29
	<i>Sphingomonas</i> sp. NBRC 15917
1.5	Uncultured <i>Pseudomonas</i> sp.
	<i>Pseudomonas mendocina</i> strain S100E
2.5	<i>Agrobacterium</i> sp. PNS-1
	<i>Novosphingobium subterraneum</i> strain B1AT14
	<i>Achromobacter</i> sp. OPB3



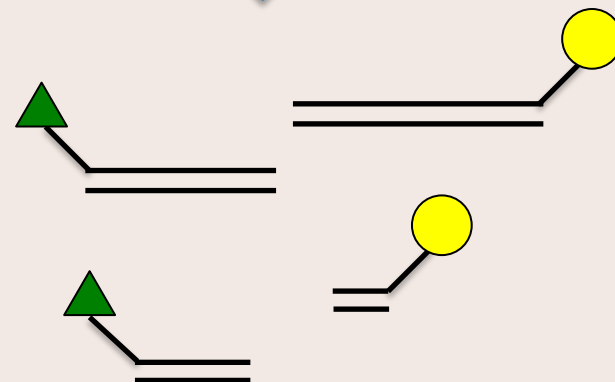
T-RFLP



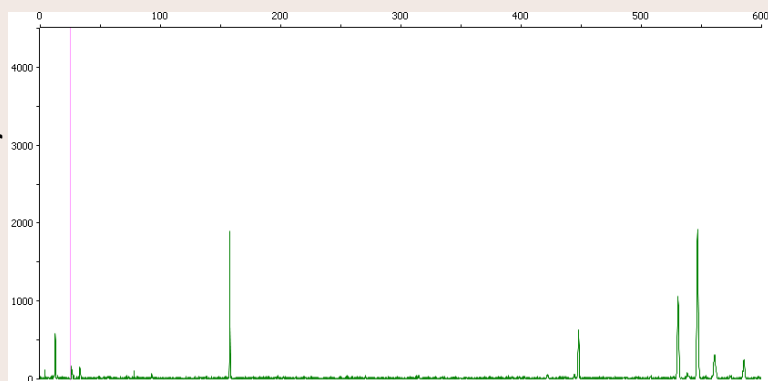
**Amplify 16S
rDNA with
labeled
primers**



**Digest PCR
amplicons**



**Analyze size
of fragments**

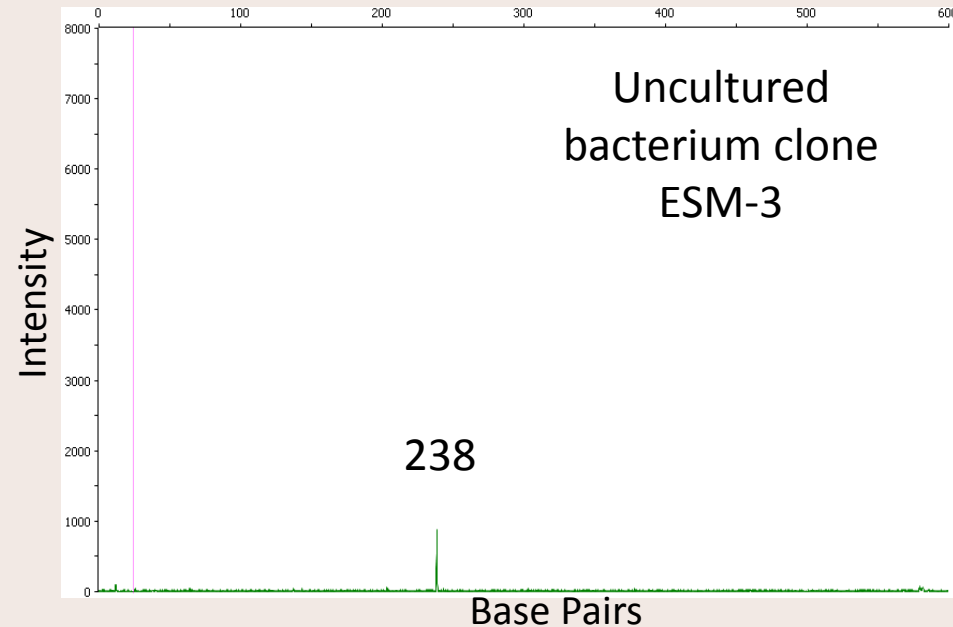
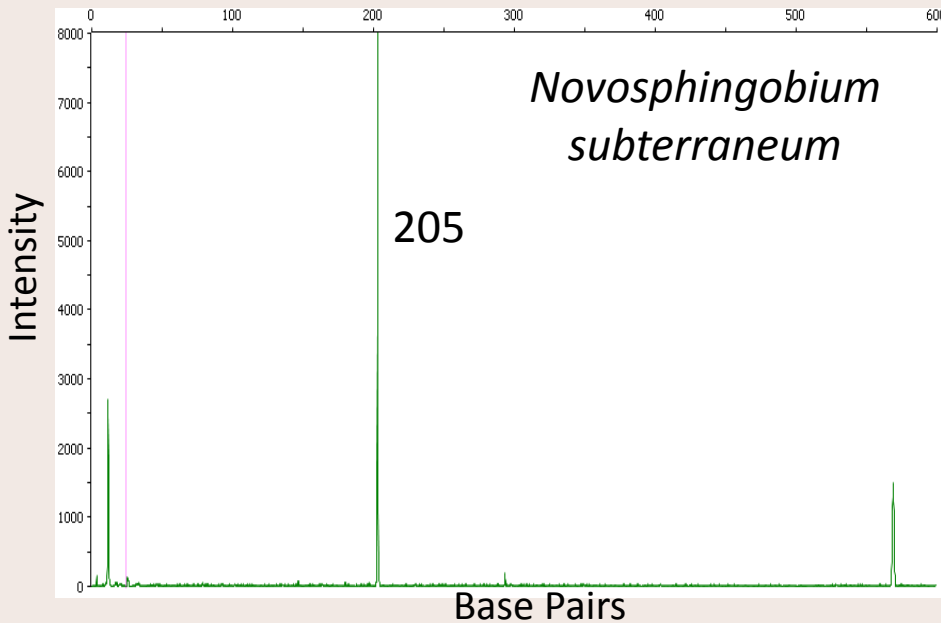


Base Pairs



Studying the Microbial Community

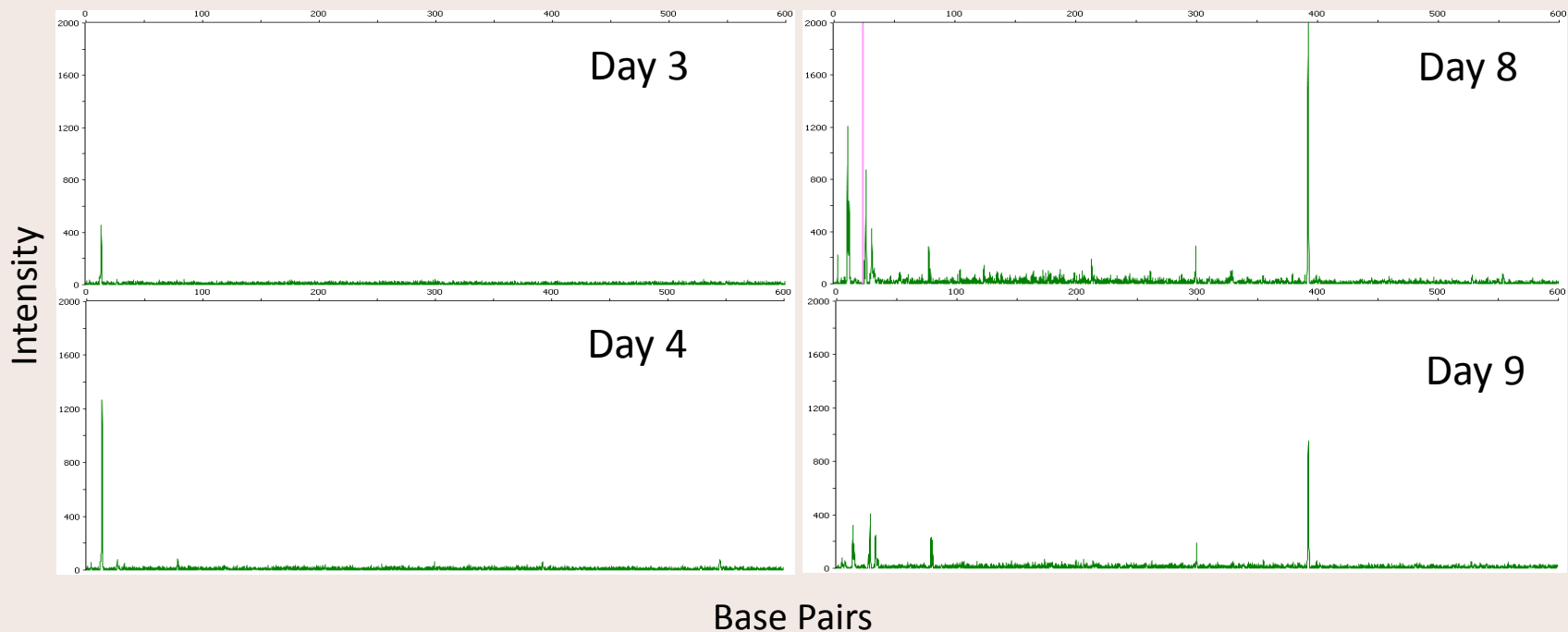
Each species produces a unique fragment length





Studying the Microbial Community

- Monitoring the community across time:

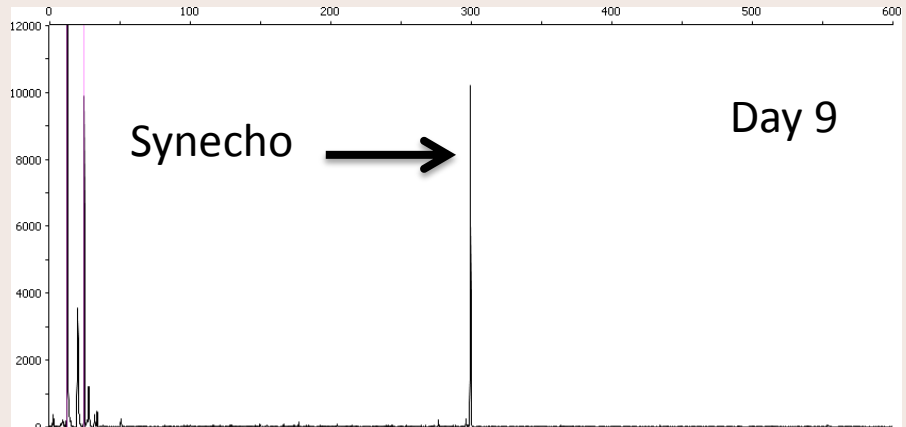
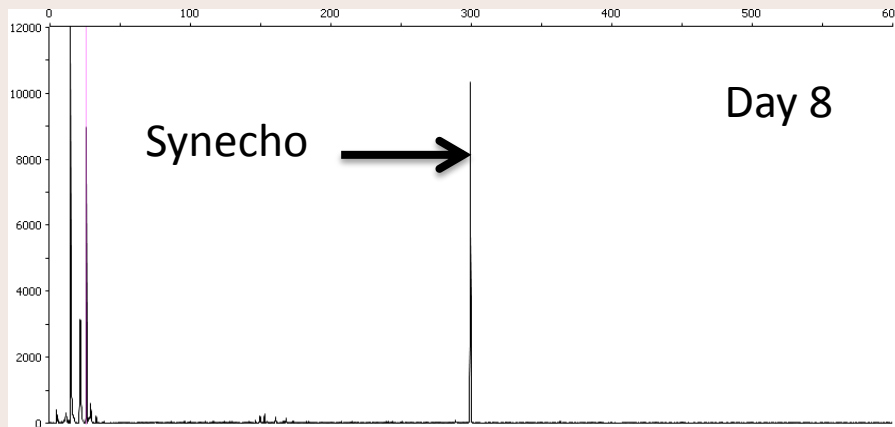


Synechocystis does not produce a peak in this window, allowing us to “magnify” the other bacterial species present in the PBR culture



Studying the Microbial Community

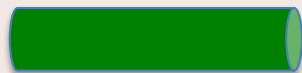
1. The culture is predominantly *Synechocystis*



2. Everything else gets “washed out”



PBR Culture



Extract DNA
from sample
of PBR
culture



Add biotin tagged
Synecho specific probe
and mix



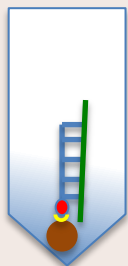
Probe hybridizes
specifically to
Synechocystis
16S DNA



Add streptavidin coated magnetic
beads and mix

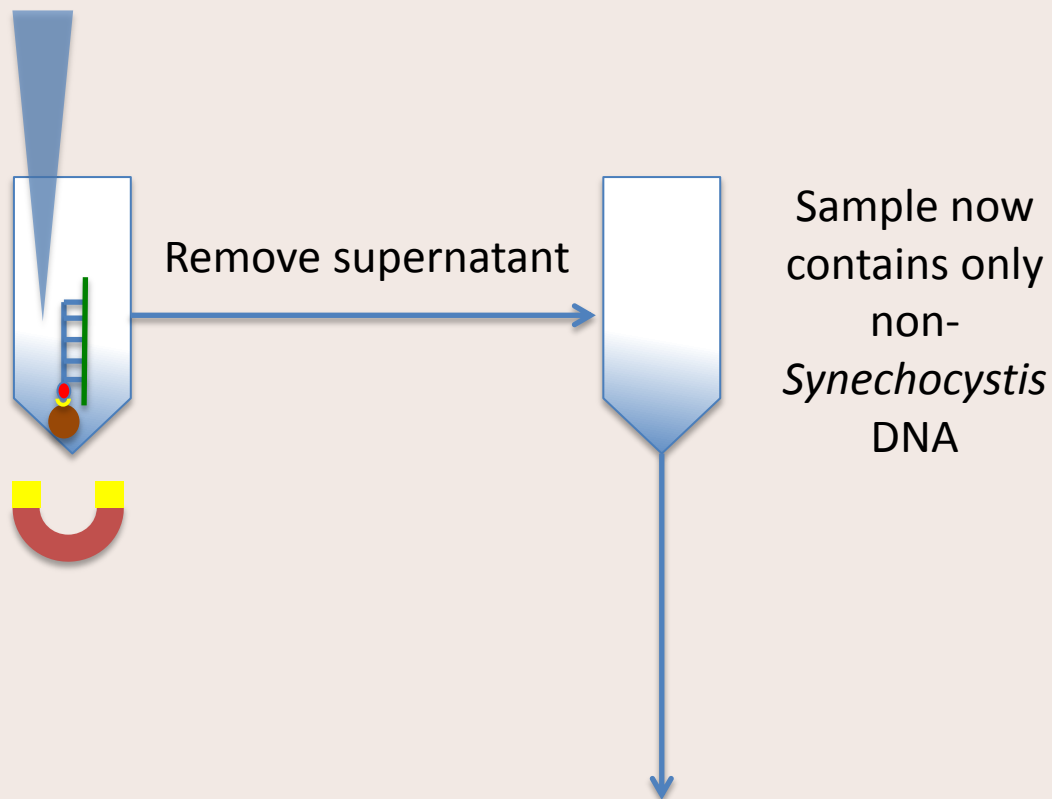


Streptavidin binds to
biotin



Apply magnet to pull beads down

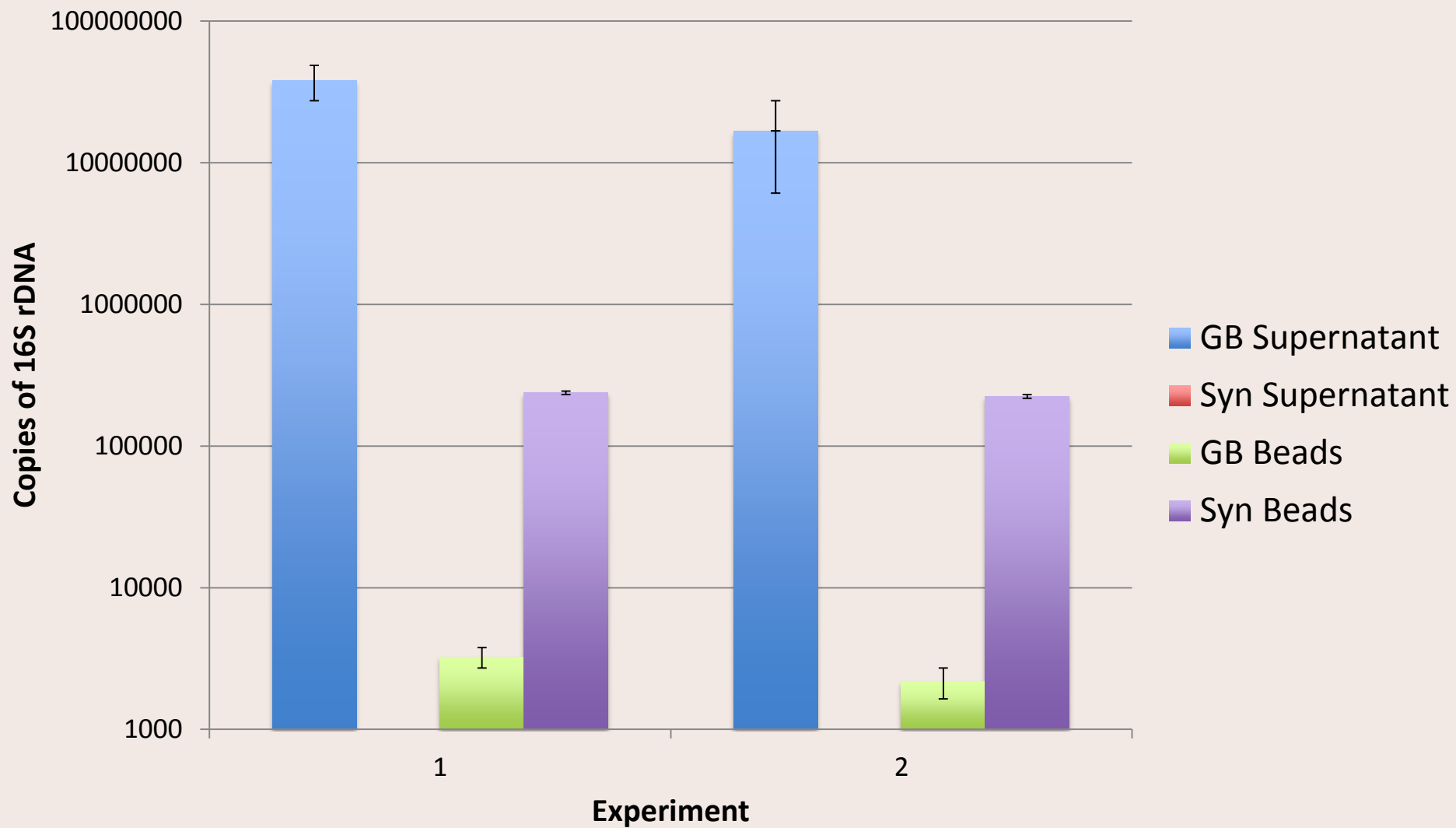




1. Verify that *Synechocystis* DNA has been removed using qPCR
2. Submit samples for pyrosequencing to analyze non-*Synecho* community members



Removal of *Synechocystis* DNA

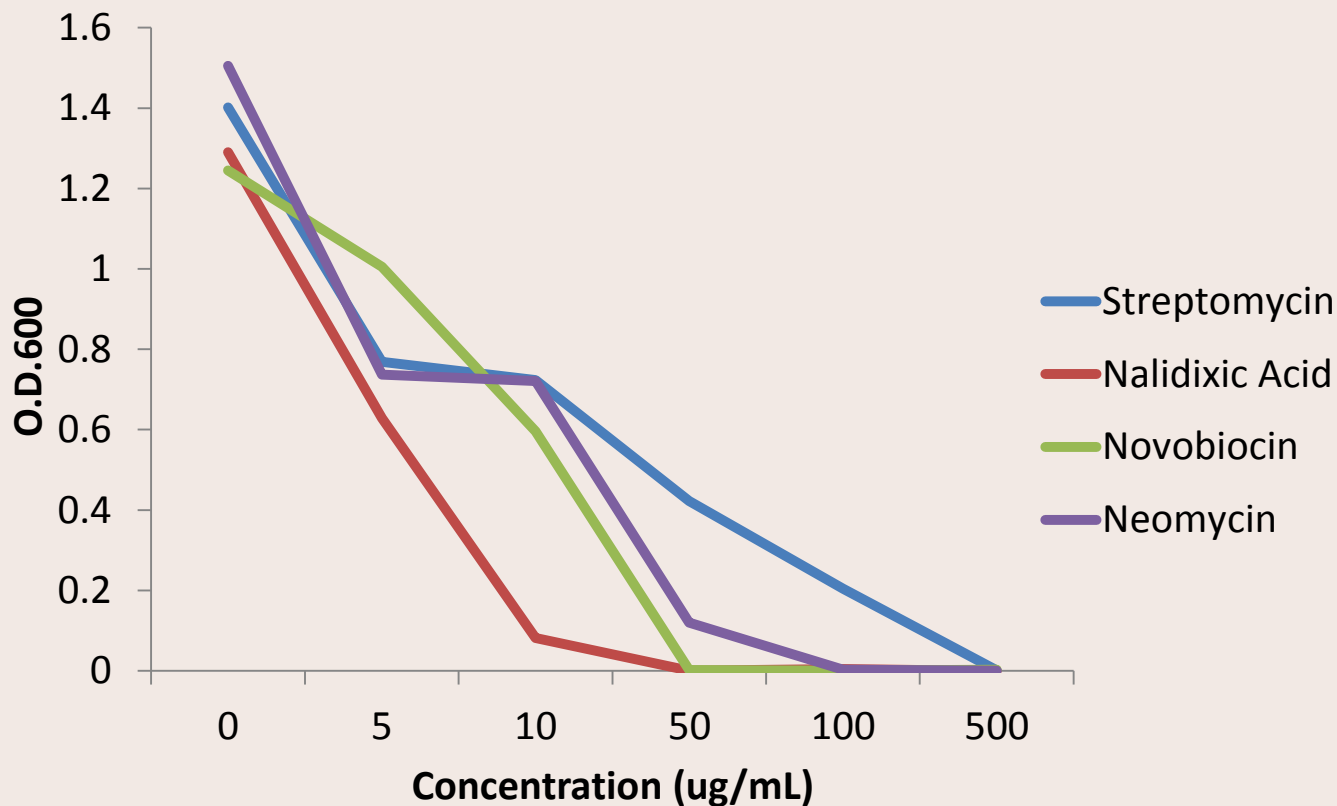




Strategies to control scavengers

- pH>10
- High salt
- Antibiotics

Effects of Antibiotics on Isolated Pseudomonas





Future Directions

- Employ deep sequencing techniques to determine phylogenetic distribution of heterotrophic bacteria in the PBR culture
- Use flow cytometry to determine total contamination levels
- Continue searching for effective and sustainable scavenger mitigation strategies



Thank You

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- EB
- You guys



Questions?

