Treatment against the predatory bacterium
Vampirovibrio chlorellavorus

Eneko Gauza
Charles E. Sellers
Braden W. Bennett
Eric M. Lyons
Laura T. Carney
Presentation outline

1. The predatory bacterium.
2. Infectious detection & identification.
3. Treatment development.
4. Treatment validation.
5. Concluding remarks

For more detailed information please check:
1. The predatory bacterium: *Vampirovibrio chlorellavorus*

- **Genus:** *Bdellovibrio* → *Vampirovibrio* (Gromov & Mamkaeva, 1980).
  - Epibiotic predation.

- **Phylum:** Proteobacteria → Cyanobacteria (Soo et al., 2014).
  - Melainabacteria (16S phylogeny)

- **Predatory lifestyle** on the genus *Chlorella*.
  - Maintained only in co-cultured with a very narrow host range (Coder et al. 1989).
1. The predatory bacterium: Infectious prognosis
1. The predatory bacterium: Widespread incidence

- Globally distributed, any *Chlorella* culture in the world could be exposed.
- *Chlorella* is a strain of reference in microalgae biotechnology due to its “robustness”.

Algae cultures

Environmental samples: reservoir, amphibian pond, bovine rumen, soil, biofilm.
2. Predatory bacterium:
Crop protection and scale up

Heliae Mixotrophic Platform

The scale up (130 000 L reactor).
3. Infectious identification & detection: Diagnosis

1. **Chlorella Growth**

3. **V. chlorellavorus** qPCR results
   6-carboxyfluorescein based (FAM) qPCR assay:
   - Early detection
   - Infectious Progression

2. **Bacterial community structure**
   16S rRNA sequencing:
   - Early diagnosis
3. Infectious Identification & Detection: Phycosphere

Bacterial community structure:
Sanger 16S rRNA sequencing.
Phycosphere by centrifugation (1000 g)

- The proportion of reads assigned to this predatory bacterium was much more abundant in the **phycosphere** sample (0.47 versus 0.07), in agreement with its predatory behaviour.
Figure 1 Intracellular pH regulation in chlorophyta vs. cyanobacteria.
4. Treatment Development: Literature review

Acetate is not a heterotrophic substrate for cyanobacteria.
4. Treatment Development: The pH shock methodology

The pH shock treatment in the presence of 0.5 g/L acetate!!
4. Treatment Development
Microalgae tolerance

- *Chlorella* growth following the pH-shock treatment.
- Growth was evaluated in shake flasks cultures (250 mL, n=2).
4. Treatment validation
Treated vs untreated (1000 L)

Attachment

Clumping

Discoloration

Untreated | pH-treated

![Images showing treatment validation with私下content]
4. Treatment validation
Treated vs untreated (1000 L)

Pullbacks from an industrial scale reactor (130,000 L).
Figure x Simultaneous side by side comparison in raceways (1000 L, n=1) of pH-treated and untreated culture originated from an industrial scale reactor (130,000 L).
Figure x Longevity of a Chlorella culture was prolonged by applying the pH treatment repeatedly to the same culture upon transfer, at the beginning of consecutive batch cycles.
5. Concluding remarks

A case study for microalgae crop protection:

- Crop protection & scale up.
- *Chlorella*, keystone algae in industry.
- *V. chlorellavorus* had no treatment.
- Rescue treatment (24 h).
- Advanced detection techniques.
- Easy and cheap to scale.
- Repetition.
- Resistance to a discrete treatment.

Validated in mixotrophic cultures.

- One order of magnitude more productive.
- The same crash agent as in photoautotrophic cultures.
- Expanded host range to *Micractinium inermum*. 
Koch Postulate

Four criteria that were established by Robert Koch to identify the causative agent of a particular disease, these include:

- the microorganism or other pathogen must be present in all cases of the disease
- the pathogen can be isolated from the diseased host and grown in pure culture
- the pathogen from the pure culture must cause the disease when inoculated into a healthy, susceptible laboratory animal
- the pathogen must be reisolated from the new host and shown to be the same as the originally inoculated pathogen

<<Koch postulate could not be fulfilled because the V. chlorellavorus could not be isolated in a pure culture (is an obligate predator)>>
The GP2 team (Mike Lamont, Alex Sitek, Jenna Lloyd-Randolfi, Kevin Boyd, Brandi Alderson, Steven Ventre, Ravi Vannela and Luke Cizek) for helping to develop our mixotrophic platform.

Kristine Sorensen for assistance with molecular work.

Justin Kniep for the patenting process leading to this presentation.

Shan Qin and Josh Wilkenfeld for monitoring our cultures.

Barbara Melkonian and Michael Melkonian for the algae molecular work.

R.A. Andersen for his microscopy work and his invaluable suggestions that greatly improved this manuscript.