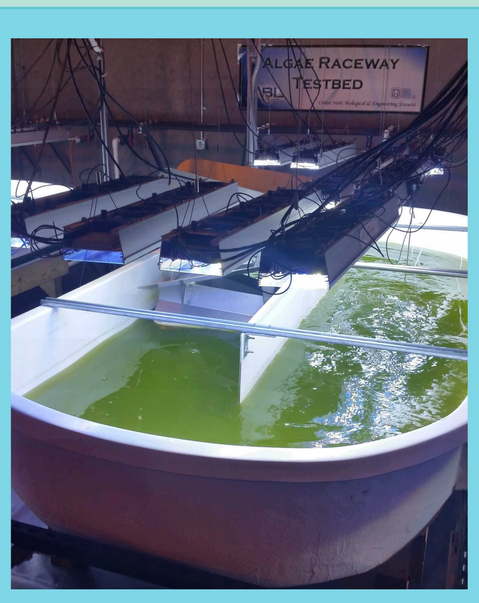
Sandia National Laboratories

SAND2019-8555C

Inhibition of Industrially Relevant Algae by *Bizionia echini*

Adison R McLaggan, Krissy M Mahan, Colleen M Courtney, and Todd W Lane

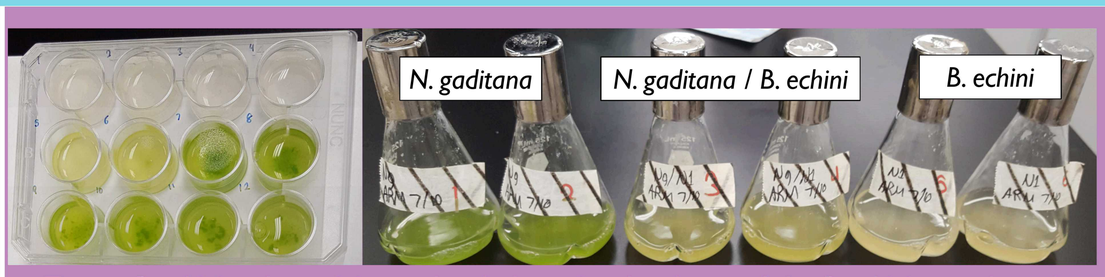
Algal biofuels are the alternative to petroleum based fuels.



Bacterial contamination is an inevitability in open raceway ponds especially when growing monocultures. Knowing more about the bacterial species that are pathogenic to highly productive algal strains can allow us to create preventative measures to maximize growth and cultivation.

Bizionia echini on marine agar

Bacterial strains were isolated from an open raceway pond in Texas that was growing *Michrochloropsis salina and Phaeodactylum tricornutum*. One marine isolate was a yellow-pigmented flavobacterium with 16S rRNA gene sequence similarity of 100% to *Bizionia echini*[1]. Experiments investigated the magnitude of inhibition as well as the potential mechanisms by which the *B. echini* inhibits the growth of and kills industrial strains of marine algae.



To test *B. echini* pathogenicity to the algal species the algae and the *B. echini* are cultured separately and then the algae is infected with *B. echini* in welled plates or shake flasks and algal growth is determined by monitoring chlorophyll fluorescence over time.

1a. *M. salina* and 1b. ESAW	2.*a M. salina* and 2b. *B. echini* (peptone)	3a. *M. salina* and 3b. *B. echini* (ESAW + NPM)	4a. *M. salina* and 4b. *M. salina* / *B. echini*

To determine if the *B. echini* kills algae through the bacterial production of toxic chemicals, the *B. echini* and *M. salina* were grown in bottles connected by a bridge and separated by a 0.22 µm filter allowing chemicals but not cells to pass through. The bottles were placed on magnetic stirrers to allow for gas exchange and fluorescence was monitored daily. In the pilot study above, no chemicals that passed thru the filters impacted algal growth.

To determine if the *B. echini* inhibits and kills the algae through attachment the samples were grown and then combined in flasks. These samples were then stained and observed under a microscope. The *N. gaditana* cells autofluoresce pink. To determine if the algae were dead, the cultures were stained with SYTOX green, a dye that stains DNA green but is not membrane permeable, thus any dead green algae or bacteria can be seen as green. Bacteria were also stained blue with Hoechst dye (live stain) so that they could be observed interacting with the algae.

Live/Dead Imaging

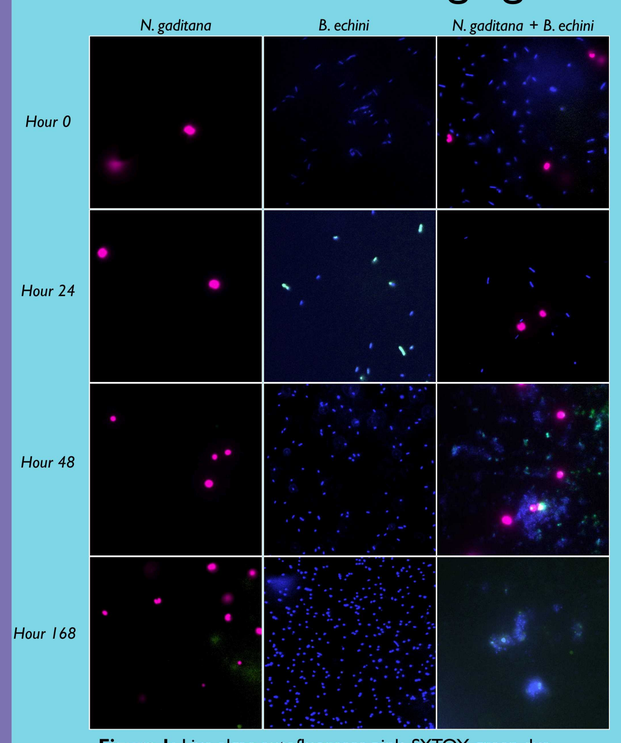
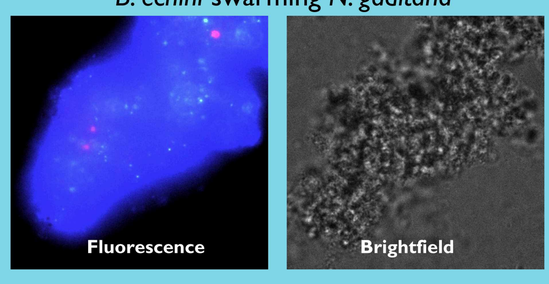


Figure 1: Live algae autofluoresce pink. SYTOX green dye stains DNA of permeabilized (dead) cells. Bacteria are stained blue by the live stain Hoechst dye. At 48 hr the bacteria begin to blanket live algae and at later times, live bacteria can be seen on dead algae.

B. echini swarming *N. gaditana*



A large mass of *B. echini* is completely overwhelming the *N. gaditana* which will likely soon be dead.

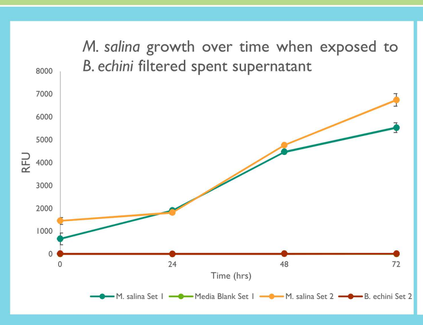


Figure 2: Chemicals in the media supernatant of *B. echini* cultures do not have a huge impact on *M. salina* growth.

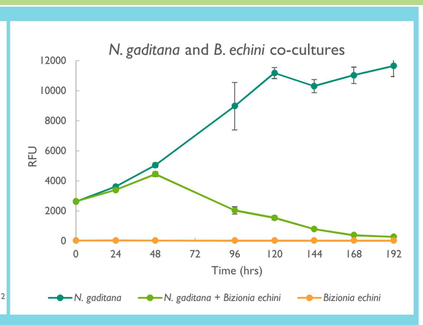


Figure 3: *B. echini* co-cultured with *N. gaditana* inhibits algal growth.

Survivability of various strains of algae when exposed to *B. echini*

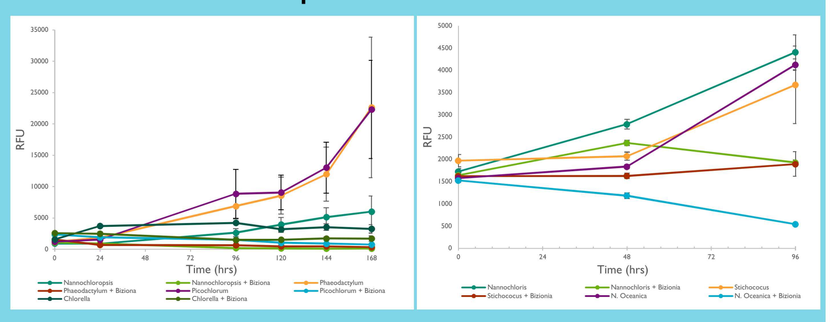


Figure 4: When exposed to a significant amount of *B. echini* no algae seems to be able to grow or even survive aside from perhaps *Stichococcus*.

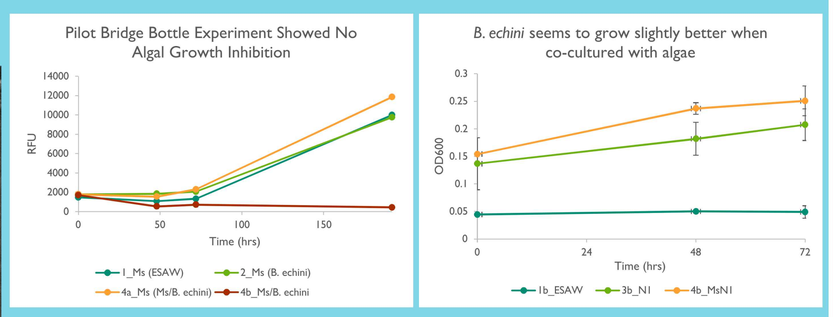


Figure 5: Pilot bridge bottle experiments showed that attachment of *B. echini* seems to be required for growth inhibition and not chemicals released from bacteria. *B. echini* cannot grow on algae alone. Additional nutrients are required. *B. echini* does seem to grow better when co-cultured with algae.

FURTHER INVESTIGATION

- Gain insight into the mechanism for *B. echini* pathogenesis via transcriptomics, proteomics, and microscopy.
- Test closely related bacterial strains for algal growth inhibition. Use comparative genomics to minimize protein targets.
- Test the survivability of more marine algal strains and determine the mechanisms of their resistances if any.
- Identify any proteins that may be produced by algae as a defense mechanism.

CONCLUSIONS

Bizionia echini inhibits the growth and decimates the population of most marine algal strains through a possible attachment mechanism and not by producing algicides as observed through microscopy, co-culture, and filter experiments. *B. echini* can inhibit algae at very low bacterial concentrations and during any growth stage but cannot grow without additional carbon sources available.

[1]Nedashkovskaya, Olga I., Marc Vancanneyt, and Seung Bum Kim. "Bizionia echini sp. nov., isolated from a sea urchin." *International journal of systematic and evolutionary microbiology* 60.4 (2010): 928-931.

U.S. DEPARTMENT OF ENERGY
National Nuclear Security Administration

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.
SAND No. _____

Sandia National Laboratories