



Reporter Constructs Based on • *N. europaea* to identify nitrification inhibitors in Wastewater

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Goals

- Use the reporter constructs in *N. europaea* to detect stress in the nitrification process by measuring fluorescence.
- Test the bacteria against known inhibitors of nitrification such as copper chloride, chloromethane gas, chloroform, hydrogen peroxide, and household bleach.
- Potentially use the constructs as indicators of biological stress.

Background

- ***N. europaea*** - Ammonia oxidizing bacteria
 - Sensitive to inhibitors
- **Green Fluorescent Protein (GFP)**
 - extracted originally from jelly fish
 - emission peak at wavelength 510 nm
- **Cell lines** - The constructs in *N. europaea* were based on the GFP with promoters of genes that were highly expressed in chloroform incubations. I explored the CLPB, mBLA clone 3, B10S2, and NTGR reporter Strains.

Procedure

- Grow culture in growth medium for 2-3 days.
- Harvest cells by spinning in the centrifuge.
- Put cells into a fresh growth medium.
- Incubate cells for one hour.
- Treat cells.
- Incubate cells for one more hour.
- Wash cells and spin them down.
- Incubate for one more hour and then pipette cells into a plate for the plate reader.

How to test the culture for inhibition

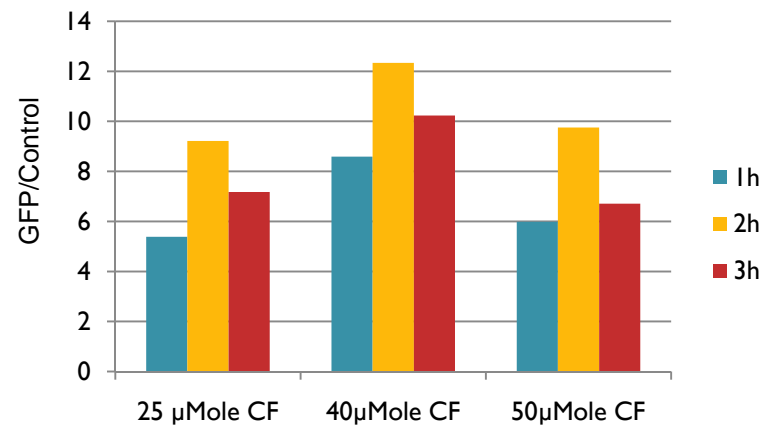
- Test inhibition of nitrite production

- Oxygen electrode
- Fluorescence

Results- CLPB Cell line

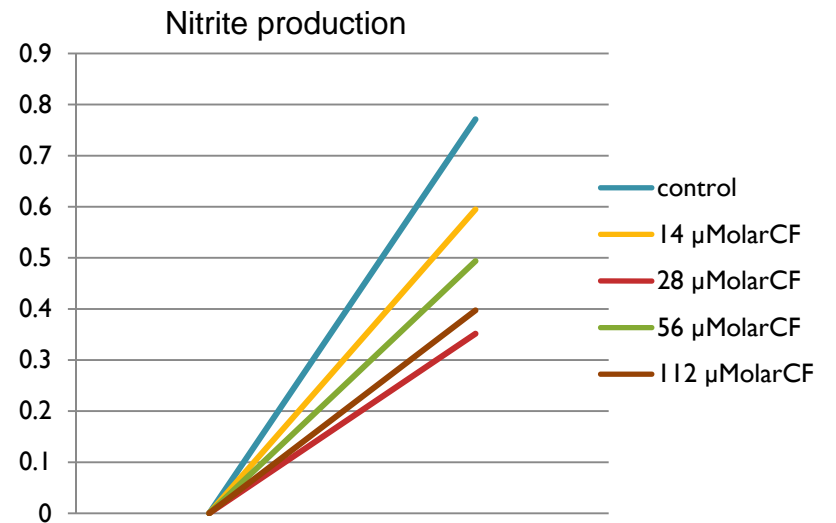
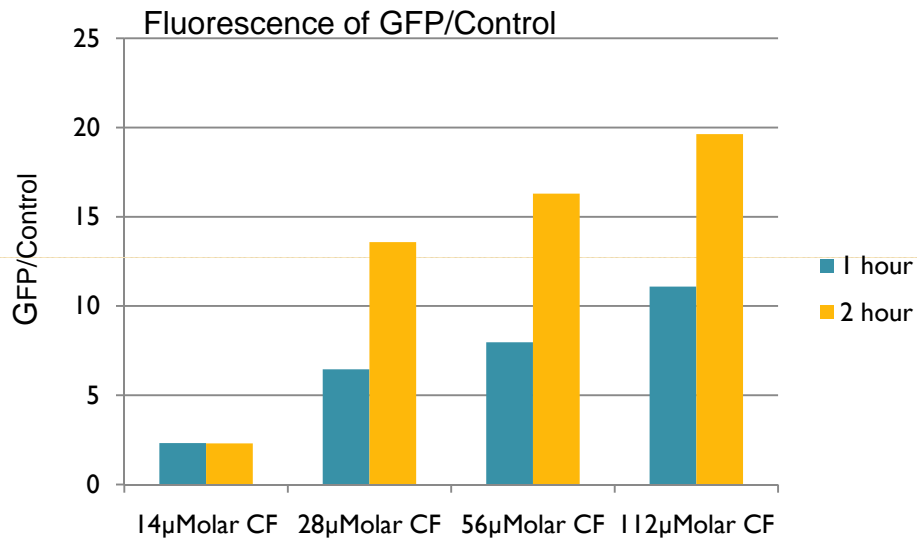
Chloroform treatments

Fluorescence of GFP/Control



Results- NGTR Cell line

Chloroform Treatment



Conclusion

- Chloroform can be used as a positive control in experiments
- All cell lines fluoresced about the same when treated with chloroform, and also did not fluoresce when treated with other chemicals.
- B10s2 and NGTR take longer to grow.
- Based on my experiments, CLPB and mBLA clone 3 are the most efficient reporter strains for chloroform.
- Many more experiments would need to be done to correctly identify the best reporter strain.

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