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

- 25 µMole CF
- 40 µMole CF
- 50 µMole CF

- 1h
- 2h
- 3h
Results- NGTR Cell line

Chloroform Treatment

Fluorescence of GFP/Control

Nitrite production
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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