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Creating a microcosm to examine salinity tolerance of Escherichia coli in beach sand

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ABSTRACT

Escherichia coli (*E. coli*) is a bacteria species that thrives in a variety of environments. Due to its widespread prevalence, it is commonly used as an indicator for pollution and other pathogens. One place where it is not often looked for is oceanic beaches because *E. coli* is inhibited by salt. However, recent research has shown that *E. coli* often thrives in sand at beaches. To determine how it persists in sand, we created a microcosm simulating the intertidal zone of a beach. Using this microcosm, we are testing how varying salinity levels affect persistence of *E. coli* in sand. Collectively, our findings suggest that *E. coli* may be able to persist on sandy beaches despite the stress of salinity and may be a useful tool in the future for assessing these ecosystems for fecal contamination levels.

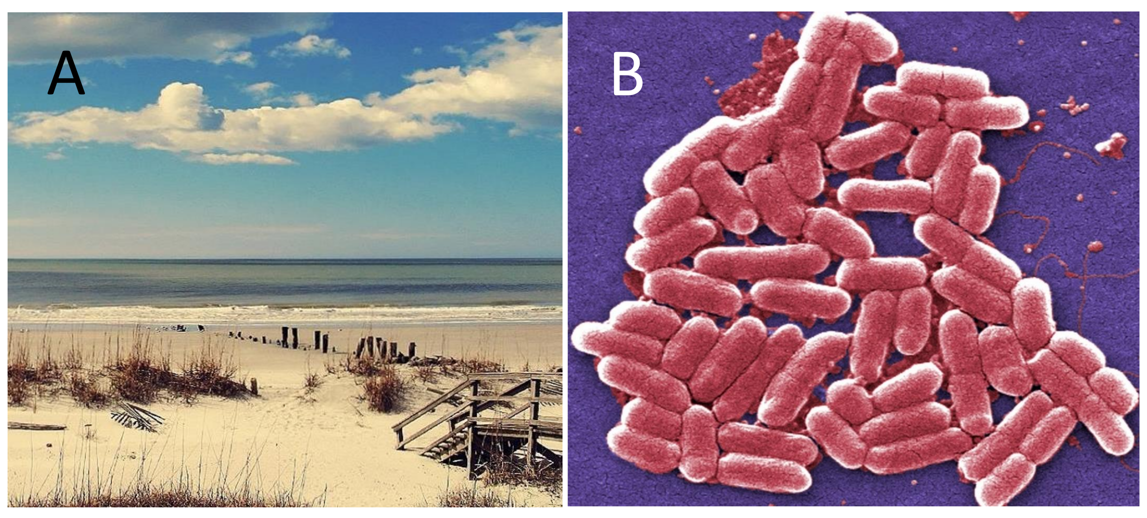


Figure 1. (A) depicts an example of a marine oceanic beach where microbes such as *E. coli* can persist (HD Folly Beach 2015). (B) contains *E. coli* O157:H7 under a magnification of 6836x using a Scanning Electron Microscope (SEM) that has been gram-stained (CDC 2006).

INTRODUCTION

- *E. coli* are a fecal indicator bacteria, which can be used to measure the presence of fecal material and associated pathogenic bacteria. This can be used to assess ecological quality and sanitation (Brady et al. 2014).
- *E. coli* are found in a variety of environments and locations including oceanic beaches where they face many stressors that can threaten their survival.
- One important stressor at oceanic beaches is salt, which can inhibit the growth and survival of *E. coli* (Ortega et al. 2009).
- However, our research and other recent research has shown that *E. coli* can tolerate salinity and persist to some extent in ocean environments (Ortega et al. 2009; How et al. 2012; Lewis et al. in prep).
- One potential issue that arises from this is that the Environmental Protection Agency (EPA) does not test for *E. coli* on oceanic beaches (EPA 2014).
- In our study, we created a microcosm of the intertidal beach zone to assess how *E. coli* can persist in a sandy environment with varying levels of salinity.
- Our goal is to better understand how salinity affects growth and persistence of *E. coli* in beach sand.

METHODS

- We set up an intertidal microcosm with sterilized materials using 96.25g of play grade sand that was placed in 600mL beakers.
- To obtain standardized levels of *E. coli,* we created a McFarland Standard (1.5 X 10^8 CFU/mL) (*Thermo Scientific Remel*™, KS, USA 2015) of K-12 *E. coli*, a commonly used lab strain.
- We diluted the McFarland standard and added 1µL to 25mL of nutrient broth, which was mixed with varying concentrations of salt (NaCl; 0%-6%) and replicated our experiment in triplicate.

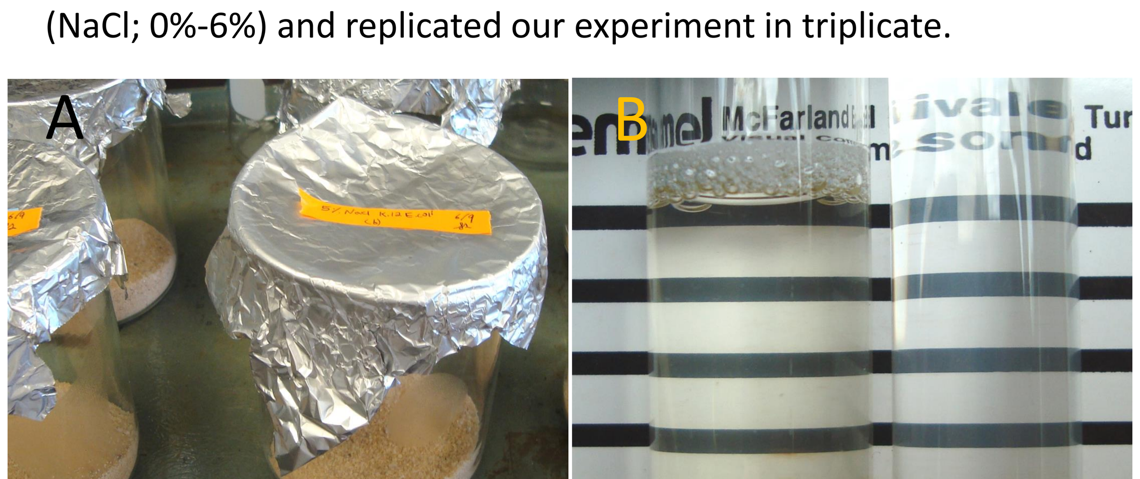


Figure 2. (A) shows the simulated microcosm with sand without the *E. coli* added yet. (B) compares the *E. coli* and McFarland standard. When a match in clarity and turbidity is achieved, the McFarland standard is reached, signifying a certain amount of *E. coli*.

METHODS CONTINUED

- *E. coli* was extracted from sand after 0 hours and 6 hours to determine the change in *E. coli* growth in response to salinity.
- *E. coli* was extracted by removing 7.0g of sand via a sterilized spatula and placing it into 35mL of water. The mixture was then shaken for two minutes and allowed to settle for 30 seconds (Boehm et al. 2009; Velonakis et al. 2014).
- For each mixture, 300µL was micropipetted into each well of a 96-well Coliplate in triplicate for each salinity concentration.
- Coliplates were incubated for 48 hours at 37°C. Blue wells and fluorescent wells were then counted (Bluewater Biosciences Inc., Mississauga, ON, Canada 2015).
- We examined whether salinity inhibited the growth of *E. coli* using linear regression.
- We analyzed changes in the abundance of *E. coli* over the six hours using ANOVA and Pairwise t-tests.

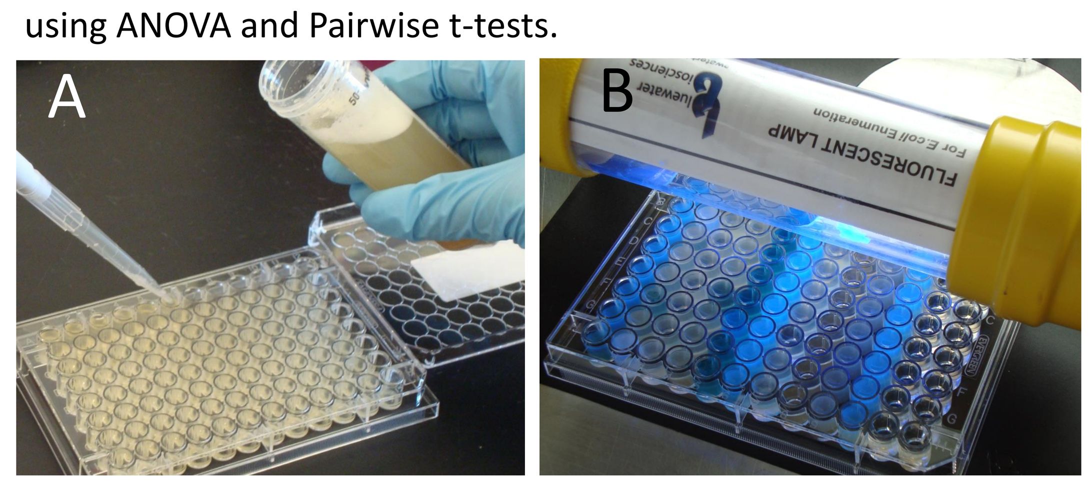


Figure 3. (A) the extracted *E. coli* from the sand is being micropipetted into the Coliplate. (B) UV light is being held over a Coliplate containing *E. coli*, prompting the *E. coli* filled wells to fluoresce a bright blue color.

RESULTS

- Salinity had a significant effect on the growth of *E. coli*. We observed a significant decrease in the amount of E. coli with increasing concentrations of salinity between 0 and 6 hours.

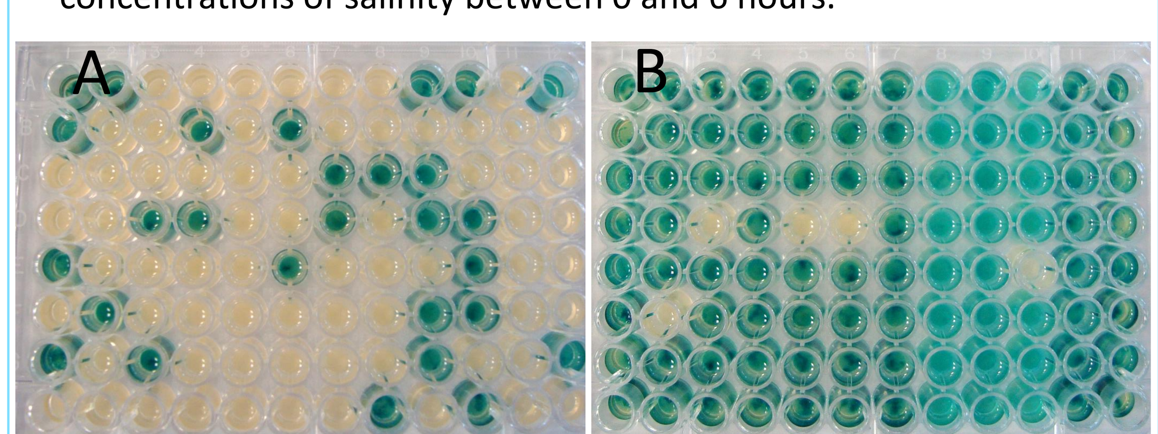


Figure 4. (A) contains 1% salinity at 0 hr. (B) contains 1% salinity at 6 hours. As shown, there is an increase in blue wells and MPN (most probable number) of *E. coli* at this low concentration of salinity.

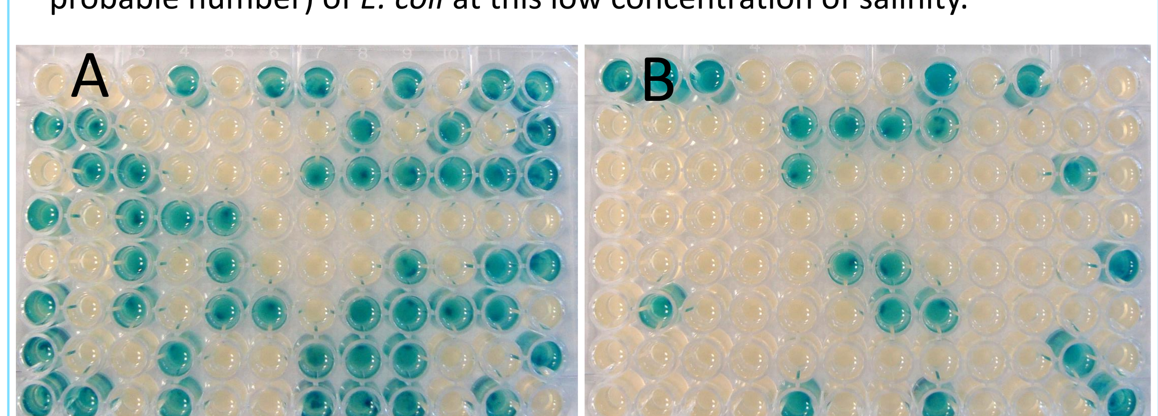
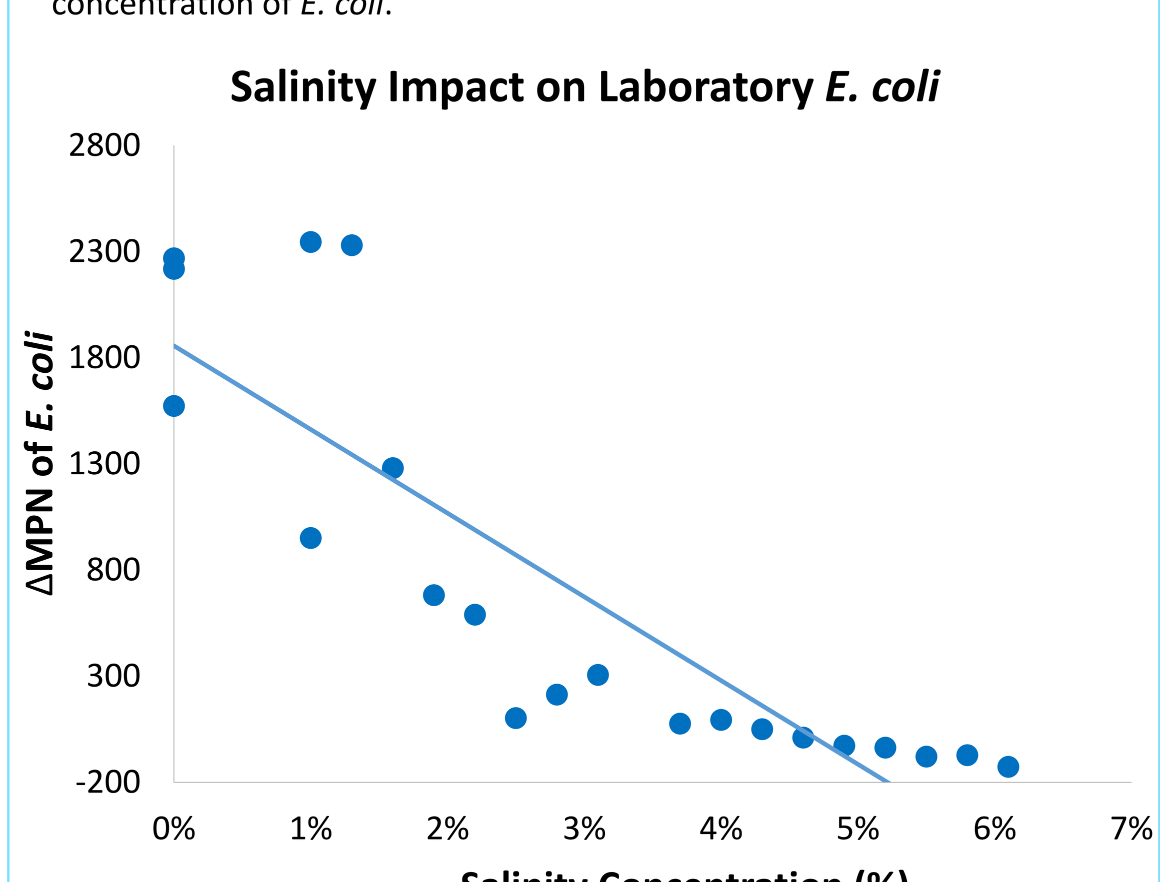
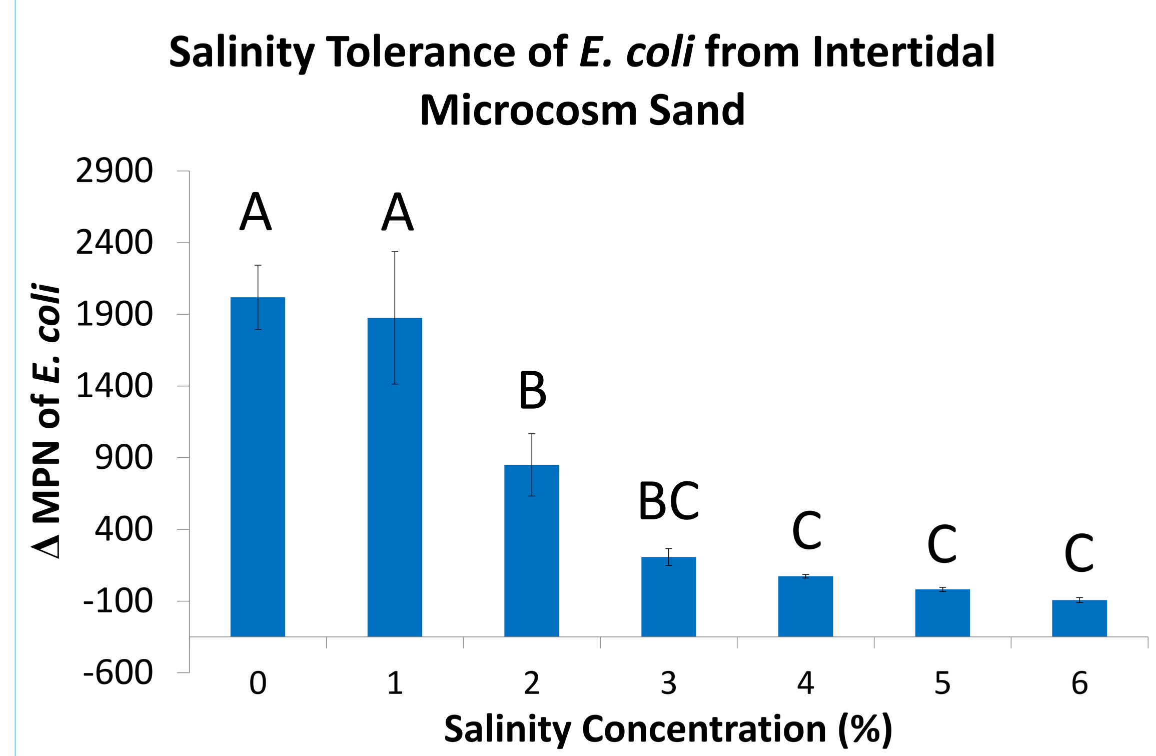


Figure 5. (A) contains 6% salinity at 0 hr. (B) contains 6% salinity at 6 hours. There is a decrease in blue wells and MPN of *E. coli* at this higher concentration of *E. coli*.



Graph 1. We observed a significant effect of salinity concentration on the growth of *E. coli* (R^2= 0.764; p<0.0001). Our linear regression analysis showed that inhibition of growth increased as salinity concentration increased.

RESULTS CONTINUED



Graph 2. We observed a significant effect of salinity concentration on the growth of *E. coli* ($F_{6,14}$=18.2553; p<0.0001). Our Pairwise t-tests showed that inhibition of growth increased as salinity concentration increased and which salinity concentrations varied from each other.

DISCUSSION

- We found that *E. coli* is capable of surviving at salinity levels greater than present in most oceanic ecosystems.
- Our microcosm findings imply that *E. coli* is capable of persisting on oceanic beaches in beach sand.
- Our findings also suggest that when health agencies like the EPA test for fecal contamination on oceanic beaches, they may want to consider including tests for *E. coli.*
- Further research may include microcosm testing of other beach zones, environmental *E. coli* strains, and temperature.

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Figure 7. Leigha Stahl preparing the simulated beach microcosm by measuring the specific sand amount for each beaker.

Figure 8. Creating the McFarland standard for the laboratory *E. coli* using a micropipette, vortex, and Bunsen Burner.

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