Monitoring Changes in Microbial Composition and Density in Coastal Marine Waters in Essex County, MA: Part I

Ethel J. Gordon, Mark Fregeau, Ted Maney, Jason Brown, Thomas Scottgale, Sheila M. Schreiner, and Peter Alachi

Salem State University, Dept. of Biology

Abstract

This research tests the hypothesis that periodic assessment of the variety and density of coastal microbes will coincide with the safety or danger of these waters for public use. We will use sterile containers to collect samples from surface water near the Salem State University mussel farm and assess microbes by conventional and genetic reactions. A prolonged study of microbial life in this area may have implications for understanding the impact of current environmental changes.

Background

- Salem State University has a unique coastal location and a unique facility where Marine Biology and Aquaculture faculty not only study chemical, physical, and biological properties of Essex County waters, but also develop ways of propagating species of shellfish that are important to the fishing industry (1). Until now, there has not been an active scholarly interaction between our Marine Biology faculty and our Microbiology or Molecular Biology faculty.

Background (continued)

- While discussing my colleagues' proposal for new buoys to monitor marine conditions and larger organisms, it became evident that there were no scientists at Salem State or other regional institutions who were studying the presence of bacteria in the same waters (2). This revelation indicated a gap in our knowledge and understanding of the changes in microbial presence in our immediate coastal waters. The project we describe here aims to diminish the gap.

Materials and Methods

1. Sample Collection.
Collect sea water samples in sterilized one liter (1 L) jars and store at the Cat Cove Marine Laboratory and Aquaculture Center or suitable refrigerator pending analyses.

2. Sample Concentration.
Pour the sample into the top of the flask above the membrane and apply suction to pull the water through the pores of the filter, leaving the bacteria trapped on the membrane (3).

1 Place a sterile filter on a support above this section to capture all of the bacteria. The water that is drawn into the bottom should be sterile. A pump is connected to one of the side ports to create a vacuum to draw the water through the filter between sections 1 and 2.

2 Invert part #2 and screw it onto the bottom. Pour the water sample into this chamber.

3 Attach the lid to section #2 to create a sealed chamber.

Materials and Methods

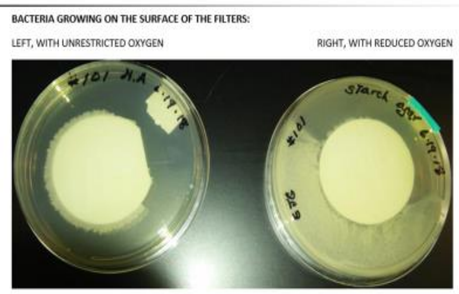
- 3. Conventional Identification of Bacteria.

We observed the appearance, growth, and phenotypic characteristics of the sea water bacteria grown on the nutrient agar under unrestricted oxygen tension. We plated duplicate samples on starch agar and incubated the plates in a candle jar to detect organisms that grow under reduce oxygen tension.

- 4. Determination of Bacterial Density.

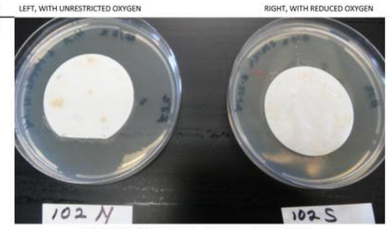
We counted the number of colonies that form on the surface of the agar plates as indicating the number of microbes per volume of sea water filtered.

Results 1.



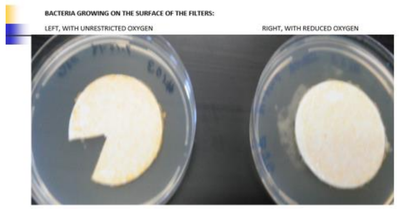
101 N.A., nutrient agar; 101 S.A., starch agar

Results 2.



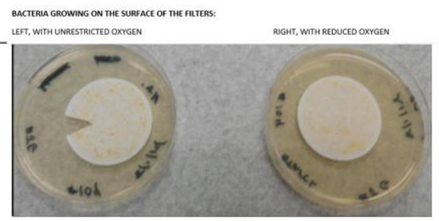
102 N.A., nutrient agar; 102 S.A., starch agar

Results 3.



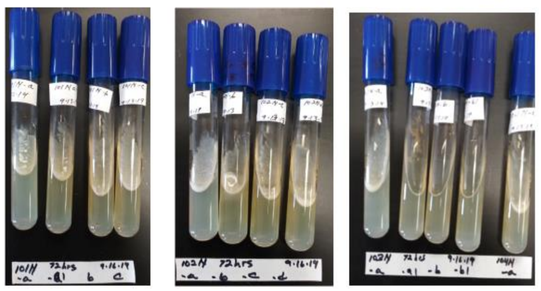
103 N.A., nutrient agar; 103 S.A., starch agar

Results 4.



104 N.A., nutrient agar; 104 S.A., starch agar

Results 5. Subcultures of isolates grown on nutrient agar



Discussion

1. Results 1 – 3 show that it is possible to cultivate microbes from sea water on conventional media under aerobic conditions and reduced oxygen levels.
2. Post incubation, one to four different colonial morphologies were evident. The number of each type varied greatly (data not shown).
3. We sub-cultured colonies demonstrating different morphologies on nutrient agar slants for future assessment.

Discussion (continued)

- 4. We detected no Gram negative rod shaped organisms in initial screenings (data not shown). We will use media selective for the coliform bacteria to confirm or reject these findings. We will use a medium selective for Vibrio species to determine their presence.
- 5. We preserved Sixteen (16) samples of original colonies and sub-cultures as glycerol stocks using a proven method (6).
- 6. We will include incubation temperature variables, DNA extraction, and PCR identification in future experiments.

Conclusion

We have demonstrated our ability to cultivate microorganism from coastal sea water under aerobic and reduced oxygen conditions using conventional techniques. Organisms showed different colony size and morphology. We were able to subculture the organisms and isolate some cultures for as glycerol stocks for future use.

References

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