

Identification of Activity Characteristics of Bacteria Isolated from the Continental Shelf in the Middle Atlantic Bight

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INTRODUCTION

The microbial life that makes up the ecosystem on the continental shelf in the Middle Atlantic Bight is poorly known. As a result, many microbial ecologists have rarely been able to formally draw the link between ecological processes and the bacteria. The lack of a connection may be in part because many methods focus on bacteria as a uniform group, rather than a community consisting of many different, individual organisms¹.

OBJECTIVES

- Isolate strains of bacteria present at the LEO-15 site on the continental shelf in the Middle Atlantic Bight
- Establish pure strains of bacteria
- Obtain a growth curve for each isolated strain of bacteria
- Measure extracellular enzyme activity rates for each strain

SAMPLING

Water samples were collected off the New Jersey coast at LEO-15, approximately 1.2 km southeast of Node B, in the Middle Atlantic Bight. Samples were captured using a boundary layer sampler, the Remote Access Sampler (RAS), on June 13th, 2005. The intake was located 5cm above the sediment bed and was programmed to collect the samples at various times over an approximately 24-hour time period. Water sample collection times were as follows beginning June 13th and ending June 14th: 4:00pm, 5:30pm, 7:00pm, 10:00pm, 1:00am, 4:00am, 7:00am, 8:30am, and 10:00am.



The location of LEO-15.



The Remote Access Sampler aboard ship.



The Remote Access Sampler resting on the sediment bed.

METHODS

- Each water sample was streaked onto nutrient rich-agar (1.5% agar and 5g peptone/L in filtered seawater) in petri dishes using the quadrant method of streaking
 - A duplicate was streaked for each sample in order to obtain the most abundant bacteria present per sample
- Bacterial strains were isolated based on color and morphology: White, Peach, Yellow, Red, and Rough
- Each isolated strain was then transferred to liquid media (1% peptone in filtered seawater)
- Each strain was again streaked onto agar in petri dishes using the quadrant method in order to ensure pure cultures
- Each strain was verified to be a pure culture and was then placed into liquid media as a stock of each strain
- A timed series of each strain was measured for growth using a Spectrophotometer at A(660nm) in order to obtain an approximate growth curve for each sample
 - Sacrificial sampling was used in order to avoid contamination of the pure cultures
- Each strain was then freshly inoculated and the growth was followed
 - Measurements and samples were taken for A(660nm), Protein Concentration, and Bacterial Activity
 - BCS Protein Assay developed by Pierce Chemical Company, Technical Note 23225 performed
 - Extracellular Enzyme (Aminopeptidase) Activity was measured using the method of Mayer²



Isolation of colonies.

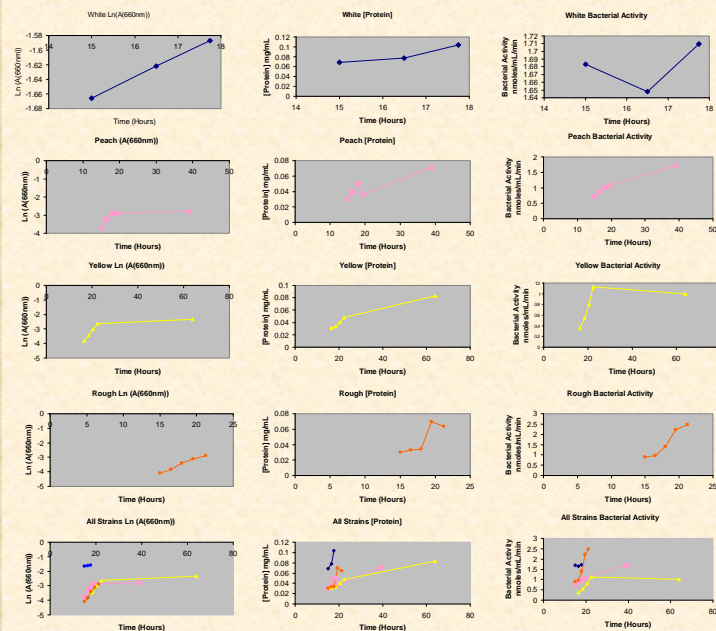


Inoculation of the T-series.



One of many Protein Assays performed.

RESULTS



Graphical representation of measurements of (left to right): Ln (A(660nm)), Protein Concentration, and Bacterial Activity for (top to bottom) White, Peach, Yellow, Rough, and all the strains, versus time.

DISCUSSION

There were notable differences in growth rates of the strains. The growth curve of the white strain, White Ln(A(660nm)), has readings much higher than compared to the other strains. It is believed that the White strain had already grown up and the measurements that were taken were late in its growth cycle when compared to the other strains. The Peach, Yellow, and Rough strains all showed relatively similar growth early in their cycles. The Yellow and Rough strains were notably similar when comparing the growth curves. The Red strain's growth was the slowest of all and could not be accurately measured. Thus, it was taken out of consideration for comparison.

The White strain again was different when compared to the rest of the strains in protein concentration. The White strain showed a high protein concentration whereas both the Peach and Rough strains had low concentrations that increased and then decreased. The Peach continued to again increase after the decrease in protein concentration but contrastingly, the Yellow strain began low with a slow but steady increase in protein concentration.

The Bacterial Activity of all four strains was very variable. The White strain, which was most likely late in its growth cycle, showed high activity initially with little variance. Both the Peach and Yellow demonstrated a steady increase in activity but the Yellow decreased slightly for the last time point, whereas the Peach increased greatly. The Rough strain was observed to increase in its activity significantly throughout all of the time points.

CONCLUSION

When taking into account all of the variations seen, it can be concluded that the bacterial strains that I was able to isolate do vary in their response to the fluorochrome, L-leucine 7-amido-4-methylcoumarin, used to measure their activity, depending on their stage of growth. The White strain showed increasing bacterial activity per protein concentration, even late in its cell growth. This was different from the Yellow strain, which showed a general decrease in bacterial activity per protein concentration early in its growth. Both the Peach and Rough strains had a general trend of increasing, decreasing, and then again increasing bacterial activity per protein concentration along their growth cycles. The goal from this point is to determine the conditions that are optimal for each strain which can be considered representative of conditions seen at LEO-15.

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