2006

Contribution of Sediment to High Enterococcus Counts Along the Northern Gulf of Mexico

Jason Townsend  
University of Southern Mississippi

Jennifer A. Ufnar  
University of Southern Mississippi

David F. Ufnar  
University of Southern Mississippi

Shiao Y. Wang  
University of Southern Mississippi

R.D. Ellender  
University of Southern Mississippi

Follow this and additional works at: https://aquila.usm.edu/mst_presentations

Recommended Citation
https://aquila.usm.edu/mst_presentations/6

This Poster is brought to you for free and open access by the Microbial Source Tracking at The Aquila Digital Community. It has been accepted for inclusion in Presentations by an authorized administrator of The Aquila Digital Community. For more information, please contact Joshua.Cromwell@usm.edu.
Contribution of Sediment to High Enterococcus Counts Along the Northern Gulf of Mexico

Jason Townsend, Jennifer A. Ufnar, David F. Ufnar, Shiao Y. Wang, R.D. Ellender
University of Southern Mississippi, Hattiesburg, MS 39406

Abstract
Enumeration of enterococci (EN) bacteria in water is an USEPA approved indicator of fecal pollution and the possible presence of enteric pathogens. Along the northern Gulf of Mexico, the water is shallow with a high organic and particulate load because of the Mississippi River discharge. Disturbance of coastal sediments during windwave action caused either by the weather or human activities may increase bacterial counts as a result of increased EN particle concentrations on the water column and/or resuspension of EN in the sediment. The goals of this project are to determine the relationship between organic content and EN counts in the water and whether bacterial resuspension from the sediment contributes to elevated EN counts in the water. EN counts in the water were correlated with wave conditions at seven sites along the Mississippi Gulf Coast. During calm wave conditions, low bacterial levels (1.0 – 227 CFU/100mL) were observed in the water with higher counts in the sediment; the reverse was observed (10 – 351 CFU/100mL) during rough wave conditions. EN counts were positively correlated with organic content of the sediment. Wave activity to keep EN in suspension was apparently critical for high counts. EN counts decreased by 50% in 4 hrs from 38 to 17 CFU/100mL, in the absence of resuspension and decreased to 1 CFU/100mL after 48 hrs. EN in the sediment are not stationary as genetical fingerprinting using REP-PCR showed low persistence of specific isolates over time. Jackknife analysis revealed low similarity among EN isolates from the water and sediment collected on the same day and site during calm wave conditions. This shows that EN are not persisting for long periods in the same area but instead are resuspended and redistributed along the coast. Results from this study provide evidence that high organic content and resuspension of isolates from the sediment during periods of strong wave action contribute to high EN counts. Current research on the survival of EN in eutrophic habitats will provide insight on the balance between environmental persistence and fecal pollution in causing high EN counts along beaches in the northern Gulf of Mexico.

Introduction
Enterococci are defined as Gram (+), coccaceae, non-spor-forming facultative cocci able to grow at 41°C. Regulatory agencies use enterococci counts as the standard for monitoring fecal pollution levels in coastal recreational water. Ocean sediments may serve as a reservoir for enterococci and other indicator bacteria; environmental scientists often suggest these microorganisms have a better chance of survival in sediment than in the overlying water. Furthermore, human pathogens and fecal indicators are thought to persist in the environment by attaching to biofilm, clay or algae, and by entering into the visible but non-cultivable state. Previous work has shown that the enterococci are found in both the water and the fluffy sediment and researchers have examined the significance of sediments, marsh, and seaweed as sources of enterococci in water. Bacteria react to particles in the water column, become part of the sediment surface, and can enter the water column in the attached state. Enterococci in/on sediment are protected from UV inactivation, bacteriophage and toxins and sediments may also provide nutrients that originate from algae, plankton, and other organic debris. Enterococci abundances in long term sediments may be due to desiccation. This occurs when sediments are exposed to fecal contamination from high tides or storm flow events. After the water level drops, the exposed sediment dries and the bacteria become desiccated. Enterococci are known to survive for weeks in the desiccated state in both salt and fresh water and growth can occur once the sediment is rewetted. The organic content in the sediment may also play a protective role in bacterial counts in sediment. Sediment with elevated levels of organic matter generally have a higher bacterial level than those with low organic content.

The predominant enterococcal species found in the sediment and in water are Enterococcus faecalis, Enterococcus faecium, Enterococcus hirae, Enterococcus casseliflavus, and Enterococcus mundtii. Enterococci may be more abundant in marine sediment than other indicator organisms, including E. coli, and they may able to grow at a wider range of temperatures (10 – 45°C), pH (6.8 – 9.6), are more resistant to saline, and are not easily inactivated by sunlight.

Objectives of this study
To determine if the levels of organic material in the sediment correlate with the levels of enterococci in sediment.
To determine how long sediment enterococci stay suspended in the water column after it is disturbed.
To determine if enterococci found in the water column produce fingerprints that positively correlate with beach sediment isolate fingerprints.

Materials and Methods
Mississippi Department of Environmental Quality sampling sites 7A, 8, 9, 10, 11 and 12A were tested during this study (Figure 1). Water (20, 40, and 80mL) and sediment (15, 25, 50mL) samples were filtered through a 0.45um, 47mm glass containing mEL agar (BD Bioscience, Sparks, MD.) and incubated at 41°C for 24 hours. Counts of sediment from an individual station were mixed using a sterile spatula. 10 grams of sediment was blended with 200mL of phosphate buffer saline, and the mixture was vortexed vigorously for two minutes followed by settling for 1 minute. Sediment supernatant was filtered through a 0.45um, 47mm nitrocellulose membrane, placed on mEL agar, and incubated at 41°C for 24 hours. Colonies with raised blue halos (0.5mm) were considered enterococci. Isolates were picked into 3mL of brain heart infusion broth (BHIB) and incubated for 24 hours at 37°C.

Enterococcus Suspension: Sediment containing enterococci was suspended as a slurry in 5L of sterile coastal water and sampled immediately for enterococcal counts. The water was sampled at 1, 2, 4, 8, 24, and 48 hours.

Organic Matter: 15g wet sediment sample was added to 120mL of sterile water, shaken vigorously for two minutes, and the supernatant filtered through a 4.25cm, 934AH grade, glass fiber filter (Reeve Angel, Chelmsford, New Jersey, USA). 100mL of water was added two additional times and filtered to collect the remainder of the organic matter. After filtering, sediment was dried, weighed, and transferred to a 450°C muffle furnace for 5 hrs. The ash weight was measured and recorded. The weight of organic matter was calculated as: Dry weight – Ash weight = Weight of Organic matter

Enterococci's ability to survive for long periods in sediment may be due to desiccation. The predominant enterococcal species found in the sediment and in water are Enterococcus faecalis, Enterococcus faecium, Enterococcus hirae, Enterococcus casseliflavus, Enterococcus mundtii. Enterococci may be more abundant in marine sediment than other indicator organisms, including E. coli, and they maybe able to grow at a wider range of temperature (10 - 45°C), pH (4.8-9.6), are more resilient in seawater, and are not easily inactivated by sunlight.

Results & Discussion
Wave action along the Mississippi coast readily suspends the upper, fluffy sediment layer releasing enterococci (EN) into the water column. During calm wave conditions, we observed low bacterial levels (1.0 – 227 CFU/100mL) in the water, and higher counts were observed in the sediment; 10 – 351 CFU/100mL, during rough wave conditions. Rough wave conditions (>12" wave height) cause continuous resuspension of the sediment. Mississippi coasts experiences this when sediments are exposed to fecal contamination from high tides or storm flow events. After the water level drops, the exposed sediment dries and the bacteria become desiccated. Enterococci are known to survive for weeks in the desiccated state in both salt and fresh water and growth can occur once the sediment is rewetted. The organic content in the sediment may also play a protective role in bacterial counts in sediment. Sediment with elevated levels of organic matter generally have a higher bacterial level than those with low organic content.

Table 1. Comparison of enterococcal counts and organic matter
<table>
<thead>
<tr>
<th>Station</th>
<th>Organic Matter (g)</th>
<th>#EN/100mL</th>
<th>Water</th>
<th>Sediment</th>
</tr>
</thead>
<tbody>
<tr>
<td>5/31/2005</td>
<td>0.0136</td>
<td>61</td>
<td>0.0019</td>
<td></td>
</tr>
<tr>
<td>6/13/2005</td>
<td>0.0019</td>
<td>71</td>
<td>0.0009</td>
<td></td>
</tr>
<tr>
<td>6/15/2005</td>
<td>0.0009</td>
<td>71</td>
<td>0.0009</td>
<td></td>
</tr>
<tr>
<td>7/12/2005</td>
<td>0.0019</td>
<td>71</td>
<td>0.0009</td>
<td></td>
</tr>
</tbody>
</table>

Objectives of this study
To determine if the levels of organic material in the sediment correlate with the levels of enterococci in sediment.
To determine how long sediment enterococci stay suspended in the water column after it is disturbed.

Table 2: Enterococci counts following suspension of sediment in marine water
<table>
<thead>
<tr>
<th>Time (Hours)</th>
<th>CFU/100mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 hr</td>
<td>24 hrs</td>
</tr>
<tr>
<td>4 hrs</td>
<td>8 hrs</td>
</tr>
</tbody>
</table>

Table 3: Jackknife analysis of sediment and water enterococcal fingerprints

<table>
<thead>
<tr>
<th>Water isolates to Sediment isolates</th>
<th>Water</th>
<th>Sediment</th>
</tr>
</thead>
<tbody>
<tr>
<td>5/31/2005 (Calm)</td>
<td>71.1</td>
<td>46.4</td>
</tr>
<tr>
<td>7/13/2005 (Rough)</td>
<td>35.4</td>
<td>47.2</td>
</tr>
</tbody>
</table>

Conclusions
• Enterococci attached to upper layer sediments are readily resuspended in the water column.
• Higher levels of organic matter appear to correlate with higher enterococci counts.
• Sediment-associated enterococci may persist in the water column for 48 hours after settling.
• Jackknife analysis demonstrates differences in sediment and water isolates.
• During rough wave conditions, sediment and water enterococci isolates show greater similarity than during calm conditions.

Acknowledgements
This project was funded by the Environmental Protection Agency, Gulf of Mexico Program Grants M98403H04-0 and M00429585.

For More Information Contact:
Dr. R.D. Ellender
University of Southern Mississippi
Department of Biological Sciences
118 College Drive #5018
Hattiesburg, MS 39406-0001
601-266-4720 or 601-266-4752

Figure 1.