Introduction

In the Mississippi Sound, water quality is routinely tested by the enumeration of the indicator bacteria. Coastal beach waters are monitored using Enterococcus (EN) species and fecal coliforms are used to test for fecal pollution in fresh water creeks and salt marshes (EPA, 2002; EPA, 2004; USEPA 2005). Enterococci and fecal coliforms are associated with a wide variety of fecal inputs from humans and animals, but if other information is available, the source(s) of pollution that can degrade water quality in coastal areas. This failure impedes the ability of regulatory agencies and managers to protect public health and remediate sources of pollution.

Of the MST methods available, those that focus on the detection of a single group or gene of host specific genes have emerged as credible measures for reasons of specificity, sensitivity economy, speed and transferability. Bacteroides (Bernhard and Field 2000a; Dick et al., 2004) was the first bacterial human marker to be used in source tracking applications. Additional microbial human markers have been developed including Bacteroides thetaiotamicron (Gottschalk et al., 2000; McQuaig et al., 2006), Methanobrevibacter smithii (Ufnar et al., 2006a; Ufnar et al., 2006b) and Faecalibacterium prausnitzii (Carson et al., 2005). Template specificity, sensitivity economy, speed and transferability are at play to create this disparity. Unquestionably differences do exist between the creek and beach environment including such variables as fresh vs. salt water, the levels of ultraviolet light, the dilution effect as creek water enters the estuary, and water transport at beach sites, as well as differences in turbidity and sediments. All of these factors may account for the lack of correlations observed.

Similarly, there was no relationship detected between EN and the presence or absence of MS or BA marker in either the creek or coastal samples. This is not unexpected since one human marker is often not enough to establish the presence of human material. Conversely, the cross-stabilisation frequencies presented a different view of the results: when MS was absent in a sample, BA was also absent 73% of the time. When MS was present in a sample, BA was present 68% of the time. This seems to imply that each marker is testing for the same parameter, i.e. fecal pollution of water.

Materials and Methods

Sampling Sites: Six Mississippi Department of Environmental Quality (MDEQ) coastal beach sites were chosen for analysis and processed with three replicates on the frequency of beach closure events. They included one site with a moderate number of beach closures (1-2 yr; 40, 96, 99, 01), two sites with an analysis of the effects of beach closures (3-5 yr; 89, 90, 96, 99), one site with high numbers of beach closures (3-5 yr; 94, 95, 96, 97), one site with 100-1000, one site with 1000-10000, and one site with 10000-100000. Six tidal creek sites that flow into the Mississippi Sound were sampled. A total of 16 sites were monitored in creek samples as well as the coastal site. Tidal creek inflows were significantly different from those sites with no associated beach.

Eighty-nine percent were collected from Mississippi coastal stations represented here are the dominant beach recreational sites along the Mississippi coast.

Abstract

The objective of this study was to determine whether statistically valid correlations could be shown between enterococcal counts of water samples from creek and coastal sites and the presence of two molecular, library-independent markers that specify human and animal fecal pollution. In addition, another sampling trip was conducted between August 2007 and April 2009. A data subset of samples collected between September 08 and April 09 were analyzed by traditional gel electrophoresis and microchip capillary electrophoresis.

Enterococci Isolation / Characterization: Enterococci were cultured and enumerated following the United States Environmental Protection Agency (USEPA) Standard Method 1600 (Messer and Dufour, 1998; USEPA, 2005). Briefly, water samples were diluted (10^-10^-6), filtered through a 0.45 µm cellufil cellulosic membrane (Pall Corporation, Ann Arbor, MI), placed on 50 mm diameter dishes containing Euglena (BD Bioscience, Sparks, MD), and incubated at 4°C for 24 hours. Colonies (0.5 mm in diameter) characterized by blue halos on m17e were designated enterococci. Counts were expressed as CFU/100 ml.

DNA Extraction and PCR Analysis: Sample volumes (500 ml) were filtered through 3 µm and 0.45 µm cellulose acetate membranes (Pall Corporation, Ann Arbor, MI) and the 0.45 µm filtered extract was used for template DNA extraction using the Mini prep kit (Bio 101, Inc., Rancho Bernardo, CA). DNA concentrations were measured in ng/ml using a NanoDrop ND-1000 spectrophotometer (Nanodrop Technologies, Wilmington, DE) and frozen (-20°C) pending analysis, generally in 2 weeks of collection. Total DNA from each water sample was amplified using a specific set of primers designed for enterococcal (Mnif142f and Mnif142r) species and fecal coliforms are used to test for fecal pollution in fresh water creeks and salt marshes (EPA, 2002; EPA, 2004; USEPA 2005). Enterococci and fecal coliforms are associated with a wide variety of fecal inputs from humans and animals, but if other information is available, the source(s) of pollution that can degrade water quality in coastal areas. This failure impedes the ability of regulatory agencies and managers to protect public health and remediate sources of pollution.

Discussion

Enterococci measurements are the standard measure of human risk from contact with enteric pathogens in coastal waters. However, recent studies have indicated that there are factors that mitigate the value of these analyses. For example, enterococci are known to exist in many animal species, and to reproduce in the coastal environment (Sigterson et al., 2004).

Furthermore, sediments and beach sand have been shown to harbor enterococci and allow them to persist in the environment (Scott et al., 2002; Hartz et al., 2008).

For this study, enterococci counts at 12 coastal sampling sites were not positively correlated, nor were the presence or absence of the human markers correlated with the concentration of this indicator bacterium. Unquestionably, creek waters contain substantial enterococcal levels and frequently show the presence of the human markers; however, these measurements did not statistically translate into associated beach water counts of enterococci or the presence of the human markers. During the same period from August 2008 to April 2009, there were 131 enterococci exceedances (Mississippi sites) at a single sample count of ≥104/100 ml to designate a polluted beach) associated with the six coastal sites tested. Forty eight exceedances occurred at station 10, followed by 26 at site 10A, 22 at site 9, 17 at site 11, 13 at site 12A and 5 at site 7A. The same data imply that a statistical correlation should occur at site 10 which is influenced by fishing, sampling sites 11 and 12. On the other hand, the sampling times were not significantly different from one another. Therefore, the impact of human markers may be a function of the number of samples tested. The lack of correlation between enterococcal counts and the presence of human specific fecal markers in Mississippi coastal waters. However, recent studies have indicated that there are factors that mitigate the value of these analyses. For example, enterococci are known to exist in many animal species, and to reproduce in the coastal environment (Sigterson et al., 2004).

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