Report for 2003NY25B: Combining an Optical Strip-Assay Biosensor with Ribotyping for Bacterial Source Tracking of Enterococcus faecalis in the Lower Hudson River Basin

- Other Publications:
    - Reilly, J.P., J.E. Thies, A. Baeumner, 2003, Development of an optical strip-assay biosensor for Enterococcus faecalis, American Society for Microbiology Annual Meeting, Washington, DC.
    - Reilly, J P and J E Thies, 2003, Emerging Water Contaminants: Preliminary findings from Ribotyping of Enterococci Isolated from Wappinger and Stony Creek Catchments. Invited talk at the Dutchess Environment Council meeting.
    - Hartel, P.G., S. Myoda, R.L. Kuntz, K. Rodgers, J. Entry, S. Ver Wey, E. Schroder, C.I. Castro, J.E. Thies, J.P Reilly and J.J. Fuhrmann, 2003, Geographic and temporal variability of Enterococcus faecalis ribotypes for bacterial source tracking, American Society for Microbiology Anual Meeting, Washington, DC.
- Articles in Refereed Scientific Journals:
    - Reilly, J.P., A.J. Baeumner and J.E. Thies, 2005, Detection of synthetic Enterococcus faecalis gene sequence by a strip-based optical biosensor, Journal of Environmental Quality, In press.
    - Hartel, P.G., S. Myoda, R.L. Kuntz, K. Rodgers, J. Entry, S. Ver Wey, E. Schroder, C.I. Castro, J.E. Thies, J.P. Reilly, and J.J. Fuhrmann, 2005, Geographic and temporal variability of Enterococcus faecalis ribotypes for facterial source tracking, Journal of Environmental Quality, In Press.
- Dissertations:
    - Reilly, Johm, 2005, Combining an optical strip-assay biosensor with ribotyping for microbial source tracking of Enterococcus faecalis in the lower Hudson River Basin, MS Thesis, Department of Crop and Soil Sciences, CALS, Cornell University, Ithaca, NY.

Report Follows

Title: Tracking of *Enterococcus faecalis* in the Lower Hudson River Basin

Overview

Determining the presence/absence of fecal bacteria and their sources continues to be important for water quality managers monitoring bacterial contamination of public water bodies. *Enterococcus faecalis* is a fecal bacterium used to indicate the potential presence of more pathogenic fecal bacteria in contaminated waterways. This bacterium is currently being tested as a means to distinguish contamination arising from humans and birds from that derived from other animals. Two complementary molecular methods for (i) bacterial detection and (ii) source tracking were developed and used in this project. An optical biosensor was developed to detect *Enterococcus faecalis* and ribotyping of isolates was used to fingerprint these bacteria and track their likely source.

Biosensor

The first project aim was to develop an inexpensive, portable, and accurate biosensor that could be used to confirm the presence of *Ent. faecalis* in impaired waterways. The biosensor developed in this project employs sulforhodamine B (SRB) dye-encapsulated liposome technology to generate a positive signal when the target 16S rRNA gene is present.

Experiments were conducted with synthetic sequences and subsequently with 16S rRNA gene sequences from environmental isolates. Two different target analytes were hybridized with liposomes that had single-stranded DNA oligonucleotides (reporter probes) attached to them. The lowest level of detection of 16S rRNA synthetic gene sequences for Analytes 1 and 2 was 14 fmol μL^{-1} and 19 fmol μL^{-1}, respectively. A dynamic range up to 5000 fmol μL^{-1} for both synthetic sequences was obtained. A genus-level primer pair was then designed to amplify a sequence of the 16S rRNA gene of *Enterococcus* spp. and used to test the ability of the biosensor to detect the target sequence in samples mimicking those from the environment.

To date, the biosensor has yet to detect successfully the target 16S rRNA gene sequence amplified from environmental isolates. Trouble-shooting involved testing for and ensuring a sufficient concentration of ssDNA for binding to the capture probe; testing for failure of the reporter probe to successfully bind to the liposome surface; and examining the sequence of the 400 bp amplicon from the environmental isolates for potential hairpins or steric hindrance that might preclude binding of the amplicon to the capture probe.

Perhaps the greatest obstacle to overcome for the practical, field-based application of any strip-based biosensor is the large amount of water that may need to be filtered to obtain sufficient quantities of DNA to detect a signal for the *Ent. faecalis* 16S rRNA gene. Despite protracted efforts to detect the target sequence from environmental samples, this is yet to be accomplished. However, the excellent success of the biosensor to detect synthetic sequences provides 'proof of concept' for the biosensor design. Once problems with detection of amplified *Ent. faecalis* isolate sequences are resolved, the biosensor should provide a powerful new tool for water quality managers to quickly and accurately detect and report water quality impairment in water bodies of interest.

Ribotyping

Ribotyping is a DNA fingerprinting method that is used to compare the genetic similarity between samples of varying origin. Ribotyping of *Ent. faecalis* was employed to determine the similarity between 16S rRNA gene restriction fragment length polymorphism (RFLP) patterns derived from isolates obtained from two wastewater treatment facilities (Tivoli, NY and Wappingers Falls, NY) and those obtained from upstream and downstream grab samples.

Ribotype patterns obtained from *Ent. faecalis* isolates from the two NY sites clustered separately from each other indicating distinct geographic separation. However, within sites, there was evidence of the presence of resident ribotypes within each sampling time, but not between sampling times. These results confirmed the need for intense targeted sampling to track the potential source(s) of fecal contamination at the two NY sites. On the first 3 of 4 sampling dates, *Ent. faecalis* ribotype patterns from the Tivoli wastewater treatment plant matched (>90% similarity) patterns generated from downstream isolates sampled at the mouth of the tributary to the Hudson River. This suggests that the plant may be a point-source of contamination in this tributary. For the Wappingers Falls site, at least one ribotype was observed downstream of the wastewater treatment plant in June, 2003, that matched (>90% similarity) to a ribotype from the plant, however, a similar ribotype pattern was also obtained from the upstream samples. Hence, the wastewater treatment plant was not the only source of human fecal contamination in this tributary.

Future efforts should be directed toward assuring that wastewater treatment plants in the region are designed to manage their current loads. When combined with intense targeted sampling, ribotyping proved to be an effective technique for detecting human fecal contamination in this watershed context.

Study Impact Summary

A prototype, species-specific biosensor that detects synthetic sequences of *Enterococcus faecalis* has been developed, providing proof of concept. Further development of this new technique should lead to production of a valuable tool for water managers that could be used to detect this indicator species in the environment. Isolating and ribotyping *Ent. faecalis* indicated the wastewater treatment plants were one, but not the only, source of these indicator bacteria in the catchments studied. This study confirms that ribotyping must be combined with a targeted sampling protocol in order to more specifically identify persistent and ephemeral sources of water contamination. This approach is a cost-effective alternative for the massive databases required by most other bacterial source tracking approaches.