N.H. Sea Grant Research Project Progress Report

**Today's date:** May 29, 2009

**Project number:** R/CE-137

**Project title:** Microbial Interactions Influencing the Emergence of Pathogenic Vibrios in Oysters

**Project initiation date:** 2/1/2008

**Principal investigator:** Whistler, CA; Cooper VC

**Affiliation:** University of New Hampshire

**Technicians, industry partners, collaborators, etc. (specify which) and affiliations:** Technician Randi Desy; University of New Hampshire

**Brief project overview/Abstract:**
Within the last two decades the number of gastroenteritis outbreaks due to Vibrio parahaemolyticus and Vibrio vulnificus has risen steeply worldwide. Consumption of raw or undercooked shellfish represents a common mechanism for infection by these vibrios. Due in part to changing patterns in human land and resource use, and variability in seasonal extremes that may represent larger changes in climate, these emergent pathogens are a public health concern in north temperate regions not previously at risk (McLaughlin et al., 2005; Fitzpatrick, 2002; DePaola et al., 2000; Abbott et al., 1989). Public health and shellfish program managers are now challenged to better understand ecological dynamics that drive shellfish-borne Vibrio illnesses.

One major question is whether changing estuarine conditions simply affect total abundance of all bacteria, including potential Vibrio pathogens, or whether certain physical or biological conditions actually may selectively amplify minority pathogenic strains. Because pathogenic biovars are typically rare and are usually not differentiated from total bacterial counts, we require a more nuanced perspective of factors influencing estuarine microbial ecology, including the role and relevance of other microbes residing in shellfish. Furthermore, the ability of bacteria to exchange genes with neighboring populations highlights the potential for spread of virulence determinants among non-pathogens. Thus, this proposal focuses on how physical factors, such as temperature, salinity, and dissolved nutrients, affect vibrios within the context of other resident microbes that may favor or antagonize potential pathogens. This proposal also will delineate in detail the genetic relationships between pathogenic and nonpathogenic Vibrio isolates and quantify the extent of their past and future potential genetic exchange. Both of these objectives will aid development of sensitive and accurate typing methods for pathogenic Vibrios in susceptible ecosystems. More generally, by examining the interplay of physical conditions and microbial community interactions on the emergence of pathogenic biovars, we will be better equipped to predict broader infectious threats and protect human health.
Objectives:
1. Map the population genetic structure and virulence potential of V. vulnificus and V. parahaemolyticus and determine the extent of recombination between strains using multi-locus sequence typing (MLST).
2. Determine the correlated effects of the resident microbial community on the abundance and distribution of Vibrio populations in oysters.

Research findings/accomplishments/progress to date:
Objective 1: Map the population structure of Vibrio parahaemolyticus and Vibrio vulnificus. From our first two seasons of sampling (May-Dec 2007 and May-Dec 2008) we have recovered 130 isolates of V. parahaemolyticus, 14 isolates of V. vulnificus, and 12 isolates of Vibrio cholerae. As expected, no pathogenic biovar genes were detected via multiplex PCR and molecular typing, indicating these are environmental and not clinical type strains. However, we have determined that many of the isolated strains are as cytotoxic to human colon cells as clinical isolates indicating they do harbor pathogenic characteristics.

Preliminary analysis of the collection indicates a direct and significant correlation of total population with temperature, and the seasonality in distribution that follows a temperature gradient is consistent with historical data. The low level of V. vulnificus detection is unusual compared to historical data, and may indicate a shift in population structure, perhaps due to an increase in salinity in the estuary, although the relationship was not significant. We have notably and unequivocally confirmed the identity my molecular typing of the 12 isolates of V. cholerae, the first on record as such for the GBE. None of the strains harbor any of the known virulence factors for cholera. These strains were collected following a rainfall event that lead to a decrease in salinity and an increase in organic matter, not unlike is observed in regions hit with monsoons where cholerae outbreaks are problematic. We will continue characterization of these strains by MLST to establish a baseline for the endemic population.

Due to the low level of V. vulnificus recovery as compared to past years, we have focused our MLST analysis on V. parahaemolyticus. We have completed locus sequencing for 9 gene loci on 108 of the 130 isolates, and performed preliminary MLST analysis with this data set. The population structure is hyper-dispersed with no clonality detected, although within the collection there were two complexes of related strains detected. However, the hyperdispersion is likely an artifact of our collection and processing plan which was devised to avoid collection of clones from the same sample. For our final season (May-Dec. 2009) we have revised our collection scheme to allow inclusion of additional strains from the same sample which could represent clones from within a population, but not resulting from enrichment following post-harvest processing. This final season we anticipate completion of our collection as planned, although we expect fewer total isolates due to the poor recovery of V. vulnificus.

Objective 2: Determine the correlated affects of the resident microbial population on abundance and distribution of Vibrios. We have only begun our sample processing for this objective. For this analysis, we have chosen to compare two unique sites within the estuary: a site that has historically had higher Vibrio loads (Oyster River) and a site that has had lower Vibrio loads (Nanni Island). These two sites are highly similar in temperature and salinity but differ in dissolved oxygen and an indicator of organic contamination at Oyster River. From each of these two sites we collected 11 oyster samples processed individually, along with overlying water in August 2008. Preliminary
MPN data indicated that none of the oysters contained Vibrios, therefore we have turned to a more sophisticated and sensitive method for detection. Individual samples are currently being analyzed by the highly sensitive method of Q-RT-PCR to separate them into oysters with low Vibrio CFUs versus oysters with high Vibrio CFUs from each site, allowing correlation of endemic population with Vibrio loads. We will collect a second sample from each site in the same manner at the same time this year (August) so that our library will contain two replicate experiments.

**Impacts to date:**
We have identified Vibrio cholerae from the GBE, and established a population structure map for Vibrio parahaemolyticus. We have also determined that Vibrio vulnificus populations are in decline compared to historical data. No pathogenic strains have been detected.

**Problems encountered:**
Our greatest problem is with the variability of detection by culture based methods including multiple tube fermentation MPN. For that reason, we have invested significant time in developing and optimizing protocols for non-culture detection of Vibrios by Q-RT-PCR. Until we have optimized this method, we cannot proceede with the metagenomic/16s library study. The delay in completing this work has led to an opportunity to develop the new method and also include a second season sample in our library. We also have encountered difficulties in balancing our goal of obtaining enough isolates with avoiding collection and sequencing of clones as a result of our collection and enrichment methods. However, we have adjusted our collection scheme for our final season to obtain multiple isolates from within a sample, allowing us to determine whether hyperdispersion is an artifact of our method or representative of the actual population.

**Publications to date (please attach PDF if applicable):**
None

**Presentations to date, with published abstract citation if applicable:**
American Society of Microbiology, 108th General Meeting, Boston MA, June 1-5, 2008

1. Evaluation of pathogenic potential of Vibrio parahaemolyticus found in New Hampshire’s Great Bay Estuary
   Jenny Mahoney, Nicole Lefebvre, Steve Jones, Cheryl Whistler

2. Identification of Vibrio species found in oysters and water from the Great Bay Estuary
   Megan Striplin, Jenny Mahoney, Steve Jones, Cheryl Whistler

**Students associated with project** (for graduate students, please provide full name, thesis title and degree being pursued; for undergraduates, please provide full name and major):
1. Megan Striplin (M.S. 2009); Thesis title: Distribution and population structure of Vibrio species in the Great Bay estuary of New Hampshire

2. Jenny Mahoney (Ph.D. 2011); Dissertation title: A comparative transcriptomics approach to understanding host-specific lifestyles in the genus Vibrio

3. Brian Schuster (M.S. 2010); Thesis title: Host-microbe and microbe-microbe interactions that influence niche specialization by Vibrios
4. Tucker Noyes, Microbiology (2011)
5. Anna Tyzick, Biology (2010)
7. Nicole Lefebvre, Biology (2008)