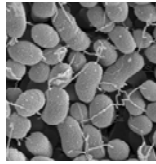


## The Colony Overlay Procedure for Peptidases: Applications for Monitoring Seafood Safety

Gary P. Richards, Ph.D.  
U.S. Department of Agriculture  
Agricultural Research Service  
Microbial Food Safety Research Unit  
Delaware State University  
Dover, Delaware



## Microbial Food Safety Research Unit



- Research Directions
  - Predictive Microbiology
  - Stress/Sensory Response
  - Interventions/Omics
  - Aquaculture Safety



## Microbial Food Safety Research Unit – Collaborators

- |   |  |
|---|--|
| <ul style="list-style-type: none"> <li>■ Hatfield Quality Meats</li> <li>■ University of Wisconsin</li> <li>■ University of Georgia</li> <li>■ University of Bologna</li> <li>■ Michigan State University</li> <li>■ Delaware State University</li> <li>■ Drexel University</li> <li>■ Rutgers University</li> <li>■ University of Delaware</li> <li>■ Purdue University</li> <li>■ Univ. of MD Eastern Shore</li> <li>■ Virginia Polytech. Inst.</li> <li>■ Teagasc - Ireland</li> </ul> | <ul style="list-style-type: none"> <li>■ J.L. Foods</li> <li>■ Pilgrim's Pride (Wampler)</li> <li>■ Institute of Food Research – UK</li> <li>■ FSIS, FDA, APHIS, CDC</li> <li>■ C.I.A.D. - Mexico</li> <li>■ American Meat Institute</li> <li>■ The Institute for Genomics Res.</li> <li>■ Qualicon/Dupont</li> <li>■ Kraft/Oscar Mayer</li> <li>■ University of Nebraska</li> <li>■ National Cattleman's Beef Assoc.</li> <li>■ Embrapa/Labex - Brazil</li> </ul> |
|---|--|



## Microbial Safety of Aquaculture Products Center of Excellence

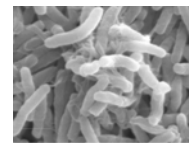
- Research on enteric virus assay methods
- Enzyme-based assays for vibrios
- Virus processing interventions (HPP)



Seafood Safety Research  
Microbial Food Safety Research Unit

## Vibrionaceae Family

- |  |   |
|--|---|
| <ul style="list-style-type: none"> <li>■ <i>Vibrio cholerae</i></li> <li>■ <i>V. parahaemolyticus</i></li> <li>■ <i>V. vulnificus</i></li> <li>■ <i>V. alginolyticus</i></li> <li>■ <i>V. anguillarum</i></li> <li>■ <i>V. fluvialis</i></li> <li>■ <i>V. harveyi</i></li> </ul> | <ul style="list-style-type: none"> <li>■ <i>Aeromonas</i></li> <li>■ <i>Plesiomonas</i></li> <li>■ <i>Photobacterium</i></li> </ul> |
|--|---|



## Shellfish Safety

- Since 1925, shellfish sanitation in the US has relied on coliform standards which have effectively eliminated outbreaks of typhoid fever
- US standards are based on sanitary surveys of water quality based on fecal coliform levels
- Testing for pathogenic vibrios is relatively recent and the assays are impractical for routine use



## Aquaculture is an Important Source of Fresh Fish and Shellfish



## Major Vibrionaceae Pathogens in Aquaculture

- *Vibrio anguillarum*, salt water
- *Aeromonas salmonicida*, salt water
- *Aeromonas hydrophila*, fresh and brackish water
- *Photobacterium damsela*, fresh and salt water

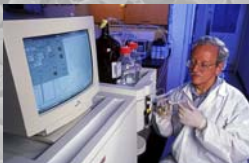


## Research Needs

Improved methods to monitor for vibrios in shellfish harvesting areas and in land-based aquaculture systems



## Vibrio Research

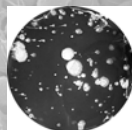


Isolated new enzyme in *Vibrio* family members

Characterized new enzyme



Developed enzyme-based assay for vibrios



New Assay Concept: Colony Overlay Procedure for Peptidases (COPP Assay)



It's so simple a Caveman could do it!



### Homogenize and Dilute



### Spread Plate on Tryptic Soy Agar



### Overlay Membrane onto Colonies on the Plate



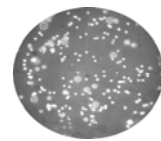
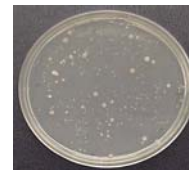
### Incubate 10 Minutes



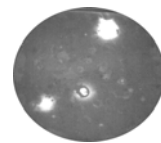
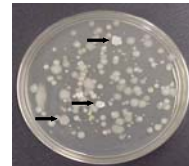
### View Membrane Under UV



Oyster

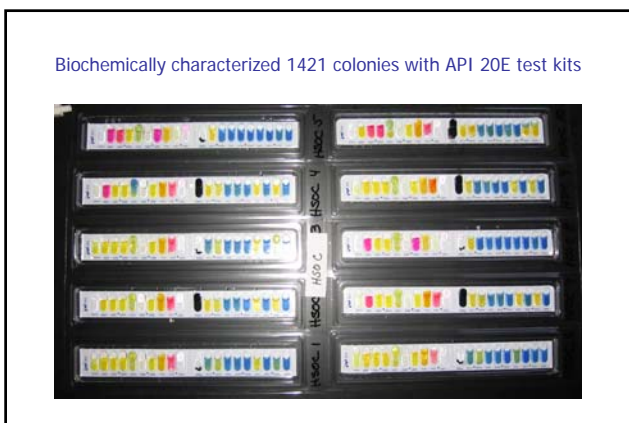
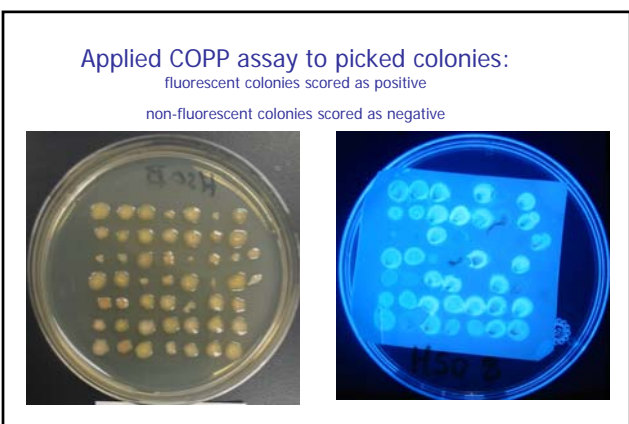
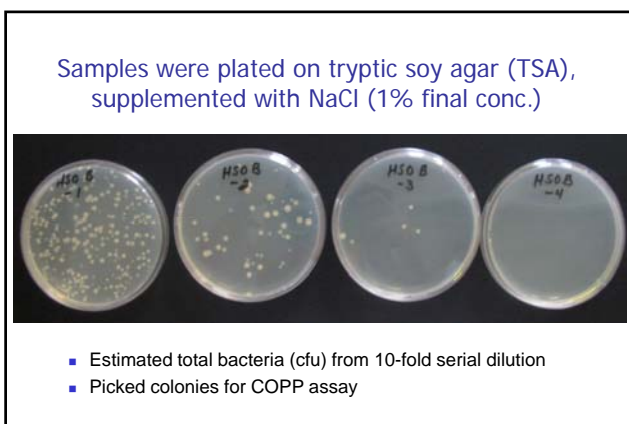
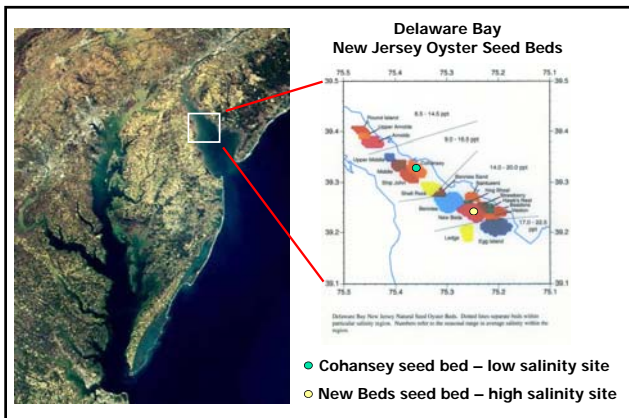


Soil



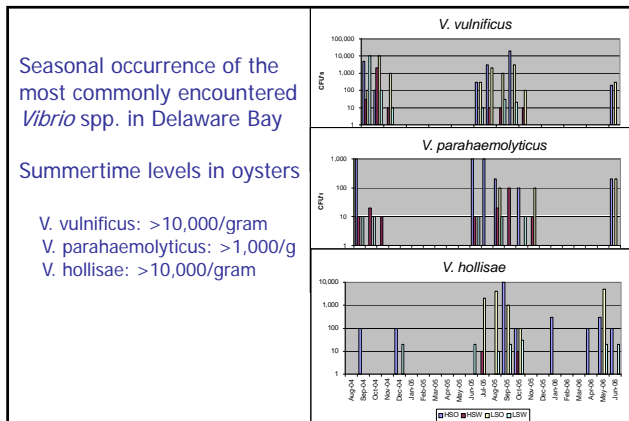
Bacterial Plates

Overlays




**Results - Overview**

- 276 samples collected and processed
- 6,416 colonies picked for COPP assay
  - 3,108 COPP+ (48.4%)
  - 3,084 COPP- (48.1%)
  - 224 COPP+/- (3.5%)
- 1,421 API's run (Aug 04 to June 06)
- Human and fish pathogenic species detected




### Colony Overlay Procedure for Peptidases

- Detected all *Vibrio* family members
- Rapid, simple, and quantitative
- Inexpensive
- Applicable for the detection of total vibrios in seawater, fish, and shellfish
- Based on the presence of an enzyme found only in *Vibrio* family members




### COPP Assay for Regulatory Use

- Determine normal, seasonal levels of total vibrios
- Monitor for spikes in levels
- Compare levels and spikes with conventional testing methods and with incidence of illness
- Identify correlations
- Determine if total *Vibrio* levels (COPP assay) may be used to regulate shellfish harvesting





### COPP assay in aquaculture and in monitoring shellfish safety

- Concept based on the premise that as total Vibrionaceae levels increase in seawater, so too will the pathogenic strains
- Similar to the way we currently regulate shellfish harvesting based on total levels of fecal coliforms
- Monitoring for total Vibrionaceae levels in market samples




### COPP Assay in a Recirculating Aquaculture System

- Determine normal levels of vibrios by COPP testing of water, fish, or shellfish
- Monitor for spikes in levels
- Compare levels with overall fish health during spike periods
- Determine if spikes serve as an index of disease
- Implement remedial actions when spikes occur

### COPP Assay for Market Surveys

- Evaluate fish and shellfish in the marketplace for total *Vibrio* levels using COPP assay
- Monitor for *V. parahaemolyticus* and *V. vulnificus* levels using cultural and PCR-based assays
- Correlate total *Vibrio* levels with specific pathogens
- Identify pros and cons of the COPP assay vs. molecular procedures





## Potential Collaborations

- Identify COPP-type assays for other enzymes/ foodborne pathogens
- Perform market surveys to validate use of COPP vs. molecular methods for vibrios
- Evaluate the use of COPP as an indicator of fish health/safety in aquaculture settings

