



- [GOAL Events](#)
- [Advocate Magazine](#)
- [Aquademia Podcast](#)
- [Blog](#)
- [Contact](#)
-
-
-
-
-
- [Log In](#)



- [About](#)
 - [Who We Are](#)
 - [Our History](#)
 - [Our Team](#)
 - [Sustainable Development Goals](#)
 - [Careers](#)
- [Membership](#)
 - [Overview](#)
 - [Our Members](#)
 - [Corporate Membership](#)
- [Resources](#)
- [Certification](#)
 - [Best Aquaculture Practices](#)
 - [Best Seafood Practices](#)

Search...



[Log In](#)

- [About](#)
 - [Who We Are](#)
 - [Our History](#)
 - [Our Team](#)
 - [Sustainable Development Goals](#)
 - [Careers](#)
- [Membership](#)
 - [Overview](#)
 - [Our Members](#)
 - [Corporate Membership](#)
- [Resources](#)
- [Certification](#)
 - [Best Aquaculture Practices](#)
 - [Best Seafood Practices](#)
- [GOAL Events](#)
- [Advocate Magazine](#)
- [Aquademia Podcast](#)
- [Blog](#)
- [Contact](#)



Health & Welfare
Health & Welfare

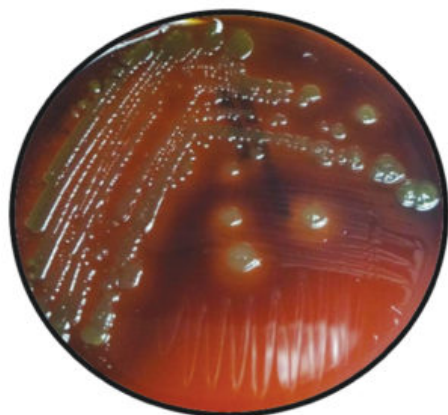
Field diagnostic tools for pathogenic vibrios



2 May 2014 Michael R. King, Ph.D. Sona Son Amy M. Lange Heather S. Behn Jim Bradley



Understanding various types of bacteria helps improve management practices



Alpha and beta hemolysis activity of *Vibrio harveyi* cultured on blood agar.

Pathogenic *Vibrio* bacteria are a major economic concern in the shrimp industry, causing global losses of approximately \$3 billion annually. *Vibrio vulnificus* and *V. parahaemolyticus* cause serious foodborne illness in humans, and other species are responsible for outbreaks of vibriosis in fish and shellfish.

Penaeid shrimp are particularly affected by luminous vibriosis caused by *V. harveyi* and *V. campbellii*. These *Vibrio* species, which are responsible for widespread outbreaks of disease with mortality of up to 100 percent, are the most important pathogens for penaeid shrimp production.

Field sample issues

As the United States represents less than 1 percent of overall aquaculture production, a global study of virulent *Vibrio* by U.S. researchers requires shipment of overseas samples. The ability to gain this big-picture view of the *Vibrio* community within shrimp production systems is often impeded by sample degradation or alteration during transport.

The authors sought to directly study *Vibrio* from producer hatchery and pond samples to determine *Vibrio*'s relationship to management practices, stressors and eventually viruses, and thus a solution for this shipment dilemma was required. Further, the authors wanted to differentiate between the pathogenic *Vibrios* and the non-pathogenic species commonly found in shrimp waters.

A common but inaccurate method used in *Vibrio* field diagnostics is to differentiate virulent from avirulent strains by plating on thiosulfate citrate bile salts sucrose (TCBS) agar, a widely used differential medium. There is often a misconception that TCBS colony color results of green or yellow are sufficient to determine the level of pathogenic *Vibrio* in the water samples. However, TCBS agar is not a differential medium for certain *Vibrio* species, including *V. harveyi* and others, which can be variable in their utilization of sucrose and can not be reliably distinguished from other sucrose-positive or sucrose-negative species.

FTA cards

Seeking solutions to overcome *Vibrio* field diagnostics obstacles, Whatman fast technology for analysis of nucleic acids (FTA) cards were evaluated as a simplified, shelf-stable collection method for the preservation of genetic material from water samples without storage concerns such as ultralow freezing or expedited transport.

With the FTA card process, cells are broken open on contact with the chemicals in the FTA card matrix, and nucleic acids bind to the fibers of the FTA matrix. When stored dry, this genetic material is safe from enzymatic degradation or hydrolysis.

DNA has successfully been amplified with polymerase chain reaction (PCR) up to 17 years after sample collection using the FTA card method. When used over shorter time frames of weeks or months, it could become a valuable tool for PCR analysis of field samples from remote locations.

It was felt that combining the use of this card with the PCR amplification of *Vibrio* pathogenesis genes could prove useful in the study of *Vibrio* pathogenesis "potential" in samples and in relating this potential to the management practices and stressors in the water environment.

Vibrio study

In the authors' study of the FTA card-sampling method, an existing PCR assay developed by Dr. S. Haldar at Osaka Prefecture University was applied to distinguish *Vibrio campbellii* and *Vibrio harveyi* species by targeting variable regions in the hemolysin gene (*hly*), a highly conserved virulence factor among the Vibrionaceae that encodes for a hemolytic toxin that attacks cell membranes, causing anion leakage, cellular lysis and cell death.

In the study, PCR targeting the *hly* gene was performed using several sources of *Vibrio* genetic material: DNA isolated from pure cultures of *V. campbellii* and *V. harveyi*, DNA bound to FTA cards spiked with *Vibrio* cells diluted in pond water, biofloc liquid or clean phosphate-buffered saline, amplified by including a punch of the FTA card in each reaction tube, or DNA eluted from the same FTA cards and repurified using nucleic acid extraction columns.

These PCR tests consistently allowed for the detection of virulence genes from as few as 100 *Vibrio* cells per PCR reaction and in all but the most heavily contaminated biofloc samples. The sensitivity of the species-specific PCR assay used remained about 100 cells/PCR reaction, no matter whether high-quality template DNA from pure liquid cultures from nucleic acid purification or FTA-bound DNA from pure culture, shrimp pond water or buffer-diluted samples was used.

However, the presence of PCR inhibitors in shrimp biofloc liquid samples reduced detection 100-fold compared to detection in pure cultures. The effect of the PCR inhibitors was removed by repurification of *Vibrio* DNA from FTA cards with the nucleic acid extraction method, allowing virulence gene detection consistently comparable to pure culture after processing.

Using the nucleic acid purification step, virulence gene detection sensitivity increased about 10-fold compared to PCR reactions with the direct FTA card method. Little to no difference was observed between the PCR amplifications of freshly inoculated FTA cards and FTA cards exposed to overseas transport simulation involving different temperatures and incubation periods. Take home message: It works!

Expression of toxin genes

Initially working only with DNA from the microbial community, the authors then sought to question if the presence of a toxin gene from *Vibrio* necessarily indicates pathogenic potential. What if the gene is never expressed? Additionally, with many genes having multiple copy numbers in the genome, how quantitative is the approach to determine pathogenic potential?

Current work involves studying the RNA from these same cards, and preliminary results suggest this is very useful. In fact, early work studying the stressors that upregulate the expression of these *Vibrio* toxins is proving quite interesting, but needs to be studied more thoroughly. The authors are also developing quantitative PCR testing to make this approach more robust, more sensitive and less labor intensive. Additionally, their work is seeking to expand the number of virulent species and virulence genes of study.

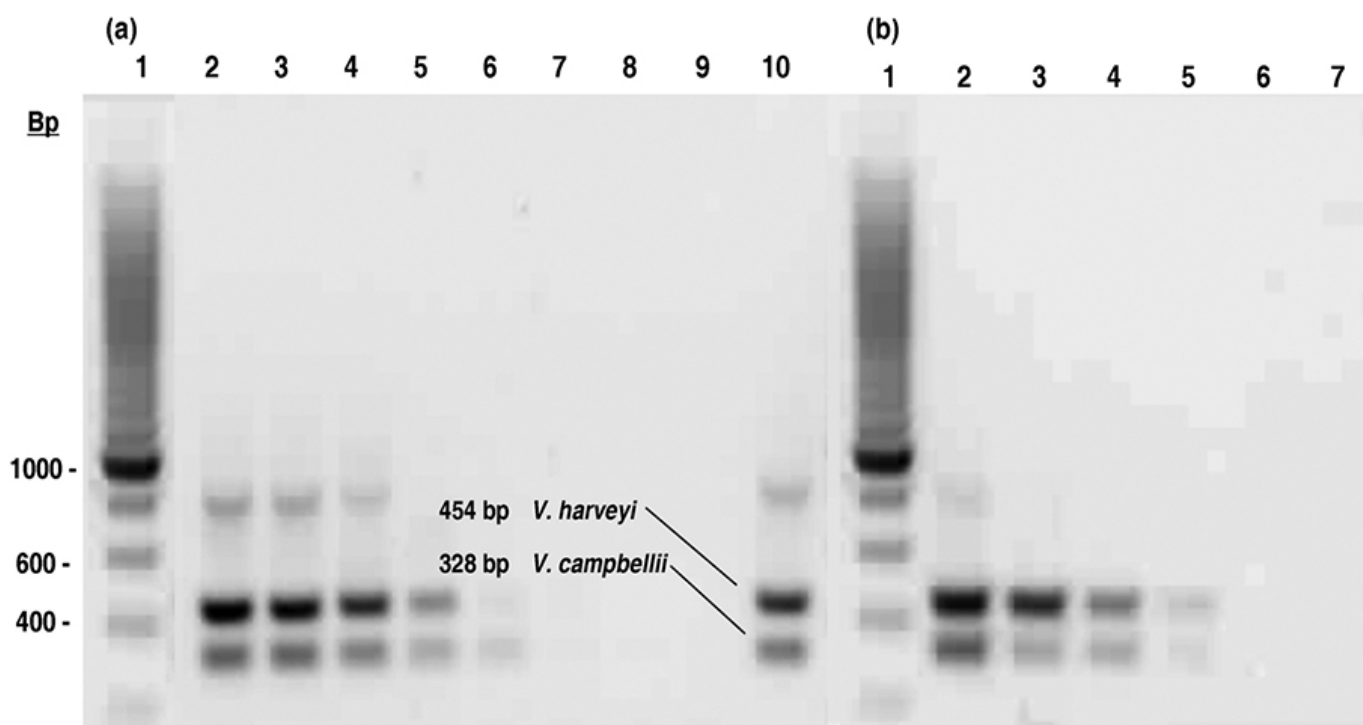


Fig. 1: Results of a multiplex PCR assay differentiating *V. harveyi* and *V. campbellii* with species-specific *hly* gene primers using high-quality DNA (left) or FTA card punches spiked with varying concentrations of *Vibrio* cells (right).

Call for field samples

With RNA and quantitative PCR work under way, the next step for this group is to start studying *Vibrio* samples from abroad, the original intent for the method development. The Microbial Discovery Group will now seek willing shrimp producers to ship nucleic acids on these FTA cards from their low- and high-producing ponds, combined with completed questionnaires relating to management practices and water chemistry data. Collection of this data will help the scientific and shrimp producer communities understand Vibrios in relation to management practices, stressors and resulting effects on shrimp economics.

Perspectives

By using FTA cards as a sampling method for remote, overseas or otherwise inaccessible water samples, one can preserve a genetic snapshot of the biological community present at the time of collection. FTA card-based sampling techniques and DNA- or RNA-based virulence gene detection and quantification can be valuable tools in the monitoring of *Vibrio* virulence potential.

The ability to detect and understand differences in the *Vibrio* community composition of shrimp production system water samples will enable researchers to understand the relationships among these organisms, pond performance, management practices and environmental stressors.

(Editor's Note: This article was originally published in the May/June 2014 print edition of the Global Aquaculture Advocate.)

Authors

-  Michael R. King, Ph.D.

Michael R. King, Ph.D.

Microbial Discovery Group/JBS United
5200 West Ashland Way
Franklin, Wisconsin 53132 USA

-  Sona Son

Sona Son

Microbial Discovery Group/JBS United
5200 West Ashland Way
Franklin, Wisconsin 53132 USA

[109,111,99,46,111,105,98,103,100,109,64,110,111,115,46,97,110,111,115]

-  Amy M. Lange

Amy M. Lange

Microbial Discovery Group/JBS United
5200 West Ashland Way
Franklin, Wisconsin 53132 USA

-  Heather S. Behn

Heather S. Behn

Microbial Discovery Group/JBS United
5200 West Ashland Way
Franklin, Wisconsin 53132 USA

-  Jim Bradley

Jim Bradley

Aqua-Manna/BDJS Farms

Share

-  [Share via Email](#)
-  [Share on Twitter](#)
-  [Share on Facebook](#)
-  [Share on LinkedIn](#)

Tagged With

[toxin genes vibrios](#) [Michael R. King](#) [Sona Son](#) [Amy M. Lange](#) [Heather S. Behn](#) [Jim Bradley](#) [diagnostic tools](#) [FTA cards](#)

Related Posts

Health & Welfare

[Low-salinity culture water controls Vibrios in shrimp postlarvae](#)

In a survey of *Vibrio* concentrations in white shrimp postlarvae at two inland farms, hatchery postlarvae were acclimated in water with salinity reduced from 30 and 5 ppt to 2 ppt at the farms. One farm stocked postlarvae in nursery ponds, and the other stocked directly into grow-out ponds.

Responsibility

[A look at various intensive shrimp farming systems in Asia](#)

The impact of diseases led some Asian shrimp farming countries to develop biofloc and recirculation aquaculture system (RAS) production technologies. Treating incoming water for culture operations and wastewater treatment are biosecurity measures for disease prevention and control.

Health & Welfare

[A comprehensive look at the Proficiency Test for farmed shrimp](#)

The University of Arizona Aquaculture Pathology Laboratory has carried out the Proficiency Test (PT) since 2005, with 300-plus diagnostic laboratories participating while improving their capabilities in the diagnosis of several shrimp pathogens.

Aquafeeds

[A look at protease enzymes in crustacean nutrition](#)

Food digestion involves digestive enzymes to break down polymeric macromolecules and facilitate nutrient absorption. Enzyme supplementation in aquafeeds is a major alternative to improve feed quality and nutrient digestibility, gut health, compensate digestive enzymes when needed, and may also improve immune responses.

About The Advocate

The Responsible Seafood Advocate supports the Global Seafood Alliance's (GSA) mission to advance responsible seafood practices through education, advocacy and third-party assurances.

[Learn More](#)

Search Responsible Seafood Advocate



Advertising Opportunities

[2022 Media & Events Kit](#)

Categories

[Aquafeeds](#) > [Health & Welfare](#) [Health & Welfare](#) > [From Our Sponsors](#) > [Innovation & Investment](#) > [Intelligence](#) > [Responsibility](#) > [Fisheries](#) > [Artículos en Español](#) >

Don't Miss an Article

Featured

- [Health & Welfare](#) [An update on vibriosis, the major bacterial disease shrimp farmers face](#)
- [Uncategorized](#) [A seat at the table: Fed By Blue team says aquaculture needs a stronger voice](#)
- [Responsibility](#) [Quantifying habitat provisioning at macroalgae cultivation locations](#)

Popular Tags

All Tags ▼

Recent

- [Fisheries](#) [Second Test: Another filler for the fisheries category](#)
- [Fisheries](#) [Test: This is filler for the fisheries Category](#)
- [Aquafeeds](#) [Test Article](#)
- [Responsibility](#) [Study: Climate change will shuffle marine ecosystems in unexpected ways as ocean temperature warms](#)
- [Health & Welfare](#) [Indian shrimp researchers earn a patent for WSSV diagnostic tool](#)



- [About](#)
- [Membership](#)
- [Resources](#)
- [Best Aquaculture Practices \(BAP\)](#)
- [Best Seafood Practices \(BSP\)](#)
- [GOAL Events](#)
- [Advocate Magazine](#)
- [Aquademia Podcast](#)
- [Blog](#)
- [Contact](#)

Stay up to date with GSA

- [@](#)
- [f](#)
- [X](#)
- [in](#)

- 

Copyright © 2024 Global Seafood Alliance
All rights reserved.

[Privacy](#)

[Terms of Use](#)

[Glossary](#)