Request for Report for Projects Awarded in 2013 and 2014 by

Mississippi Center for Food Safety and Post-Harvest Technology

Title: Development and application of quantitative PCR assays for genetic variants of *Edwardsiella tarda* for comparative evaluation of pathology, virulence and dissemination in channel, blue and hybrid catfish

Award year: 2013

PI: Matt Griffin

Co-PI: David Wise, Terry Greenway, Lester Khoo

Collaborator:

1. Objectives.
    a. Determine the prevalence of DGI, DGII, and DGIIb in commercial catfish production
        i. Survey of *Edwardsiella* sp. cultured from disease catfish phenotypically identified as *E. tarda*.
            1. Identification of specific genotypes for each genetic group based on rep-PCR fingerprints
    b. Comparative genomics to identify regions specific to each genetic group of *E. tarda* that can be exploited by PCR.
        i. Develop a qPCR assay to simultaneously differentiate and quantify different *Edwardsiella* species from fish tissues and environmental samples.
    c. Evaluate dynamics of infection of blue, channel and hybrid catfish exposed to different genetically distinct bacteria phenotypicallly identified as *Edwardsiella tarda*
        i. Determine lethal and infectious doses of genetically distinct *Edwardsiella* spp. isolates identified in Objective 1 to blue, channel and blue x channel hybrid catfish.
            1. Portal of entry and route of dissemination determined by qPCR and histopathology
            2. Immune response of blue, channel and hybrid catfish to each *Edwardsiella* spp.
                a. Cross-reactivity of blue, channel and hybrid catfish anti-body to each *Edwardsiella* spp. with all possible combinations between bacteria and fish strain.
    d. Determine potential for cross-protection against multiple species of *Edwardsiella* spp.
        i. Fish that survive challenge by one genetic group will be re-challenged with other genetic groups, with all possible combinations between strain of fish and genetic group.

2. New Accomplishments toward objectives. Please indicate if all objectives listed were completed.

This project has identified *E. tarda* DNA Group I from Griffin et al. (2013) as *E. tarda*, DNA Group II as *E. piscicida* and DNA Group IIb as the newly proposed *E. anguillarum.* PCR primers specific to each group were developed in our laboratory and a survey of archived *E. tarda* isolates from diseased catfish housed at the Aquatic Research and Diagnostic Laboratory in Stoneville, MS was performed. Survey results demonstrated that most isolates from diseased catfish in the southeastern United States phenotypically classified as *E. tarda* are in fact the newly adopted *E. piscicida* (Griffin et al. 2014).

With the assistance of Dr. Geoff Waldbieser at the USDA-ARS Warmwater Aquaculture Research Unit, the genomes of *E. tarda* (FL95-01), *E. piscicida* (S11-285), *E. piscicida*-like sp. (syn. *E. anguillarum*; LADL 05-105) and *E. hoshinae* (ATCC 35051) have been sequenced, closed, annotated and are ready for release. At present, the only means of differentiating between these phenotypically ambiguous bacteria is by molecular techniques. These sequenced genomes will be used in an attempt to identify biochemical pathways unique to each organism that can be exploited to develp reliable, differential phenotypic tests to differentiate between these phenotypically ambiguous bacteria.

A qPCR assay has been developed and validated for *E. tarda*, *E. piscicida* and *E. piscicida*-like sp. (syn. *E. anguillarum*) (Reichley et al. 2015). Current research is focusing on the using these assays, in conjunction with published qPCR assays for *E. ictaluri*, in a multi-plex format to develop an assay that can identify and differentiate between these four organisms in a single reaction.

Intraperitoneal injection LD_{50} doses in channel catfish have been established for *E. tarda*, *E. piscicida* and *E. piscicida*-like sp. (syn. *E. anguillarum*). These studies have identifed that *E. piscicida* is much more virulent ($LD_{50} \sim 4x10^5$) to channel catfish than either *E. tarda* ($LD_{50} \sim 6x10^7$) or *E. piscicida* ($LD_{50} > 4x10^8$).

3. Objectives not accomplished and impediments to meeting objectives.
    a. Evaluation of the catfish immune response to infection by these *Edwardsiella's* have been hindered by tank availability and a faulty hot water heater. These issues have been resolved and these studies are currently underway and should be complete by the end of 2015.
    b. Studies involving blue catfish and hybrid catfish are slated for Spring 2016 and have been limited by availability of blue catfish, hybrid catfish and channel catfish fingerlings of comparable ages and appropriate size. In collaboration with Brian Bosworth of USDA WARU, we anticipate having adequate supplies of similar aged fish to perform these challenges in Spring of 2016.

4. If continuing project, when will new and/or long term objectives be completed?
We anticipate all objectives proposed in this project being completed by Spring of 2016.

5. Students supported

    a. PhDs (% FTE and name)
    b. M.S. (% FTE and name)
    c. Undergraduate (number of students)

6. Leveraged Funds: External Competitive Funding Applied and Awarded based on findings from this project.
    a. Applied for:
        i. Funding agency
        ii. Program
        iii. Funding request ($$)
    b. Awarded:
        i. Funding agency
        ii. Program
        iii. Funding awarded ($$)

7. Outputs – In addition to the above, please populate the following sections to be included in a report to be compiled in a FSI Research Accomplishment Booklet. The project report will also be posted in a FSI website to be developed.

Please submit reports in Microsoft Word Document (except the published journal articles in pdf format) to Ms. Kaila Peggs by May 15.

Project Summary (Issue/Response)

The gram-negative bacterium *Edwardsiella tarda* is the most widespread member of the *Edwardsiella* genera, having been reported from over 20 species of freshwater and marine fish from 25 countries worldwide. Until recently, *E. tarda* was considered a rare disease of catfish that seldom made the annual reports of fish diagnostic laboratories in the southeast. As such, little is known about *E. tarda* in catfish and there are no approved antibiotics for its treatment. However, there has been a putative emergence of *E. tarda* in catfish aquaculture in the last 7 or 8 years. Previous research has demonstrated there to be significant genetic variability amongst strains of *E. tarda* isolated from cultured freshwater fish systems in Europe and India. Moreover, researchers in Japan have identified *E. tarda* strains of high and low virulence. We have documented intra-specific variability of *E. tarda* isolates from 4 different fish species in the eastern United States and identified two distinct genotypes of *E. tarda* (DNA group I (DGI); DNA group II (DGII) with a subgroup within DNA group II (DGIIb). Further work identified these genetic groups as *E. tarda*, *E. piscicida* and *E. piscicida*-like sp. (*E. anguillarum*). The biological consequences of these genetic differences remain unclear. Prior to this work, the prevalence of these different *Edwardsiella's* in catfish aquaculture was unknown. In addition, the establishment of a consistent disease challenge model is required to adequately assess the potential for disease related losses attributed to these *Edwardsiella* species. The pathogenesis of *E. tarda* in channel catfish has been well documented, although these studies took place prior to the adoption of *E. piscicida* and the true identity of the isolate used has been called into question. Similarly, the susceptibility of hybrid catfish to these genetic variants is unknown. Controlled studies must be performed using these genetic

strains to evaluate and properly document the pathology of these organisms and their effects on blue, channel and hybrid catfish. This effort is an invaluable first step in laying the groundwork for developing and evaluating efficacious control measures to lessen the impact of this potential emerging disease.

Project Results/Outcomes

This project has identified *E. tarda* DNA Group I as *E. tarda*, DNA Group II as *E. piscicida* and DNA Group IIb as the newly proposed *E. anguillarum.* PCR primers specific to each group were developed in our laboratory and a survey of archived *E. tarda* isolates from diseased catfish housed at the Aquatic Research and Diagnostic Laboratory in Stoneville, MS was performed. Survey results demonstrated that most isolates from diseased catfish in the southeastern United States phenotypically classified as *E. tarda* are in fact the newly adopted *E. piscicida*. This survey consisted of 44 archived specimens collected from 2007-2012, none of which were identified as *E. piscicida* or *E. piscicida*-like sp. (syn. *E. anguillarum*). All were identified as *E. piscicida.* This would suggest that *E. tarda* (A BL-2 organism) and *E. piscicida*-like sp. (syn. *E. anguillarum*) are not pathogens of major concern in catfish aquaculture.

With the assistance of Dr. Geoff Waldbieser at the USDA-ARS Warmwater Aquaculture Research Unit, the genomes of *E. tarda* (FL95-01), *E. piscicida* (S11-285), *E. piscicida*-like sp. (syn. *E. anguillarum*; LADL 05-105) and *E. hoshinae* (ATCC 35051) have been sequenced, closed, annotated and are ready for release. At present, the only means of differentiating between these phenotypically ambiguous bacteria is by molecular techniques. These sequenced genomes will be used in an attempt to identify biochemical pathways unique to each organism that can be exploited to develp reliable, differential phenotypic tests to differentiate between these phenotypically ambiguous bacteria.

A qPCR assay has been developed and validated for *E. tarda*, *E. piscicida* and *E. piscicida*-like sp. (syn. *E. anguillarum*) (Reichley et al. 2015). Current research is focusing on using these assays, in conjunction with published qPCR assays for *E. ictaluri*, in a multi-plex format to develop an assay that can identify and differentiate between these four organisms in a single reaction.

Various challenge models have been evaluated (immersion, immersion with feed, intraperitoneal injection[IP]) have been evaluated. It has been determined that IP injection yields the most consistent results. LD_{50} doses in channel catfish have been established for *E. tarda*, *E. piscicida* and *E. piscicida*-like sp. (syn. *E. anguillarum*). These studies have identifed that *E. piscicida* is much more virulent ($LD_{50} \sim 4x10^5$) to channel catfish than either *E. tarda* ($LD_{50} \sim 6x10^7$) or *E. piscicida* ($LD_{50} > 4x10^8$). This work is consistent with work out of Japan which identified what they deemed to be high and low virulent *E. tarda* strains. Our work suggests these Japanese strains of varying degrees of pathogenicity, and others like it in Europe and the Middle East, are more likely different species of *Edwardsiella* rather than variably pathogenic strains of the same organism.

Project Impacts/Benefits

This work has offered some clarity to the taxonomic chaos surrounding *E. tarda*. We have identified that *E. piscicida* is more prevalent than *E. tarda* in catfish aquaculture. In addition, we have developed molecular tools that offer rapid identification of these phenotypically ambiguous organisms. Moreover, we have sequenced the genomes of these organisms, which should offer even more insight into the biology and pathogenesis of these economically important fish pathogens. Lastly, we have evaluated several challenge models and established protocols to allow for more controlled studies of these organisms in their fish hosts and will identify the feasibility of a multivalent vaccination strategy against the *Edwardsiellas* in catfish aquaculture.

Project Deliverables

In this box list complete citations for all publications, presentations, workshops, field days, and other deliverables that came out of this project. Please use the following style (J. of Food Sci):

Reichley, S. R., G. C. Waldbieser, H. C. Tekedar, M. L. Lawrence and M. J. Griffin. 2015. Complete genome sequence of *Edwardsiella tarda* isolate FL95-01 recovered from channel catfish. Genome Announcements. In press.

Reichley, S. R., C. Ware, T. Greenway, D. Wise and M. Griffin. 2015. Real-time PCR assays for detection and quantification of *Edwardsiella tarda*, *Edwardsiella piscicida*, and *Edwardsiella piscicida*-like sp. in catfish tissues and pond water. Journal of Veterinary Diagnostic Investigation. **27**: 130-139.

Griffin, M. J., C. Ware, S. Quiniou, J. Steadman, P. Gaunt, L. Khoo and E. Soto. 2014. *Edwardsiella piscicida* identified in the southeastern United States by *gyrB* sequence, species-specific and repetitive sequence mediated PCR. Diseases of Aquatic Organisms. **108**: 23-35.

Reichley, S. R., H. C. Tekedar, G. C. Waldbieser, M. M. Banes, D. J. Wise, T. E. Greenway, L. H. Khoo, A. Karsi, M. L. Lawrence, and M. J. Griffin. Investigations into the new taxa *Edwardsiella piscicida* and comparative genomic analysis with *Edwardsiella tarda* and *Edwardsiella piscicida*-like sp. *In* proceedings of the 7th International Symposium on Aquatic Animal Health. Portland, OR. September 2014.

Griffin, M. J., S. M. Quiniou, C. Ware, and S. R. Reichley. *Edwardsiella piscicida* in Mississippi catfish aquaculture: An update. *In* proceedings of the 39th Eastern Fish Health Workshop. Shepherdstown, WV. April 2014.

Reichley, S., T. Hasan, G. Waldbieser, M. Banes, A. Karsi, M. Lawrence and M. Griffin. Comparative genomic analysis of *Edwardsiella piscicida, Edwardsiella piscicida*-like sp. and *Edwardsiella tarda* isolates from fish in the southeastern United States. *In* proceedings of the Midsouth Computational Biology & Bioinformatics Society Annual Bioinformatics & Computational Biology Conference; Stillwater, Oklahoma March 06-08, 2014. 1st Place, Best Student Presentation; Computational Biology section.

Griffin, M., C. Ware, S. Quiniou, J. Steadman, and E. Soto. Discriminatory PCR assays differentiate between *Edwardsiella tarda* and *Edwardsiella tarda*-like species and identify the predominant species in catfish aquaculture. *In* proceedings of the 38th Eastern Fish Health Workshop. Gettysburg, PA. April 2013.

Graphics

Include one or two graphics (picture or figure, colored preferred) that illustrate project outcomes. Please make sure you provide labels and appropriate units for all dimensions, and a title with a brief explanation for each figure/graph.

Attached Refereed Journal Publications in Separate Files

Please attached published journal articles (in pdf format if available) for this project. Manuscripts accepted or in review process may be submitted in word files. Thank you very much for your cooperation.