

FY 2016-17 annual report

MS Center for Food Safety and Post-Harvest Technology Initiative

Title: Phenotypic and molecular characterization of *Edwardsiella ictaluri*, *E. tarda*, *E. piscicida*, *E. anguillarum*, and *E. hoshinae* isolates from different hosts and geographic origins

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Objective 1 (Milestone fully met): Using established PCR assay protocols, a real-time multiplex PCR assay will be developed for rapid differentiation of the *Edwardsiella* species affecting fish. Manuscript in preparation.

Objective 2 (Milestone fully met): Genetic and phenotypic characterization of *Edwardsiella* species from different hosts and geographic origins. Manuscript in preparation.

Objective 3 (Milestone partially met): Investigation into the potential for multivalent vaccination of channel and hybrid catfish for *E. ictaluri*, *E. piscicida*, and *E. tarda*. Ongoing.

Initial studies demonstrated the potential for cross protection, as fish immunized with non-lethal exposures to *E. piscicida* were protected against subsequent *E. ictaluri* infection, although the inverse, vaccinating fish with *E. ictaluri* and challenging with *E. piscicida* has not been tested. These experiments are slated for Summer and Fall of 2017.

Progress Report:

This work established a multiplex PCR assay for rapid differentiation of the *Edwardsiella* species affecting fish, completed an exhaustive genetic and phenotypic characterization of *Edwardsiella* species from different hosts and geographic origins, sequenced several representative *Edwardsiella* spp. genomes, identified discriminatory peaks present for each *Edwardsiella* spp. using MALDI-TOF methodology, demonstrated increased pathogenicity of *E. piscicida* in hybrid and blue catfish over channel catfish and demonstrated channel catfish immunized with a sub-lethal dose of *E. piscicida* are protected against subsequent *E. ictaluri* infection. Challenges investigating the efficacy of a patented, live attenuated *E. ictaluri* in protecting channel and hybrid catfish against *E. piscicida* infection are slated for Summer and Fall of 2017.

Accomplishments

- Developed and validated a multiplex qPCR assay for rapid differentiation of the *Edwardsiella* species affecting fish.
- Sequenced and closed representative *Edwardsiella* spp. genomes to better facilitate proper taxonomic assignment and minimize erroneous classifications of *Edwardsiella* isolates in future research
- Identified reliable genetic markers for identification and classification of the *Edwardsiella* and linked historical studies to contemporary systematics.
- Identified discriminatory peaks were present for each *Edwardsiella* spp. using MALDI-TOF methodology, demonstrating that MALDI-TOF offers a reliable, cost effective alternative to molecular methods
- Comparative challenges demonstrated increased pathogenicity of *E. piscicida* in hybrid and blue catfish over channel catfish. Conversely, *E. anguillarum* and *E. tarda* appear to be of minimal concern in U.S. farm-raised catfish, with LD₅₀'s nearly 2-3 orders of magnitude higher than *E. piscicida*.

Significant Activities that Support Special Target Populations: (100 words or less)

We have made significant advances in *Edwardsiella* research and diagnostics. The multiplex qPCR offers a rapid, economical confirmatory test for the *Edwardsiella* spp. from fish and MALDI-TOF is identified as a reliable method for differentiating the *Edwardsiella*. We have identified more resolute gene targets for the identification of *Edwardsiella*, and coupled with whole genome sequencing, we have tied historical reports of typical fish pathogenic *E. tarda*, atypical fish pathogenic *E. tarda* and fish non-pathogenic *E. tarda* to current *Edwardsiella* systematics. This work brings uniformity to the scientific literature and will better facilitate research of these ambiguous pathogens moving forward.

Publications: Please use the following format, examples

Reichley, S. R., Ware, C., Steadman, J., Greenway, T. E., Gaunt, P. S., Carcia, J. C., LeFrentz, B. R., Thachil, A., Waldbieser, G. C., Stine, C. B., Arias, C. R., Loch, T., Welch, T. J., Cipriano, R. C., Khoo, L. H., Wise, D. J., Lawrence, M. L., and Griffin, M. J. Advancing our knowledge of the *Edwardsiella*: a comparative phenotypic and genotypic analysis of *Edwardsiella* spp. isolates from different hosts and geographic origins. In preparation. To be submitted to Journal of Applied Microbiology.

Reichley, S. R., Ware, C., Greenway, T.E., Khoo, L. H., Bosworth, B. G., Wise, D. J., Lawrence, M. L., and Griffin, M. J. Comparative susceptibility of channel catfish (*Ictalurus punctatus*), blue catfish (*Ictalurus furcatus*) and channel (♀) x blue (♂) hybrid catfish to *Edwardsiella anguillarum*, *Edwardsiella piscicida*, and *Edwardsiella tarda*. Submitted to Journal of Aquatic Animal Health. May 2017.

Reichley, SR, Waldbieser, GC, Soto, E, Lawrence, ML, Griffin, MJ. (2017). Complete genome sequence of *Edwardsiella ictaluri* isolate RUSVM-1 Recovered from Nile Tilapia (*Oreochromis niloticus*) in the Western Hemisphere. Genome Announcements. In press. April 2017.

Reichley, S. R., Waldbieser, G. C., Lawrence, M. L., and Griffin, M. J. (2016). Complete genome sequence of *Edwardsiella hoshinae* ATCC 35051. *Genome Announcements* 5(6), e01605-16.

Reichley, S. R., Waldbieser, G. C., Tekedar, H. C., Lawrence, M. L., and Griffin, M. J. (2016). Complete genome sequence of *Edwardsiella piscicida* isolate S11-285 recovered from channel catfish (*Ictalurus punctatus*) in Mississippi, USA. *Genome Announcements*. 4 (6), e01259-16.

Reichley, S. R., Waldbieser, G. C., Lawrence, M. L., Griffin, M. J. (2015). Complete genome sequence of an *Edwardsiella piscicida*-like species recovered from tilapia in the United States. *Genome Announcements* 3(5):e01004-15.

Reichley, S. R., Waldbieser, G. C., Ucko, M., Colorni, A., Dubytska, L., Thune, R. L., Lawrence, M. L., and Griffin, M. J. (2015). Complete genome sequence of an *Edwardsiella piscicida*-like species isolated from diseased grouper in Israel. *Genome Announcements* 3(4):e00829-15.

Reichley, S. R., Waldbieser, G. C., Tekedar, H. C., Lawrence, M. L., and Griffin, M. J. (2015). Complete genome sequence of *Edwardsiella tarda* isolate FL95-01, recovered from channel catfish. *Genome Announcements* 3(3):e00682-15. doi: 10.1128/genomeA.00682-15.

Reichley, S. R., Ware, C., Greenway, T., Wise, D. and Griffin, M. (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., Ware, C., Quiniou, S., Steadman, J., Gaunt, P., Khoo, L. and Soto, E. (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.

Presentations:

Reichley, S. R., Griffin, M. J., Ware, C., Waldbieser, G. C., Tekedar, H. C., Greenway, T. E., Khoo, L. H., Lawrence, M. L., Gaunt, P. S., and Wise, D. J. (2017, April) Expanding our knowledge of *Edwardsiella*. American Fisheries Society Fish Health Section Annual Meeting, East Lansing, MI.

Reichley, S. R., Lawrence, M. L., Greenway, T. E., Khoo, L. H., Wise, D. J., and Griffin, M. J. (2017, April). Advancing our knowledge of the bacteria formerly known as *Edwardsiella tarda*. 42nd Eastern Fish Health Workshop, East Lansing, MI.

Griffin, M. J. (2017, April). Misclassifications: Limitations of 16S Sequencing and Misplaced Reliance on Public Nucleotide Databases. 42nd Eastern Fish Health Workshop, East Lansing, MI. April 2017

Reichley, S. R., Griffin, M. J., Ware, C., Waldbieser, G. C., Tekedar, H. C., Greenway, T. E., Khoo, L. H., Lawrence, M. L., Gaunt, P. S., and Wise, D. J. (2016, June). Expanding our knowledge of the *Edwardsiella*. ASM Microbe 2016. Boston, MA.

Reichley, S. R., Griffin, M. J., Ware, C., Steadman, J., Greenway, T. E., Lawrence, M. L., Gaunt, P. S., Camus, A. C., Stine, C. B., Petty, B. D., Thune, R., Arias, C. R., Loch, T., Welch, T. J., and Cipriano, R. C. (2016, April). Phenotypic and genetic characterization of *Edwardsiella* isolates from different hosts and geographic regions. In proceedings of the 41st Eastern Fish Health Workshop. Atlantic Beach, NC.

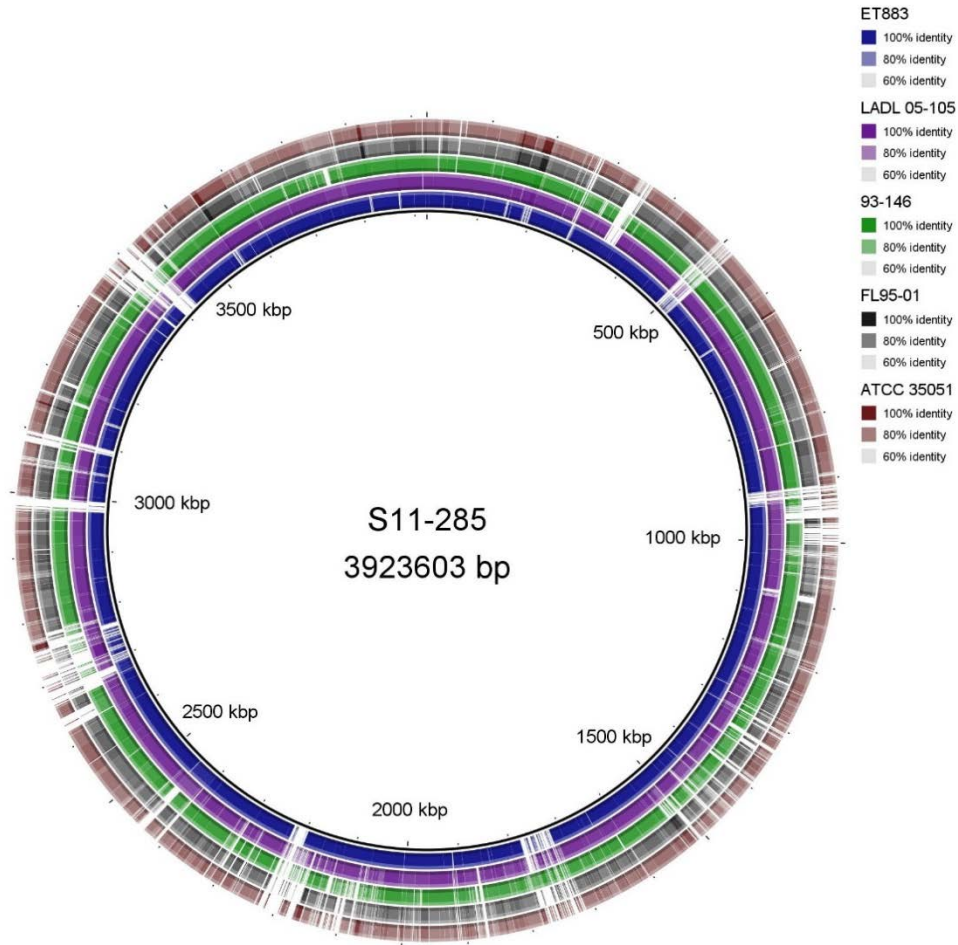
Reichley, S. R., Griffin, M. J., Ware, C., Waldbieser, G. C., Tekedar, H. C., Banes, M. M., Greenway, T. E., Khoo, L. H., Lawrence, M. L., Wise, D. J. (2015, March). Recent advancements in our knowledge of *Edwardsiella piscicida* and *E. tarda*. In proceedings of the 40th Eastern Fish Health Workshop, Charleston, SC.

Reichley, S. R., Tekedar, H. C., Waldbieser, G. C., Banes, M. M., Wise, D. J., Greenway, T. E., Khoo, L. H., Karsi, A., Lawrence, M. L., and Griffin, M. J. (2014, September). 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.

Reichley, S., Hasan, T., Waldbieser, G., Banes, M., Karsi, A., Lawrence, M., and Griffin, M. (2014, March) 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., Ware, C., Quiniou, S., Steadman, J., and E. Soto. (2013, April) 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.

Griffin, M., Quiniou, S., Cody, T., Tabuchi, M., Ware, C., Cipriano, R., Mael, M., and E. Soto. (2013, April). When is a species a species? Genetic variability of *Edwardsiella tarda* in the southeastern United States. In proceedings of the 38th Eastern Fish Health Workshop. Gettysburg, PA.



Sequence identity of *Edwardsiella* genomes, using *E. piscicida* isolate S11-285 as the reference. Consistent identification and classification of subject organisms lays the foundation for infectious disease research and fosters more reliable reporting among different laboratories and within the scientific literature. The collection of closed genomes established in this project were submitted to GenBank in line with contemporary taxonomic nomenclature consistent with current *Edwardsiella* systematics. These genomes will better facilitate proper taxonomic assignment and minimize erroneous classifications of *Edwardsiella* isolates in future research. Moreover, these closed genomes will assist in further studies investigating the biology of these important bacteria and help researchers gain a better understanding of their interactions in the environment and within different hosts.