Title: Implementation of recently developed method to detect Botulinum toxin in catfish

Project Leader (PI): Dr. Tibor Pechan

Co-PI(s): Dr. Patricia Gaunt

Collaborator(s): Dr. Brian Bosworth

Objective(s): To provide Mississippi catfish farmers and researchers with diagnostic tool do detect contamination of catfish with BoNTE.

Milestones for FY 2016-17:

1. Seeking federal funds. Fully met, proposal titled "Trial Diagnostic Services To Detect Botulinum Toxin E, The Causal Agent Of Visceral Toxicosis In Catfish" submitted to USDA-NIFA-SRGP-005776 Special Research Grants Program Aquaculture Research
2. Presentation of preliminary results at annual meeting of American Society for Mass Spectrometry. Fully met, the peer reviewed poster "Mass Spectrometry Based Method to Detect the BONT/E in Catfish Specimen" was presented at ASMS 2016, June 5 - 9, San Antonio, TX.
3. Specific goals partially met: Optimization of the method for detection of active BoNTE; Development of a user interface to automate readings of BoNTE activity from MS spectra
4. Specific goals not met: Detection of BoNTE in VTC suspected fish from commercial catfish ponds on as needed basis

The progress towards the goals was limited by lack of samples from farmer's ponds in year 2016 and first half of 2017.

Progress Report:

As mentioned above, the cases of Catfish Visceral Toxicosis were not observed (reported) in Mississippi in 2016 and 2017, yet. Therefore, the major effort was put to writing and submitting the proposal to USDA (see above). In addition, the toxin detection in laboratory condition was repeated and refined using previously developed mass spectrometry approach and a novel method using capillary-based immunoassay instrument WES (Protein Simple, San Jose, CA). It completely eliminates gels, blotting, membranes, films and imaging, while drastically lowers antibody consumption (by 3-5 orders of

magnitude). Simultaneously, WES allows for significant effort and time saving. Operator needs to spend approximately 5 minutes per sample to prepare the instrument for analysis that is completed in 3 hours.

Accomplishments

With the emergence of a new technology, we developed the detection method of BoNTE utilizing capillary based immunoassay. The analysis can be performed in one working day for the cost of $10.87 per sample, including consumables, antibody and operator time (when 24 samples are analyzed in one run). Detection limit is currently 0.2 ng of BoNTE, but is a subject to further optimization to increase method sensitivity.

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

The preliminary results were presented at annual meeting ASMS 2016, June 5 - 9, San Antonio, TX, vie poster titled "Mass Spectrometry Based Method to Detect the BONT/E.
Oral presentation was given at 2016 Annual Conference of Mississippi Center for Food Safety and Post-Harvest Technology (see below for details).

Technology Transfer:

Invention disclosure "Detection of Botulinum Toxins in Muscle, Serum, and Other Bodily Liquids" was filed with Office of Technology Management, Mississippi State University. Currently, conversation with technology licensing coordinator is taking place to determine if patenting will be pursued.

International Cooperation / Collaboration

Nothing to report

Publications:

Due to ongoing invention disclosure, publication of achieved results was limited to poster and presentation

Peer Reviewed Poster:

Arnold, J., Pechanova, O., Pechan, T. (2016). Mass Spectrometry Based Method to Detect the BONT/E in Catfish Specimen. Annual meeting of American Society for Mass Spectrometry, June 5 – 9, 2016, San Antonio, TX.

Presentations:
Oral Presentation

Pechan, T. & Gaunt, P. (2016, May). Implementation of recently developed method to detect Botulinum toxin in catfish. 2016 Annual Conference of Mississippi Center for Food Safety and Post-Harvest Technology, Mississippi State University, MS.

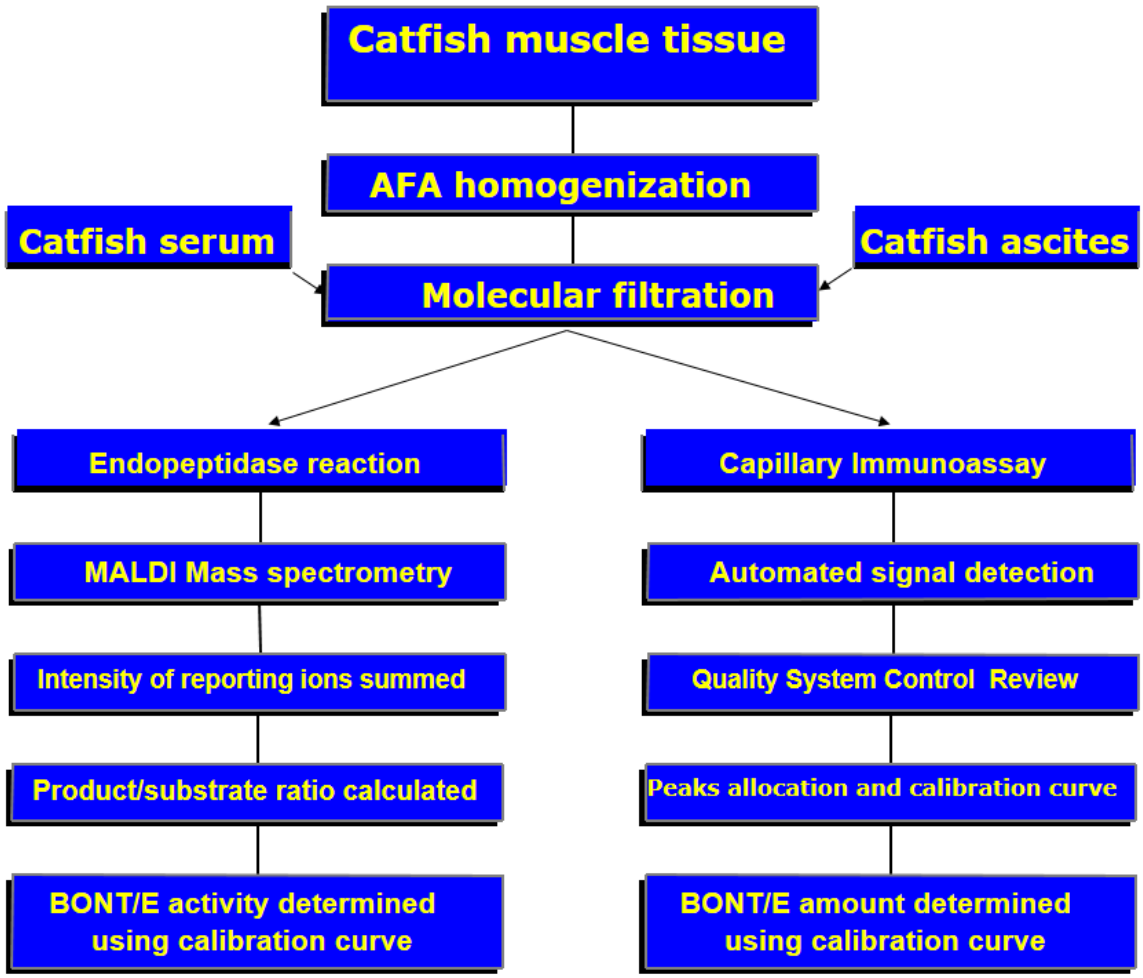

Figure 1. Workflows of novel methods to detect BoNTE toxin in catfish specimen. (A) Mass spectrometry based method to detect active toxin via quantification of products of the BoNTE endopeptidase activity; (B) Detection of BoNTE inactive traces via direct, capillary-based immunoassay.