AGRICULTURAL RESEARCH FOUNDATION
FINAL REPORT
FUNDING CYCLE 2013 – 2015

TITLE: Analyses of oyster spat pathogenic *Vibrio* species

RESEARCH LEADER: Chris Langdon, Coastal Oregon Marine Experiment Station

COOPERATORS: (if any) Claudia Häse, Dept. Veterinary Medicine

SUMMARY: Five bacterial *Vibrio* isolates were obtained from a group of dying juvenile oysters in the MBP nursery at the Hatfield Marine Science Center (HMSC) hatchery. At the time, we were working on the effects of an oyster *Vibrio* pathogen (RE 22; thought to be *Vibrio tubiashii*) on oyster larvae and spat and hypothesized that the new HMSC isolates were also a strain of *V. tubiashii*. We carried out gene sequencing of the new isolates (using primers for 16S rDNA, recA and rpoA) and compared the sequences with those of RE22 and the ATCC type isolate for *Vibrio tubiashii* (ATCC 19106). Interestingly we found that the sequence data for RE22 was not conclusively characteristic of *V. tubiashii* and that the sequence data for rpoA (RNA polymerase alpha chain) showed a more significant match with *Vibrio coralliilyticus*. The HMSC isolates were most similar to *Vibrio splendidus*, based on all the sequence data. Challenge experiments with oyster spat and developing mussel embryos indicated that none of the HMSC isolates were harmful. Furthermore, specific biochemical assays indicated that they did not secrete metalloproteases that Häse and colleagues had shown to be associated with the pathogenicity of RE22.

OBJECTIVES: The objective of the proposal was to better characterize the *Vibrio* isolates obtained from dying juvenile oysters reared at Hatfield Marine Science Center. Our goals were to understand and identify important virulence factors and determine why the juvenile oysters were dying.

PROCEDURES: We tested the pathogenicity of the HMSC isolates against oyster spat in 14 day exposure experiments and also carried out 48 h exposure experiments with developing embryos of mussels (found to be the most sensitive life stage) and compared their pathogenicities with those of RE22. We sequenced genes typically used in species determination of *Vibrio* isolates (16S rDNA, recA protein and rpoA RNA polymerase alpha protein) and developed cladograms to show relatedness. Finally, we determined B hemolysis and metalloprotease activities of the isolates.

SIGNIFICANT ACCOMPLISHMENTS: The most surprising result was that gene sequence data indicated that RE22 was more similar to *V. coralliilyticus* than *V. tubiashii*. This result has been subsequently confirmed by other researchers. Therefore, our results indicate that *V. coralliilyticus* is pathogenic to bivalves – a result that was not known at the time of writing the proposal. The absence of pathogenicity of the HMSC isolates towards oyster spat was

unexpected but was supported by their lack of secreted metalloprotease activity, although hemolysin activity was observed in some of the isolates (HMSC isolates #1, 4 and 5). Häse and colleagues have shown that the pathogenicity of RE22 culture supernatants is associated with metalloprotease, but not hemolysin, activity.

BENEFITS & IMPACT: The finding that RE22 was more closely *V. coralliilyticus* than *V. tubiashii* was unexpected. The results indicate that Pacific oyster larvae are susceptible to more than one *Vibrio* pathogen. The HMSC isolates were identified as *V. splendidus* and were non-pathogenic.

ADDITIONAL FUNDING RECEIVED: The ARF grant complemented ongoing research of Langdon and Häse funded by Oregon Sea Grant on the effects of *Vibrio tubiashii* on oyster and mussel larvae. The Sea Grant project and this ARF project were in response to high mortalities of oyster larvae reported in West Coast hatcheries that had resulted in a "seed crisis" for the industry.

FUTURE FUNDING: Häse obtained additional funding to develop "dipsticks" for determination of the presence of pathogenic *Vibrio* species in larval cultures, based on detection of metalloprotease released into the culture medium. Both PI's are working in conjunction with Dr. Gary Richards, USDA-ARS scientist, to develop bacteriophages to control both *V. tubiashii* and *V. coralliilyticus*. A Phase 2 SBIR/USDA proposal is in preparation to commercialize this method of pathogen control.