

Defense Priming from Egg to Larvae in *Litopenaeus vannamei* with Non-Pathogenic and Pathogenic Bacteria Strains

Authors : Angelica Alvarez-Lee, Sergio Martinez-Diaz, Jose Luis Garcia-Corona, Humberto Lanz-Mendoza

Abstract : World aquaculture is always looking for improvements to achieve productions with high yields avoiding the infection by pathogenic agents. The best way to achieve this is to know the biological model to create alternative treatments that could be applied in the hatcheries, which results in greater economic gains and improvements in human public health. In the last decade, immunomodulation in shrimp culture with probiotics, organic acids and different carbon sources has gained great interest, mainly in larval and juvenile stages. Immune priming is associated with a strong protective effect against a later pathogen challenge. This work provides another perspective about immunostimulation from spawning until hatching. The stimulation happens during development embryos and generates resistance to infection by pathogenic bacteria. Massive spawnings of white shrimp *L. vannamei* were obtained and placed in experimental units with 700 mL of sterile seawater at 30 °C, salinity of 28 ppm and continuous aeration at a density of 8 embryos.mL⁻¹. The immunostimulating effect of three death strains of non-pathogenic bacterial (*Escherichia coli*, *Staphylococcus aureus* and *Bacillus subtilis*) and a pathogenic strain for white shrimp (*Vibrio parahaemolyticus*) was evaluated. The strains killed by heat were adjusted to O.D. 0.5, at A 600 nm, and directly added to the seawater of each unit at a ratio of 1/100 (v/v). A control group of embryos without inoculum of dead bacteria was kept under the same physicochemical conditions as the rest of the treatments throughout the experiment and used as reference. The duration of the stimulus was 12 hours, then, the larvae that hatched were collected, counted and transferred to a new experimental unit (same physicochemical conditions but at a salinity of 28 ppm) to carry out a challenge of infection against the pathogen *V. parahaemolyticus*, adding directly to seawater an amount 1/100 (v/v) of the live strain adjusted to an OD 0.5; at A 600 nm. Subsequently, 24 hrs after infection, nauplii survival was evaluated. The results of this work shows that, after 24 hrs, the hatching rates of immunostimulated shrimp embryos with the dead strains of *B. subtilis* and *V. parahaemolyticus* are significantly higher compared to the rest of the treatments and the control. Furthermore, survival of *L. vannamei* after a challenge of infection of 24 hrs against the live strain of *V. parahaemolyticus* is greater ($P < 0.05$) in the larvae immunostimulated during the embryonic development with the dead strains *B. subtilis* and *V. parahaemolyticus*, followed by those that were treated with *E. coli*. In summary superficial antigens can stimulate the development cells to promote hatching and can have normal development in agreeing with the optical observations, plus exist a differential response effect between each treatment post-infection. This research provides evidence of the immunostimulant effect of death pathogenic and non-pathogenic bacterial strains in the rate of hatching and oversight of shrimp *L. vannamei* during embryonic and larval development. This research continues evaluating the effect of these death strains on the expression of genes related to the defense priming in larvae of *L. vannamei* that come from massive spawning in hatcheries before and after the infection challenge against *V. parahaemolyticus*.

Keywords : immunostimulation, *L. vannamei*, hatching, survival

Conference Title : ICFAS 2019 : International Conference on Fisheries and Aquaculture Sciences

Conference Location : Tokyo, Japan

Conference Dates : November 11-12, 2019