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Luminescent Vibriosis in Cultured Black Tiger Shrimp Penaeus monodon

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Shrimp monoculture, most especially grow-out culture, is a very profitable business activity, but it has been beset with economically devastating losses due to infectious diseases and environmental deterioration. In the Philippines, the most commonly cultured species is the giant tiger shrimp, Penaeus monodon. One of the most serious diseases affecting P. monodon of all stages is bacterial disease due to several species of vibrios. Infections due to these bacteria have affected both the shrimp hatchery and the grow-out sectors of the industry.

Larval mortalities associated with luminescence have been observed in epizootic proportions in *P. monodon* hatcheries in Panay Island. Luminescent vibrios, identified as *Vibrio harveyi* and *V. splendidus*, were isolated from diseased larvae and the environmental seawater. Pathogenicity tests with a *V. harveyi* isolate resulted in significant mortalities of larvae and postlarvae of *P. monodon* within 48 h of immersion challenge. Scanning electron microscopic observations showed that colonization by the bacteria occurred specifically on the feeding apparatus and oral cavity of the larvae, suggesting an oral route of entry for initiation of infection. Results of the investigations showed that the main source of luminescent bacteria entering the shrimp hatcheries is the midgut contents of the mother shrimp, from which the bacteria are shed into the water during spawning.

Mass mortality associated with luminescent vibrios started occurring in the last quarter of 1993 and contributed largely to the collapse of shrimp grow-out activities in the Philippines. It was determined that V. harveyi was the major etiological agent associated with the luminescent vibriosis in pond-cultured P. monodon and that significant histopathological changes occur in the hepatopancreas of diseased shrimp. Pathogenicity of V. harveyi was confirmed through intramuscular injection to healthy shrimp.

An ecological study on luminescent vibriosis was conducted to understand the initiation of infection. Results showed that the occurrence of mortalities was preceded by a shift of the bacterial profile of the rearing water to the dominance of luminescent vibrios. During the first 15 days, the mean luminescent bacterial load of the shrimps' hepatopancreas with disease was about ten times higher than shrimps without disease. In shrimps at 18 to 32 days of culture, the luminescent bacterial load of affected shrimp was a thousand times higher than in shrimp stocks without disease problem. This shows that monitoring the luminescent *Vibrio* population of pond-cultured shrimp during the first 30 days of culture is a valuable indicator for predicting the occurrence of luminescent vibriosis.

Because chemotherapy was a popular option to control bacterial diseases, the minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) of 24 drugs for 27 strains of *V. harveyi* and *V. splendidus* were determined. Only chloramphenicol, sodium nifurstyrenate and nitrofurans (furazolidone, nitrofurazone, nitrofurantoin and Prefuran) showed relatively low MICs and MBCs (<25 μg/ml). Shrimp larvae showed high survival rates and active

swimming movement after 24 h exposure to *in vivo* bactericidal doses of chloramphenicol, oxytetracycline, Prefuran and sodium nifurstyrenate, but these drugs caused deformities in the carapace, rostrum and setae. Chemical control of luminous vibriosis among shrimp larvae appears limited, because of the possible development of resistant strains of bacteria and the limited tolerance of the shrimp larvae to the drugs.

Manipulation of the microbial ecology of the hatchery has gained popularity, but this niche-filling approach requires thorough understanding of the epidemiology of bacterial diseases for successful implementation. It was found that *V. harveyi* has a wider range of tolerance to environmental changes (temperature, salinity and pH) than *P. monodon* larvae, and therefore control measures based on manipulation of these parameters may not be feasible. However, the natural microflora of seawater, as well as the microbial flora associated with the diatoms *Skeletonema costatum* and *Chaetoceros calcitrans* negatively affected the survival of *V. harveyi* in experimental mixed cultures. A successful manipulation of the microbial component is necessary to sustain ecological balance in the shrimp hatchery and grow-out pond environment. These approaches include the use of reservoirs for water treatment, maintenance of ecological balance through the application of probiotics, and culture system modifications such as application of polyculture system.