

---

## Characterization of Biofilm Formation by Vibrios Isolated from *Penaeus monodon*

B.V.S.M. Bopitiya<sup>a</sup>, S.U. Pathirana<sup>b</sup>, G.C.P. Fernando<sup>b</sup>, H.A.D. Ruwandeepika<sup>b</sup>,  
E.G.K.Y.C. Bandara<sup>a</sup> and K.H.M. Ashoka Deepananda<sup>a</sup>

<sup>a</sup>Department of Fisheries and Aquaculture, Faculty of Fisheries and Marine Sciences & Technology, University of Ruhuna, Sri Lanka

<sup>b</sup>Department of Livestock Production, Faculty of Agricultural Sciences, Sabaragamuwa University of Sri Lanka, Sri Lanka

corresponding author: ashoka@fish.ruh.ac.lk

Biofilm formation by pathogenic Vibrios has now become a major constrain of aquaculture causing substantial economic losses due to high virulence of those bacteria in cultured shrimps. This study assesses the biofilm formation ability of eight different *Vibrio sp.* (*Vibrio campbellii*, *Vibrio alginolyticus* (1) and (2), *Vibrio anguillarum*, *Vibrio parahaemolyticus*, *Vibrio sp.* (1), (2) and (3)) isolated from *Penaeus monodon*. The ability to form biofilms was tested at two different temperatures (27°C and 37°C) in different nutritional conditions (undiluted Tryptic Soy Broth (TSB), 1:10 diluted TSB and, 1:100 diluted TSB) at three different post-incubation periods (24h, 48h and, 72h). Selected two temperatures are ambient for shrimps and humans respectively. Diluted nutrition media were used to determine whether there is any effect of the nutrient conditions on the biofilm formation. The biofilms were quantified and categorized into strong, moderate, weak, and non-adherent biofilm formers. Phenotypic assays were done by culturing the colonies in Congo red and Coomassie Blue to detect the presence of  $\beta$ -polysaccharides and proteins respectively whilst the fluorescence microscopic observations were done after staining with Calcofluor White to detect the biogenesis of cellulose in biofilms. An antibiotic sensitivity assay was conducted against Amoxicillin, Ciprofloxacin, Enrofloxacin, Erythromycin and Oxytetracycline. The study revealed that the most *Vibrio* species were able to make strong biofilms in an undiluted TSB at 27°C over to 37°C. Only *V. campbellii* has the ability to form strong biofilms at 37 °C. According to the results of the antibiotic assay, all the tested bacterial strains showed higher resistance to Amoxicillin whilst they showed the lowest resistance to Ciprofloxacin. These results attest that the most effective antibiotic against tested Vibrios is Ciprofloxacin. Strong biofilm formers have given positive results for Calcofluor white supplemented Trypticase Soy Agar (TSA), Coomassie Brilliant Blue supplemented TSA and Congo red agar method. This study concludes that the different Vibrios have the capability of producing biofilm at varied degrees.

*Keywords: Antibiotic assay, Biofilm formation, Nutrients, Vibrio sp*