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Antiulcerative Property of *Bacillus subtilis* against *Vibrio harveyi,* A Bacterial Pathogen of *Labeo rohita* (Rohu Fish)

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Abstract

This study evaluated the antiulcerative property of *Bacillus subtilis* against *Vibrio harveyi,* a bacterial pathogen of *Labeo rohita* (Rohu Fish). About 3 fishes per group namely Normal and Induced (*V. harveyi* infected) were reared in 10 L tanks with continuous aeration. About 75 µL of *V. harveyi* bacterial suspension was injected using 1 mL insulin syringes intramuscularly in separate tanks. About 50 µL of bacterial suspension of *Bacillus subtilis* was injected in the ulcerative fish (Treated group) using 1 mL insulin syringes intramuscularly in separate tanks and analyzed for its antiulcerative property by histopathology. The small intestine of the Normal fish showed cylindrical and columnar epithelium, sub mucosa and serosa showed normal organization of intestine. Histopathology of the Induced group showed disintegration of the cell wall and shrinkage of columnar epithelial cells due to *Vibrio harveyi*. The treated group showed less damaged cell wall of the intestine columnar epithelium (EC), sub mucosa (SM) and Serosa. Hence, *Bacillus subtilis* can be used for treating the ulcerative lesions of *Labeo rohita* in near future.

Keywords: Antiulcerative property, Bacillus subtilis, Probiotic, Vibrio harveyi, histopathology

1 Introduction

Over the years there have been heavy deaths in the marine environment due to deadly diseases caused by the bacterial pathogens. These pathogenic bacterial populations exist in all the aquatic environments around the globe along with the marine habitat [1]. The bacterial pathogens are regarded as the oppurtunistic pathogens which are found along with the normal microflora of the marine environment. Fishes are important part of the marine environment and there are some bacterial pathogens which affect the fish population in greater number. Among the bacterial pathogens, *Vibrio harveyi* is mainly associated with the fish species reared in farms. *Vibrio harveyi* causes ulcerative disorders in the fish population which are reared in farms [2]. They also cause ulcers in other aquatic habitats like shrimp, lobster, seahorses, sharks and seabass in more numbers [3]. In a particular period of time, the pathogenicity rapidly spreads at a greater rate and its pathogenicity is determined by its cell concentration. Diseases like luminous vibrosis, eye lesions, vasculitis and gastro-enteritis is caused by this deadly bacterial pathogen in recent years [4]. Various symptoms like eye opacity, infections in fin rot and tail, lesions in muscle, ulcers in skin or mouth, anorexia, swollen intestine and whole fish darkening occur in the infected fish due to *Vibrio harveyi* [5].

Bacillus subtilis forms the integral part of the GI tract of the animals in common. The beneficial bacterial population is used as probiotics in various aquaculture farms for rearing marine organisms [6]. They are also commonly found in the microbiota of the marine environment and in the fresh water environment. In order to increase the productivity of the culture fish and improving its health status, *Bacillus subtilis* is widely used as an important probiotic bacterial species compared to all other species at a greater extent. In addition to that, it is also regarded as the suitable candidate for elimination of pathogenic organisms. Considering the beneficial effects of the *Bacillus subtilis* and its probiotic nature many researchers are in sought of this organism in their recent research [7]. Considering the above facts in view, this study evaluated the antiulcerative property of *Bacillus subtilis* against *Vibrio harveyi,* a bacterial pathogen of *Labeo rohita* (Rohu Fish).

2 Experimental

2.1. Collection and maintenance of experimental Rohu

The fish Rohu (*Labeo rohita*) with a 50-70 g average body weights were collected around a farm located at Chennai and was reared in tanks. The collected fish samples were transported to the laboratory in aerated bags. In the laboratory, they were maintained in 10 L fiberglass reinforced tanks containing freshwater with continuous aeration at room temperature (27-30ºC). The collected fish were fed twice a day with commercial fish feed.

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2.2. Infection by intramuscular injection

Experimental pathogenicity was conducted based on modified protocol of Ducklow *et al*. (1980). About 3 fishes per group namely Normal and Induced (*V. harveyi* infected) were taken in 10 L tanks with continuous aeration. Commercially available *Vibrio harveyi* organism was obtained and 1.0 OD concentrations of *V. harveyi* suspension were used for the experimental setup. About 75 µL of *V. harveyi* bacterial suspension was injected using 1 mL insulin syringes intramuscularly in separate tanks. Fishes were monitored twice daily for mortality and ulcerative lesions. Dead animals were removed from the tanks and recorded.

2.3. Injection of *Bacillus subtilis* extract in *Vibrio harveyi* infected fish

About 50 µL of bacterial suspension was injected in the ulcerative fish (Treated group) using 1 mL insulin syringes intramuscularly in separate tanks and analyzed for its antiulcerative property by histopathology.

3 Results and Discussion

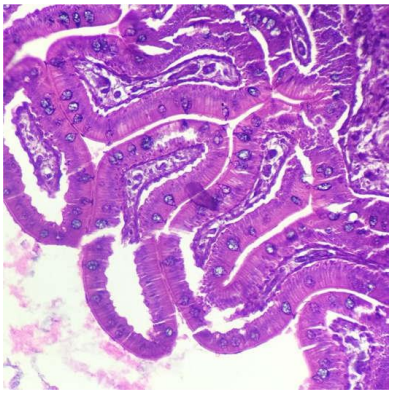

Fig. 1. Histopathology of the intestine (Normal) (X100).

Labeo rohita, commonly called as Rohu fish is reared in fresh water farms in large number in India and around the world. The nutritive value of Rohu fish is high compared to all other fish population of fresh water farms. Rohu fish has high protein content along with essential vitamins and minerals [8]. It also accounts low carbohydrate and fat content compared to other fresh water fish population. It has omega fatty acid which is regarded as the healthy fatty acid for human population. Due to high increase in population and the need for fish diets and the demand for Rohu fish increases day-by-day. The bacterial diseases of the Rohu fish greatly affect its population in recent years [9]. Among the bacterial pathogens, *Vibrio harveyi* plays a devastating role in killing the Rohu fish population due to its ulcerative properties [10]. The changes in the organs were examined in the randomly selected sectors of a fish by histopathology (Fig. 1-3). Figure 1 shows the histopathology of the normal fish group. The small intestine showed cylindrical and columnar epithelium, sub mucosa and serosa showing normal organization of intestine. Figure 2 shows the histopathology of the Induced group showing disintegration of the cell wall and shrinkage of columnar epithelial cells due to *Vibrio harveyi*. Figure 3 shows the treated group showing less damage cell wall of the intestine columnar epithelium (EC), sub mucosa (SM) and Serosa.

Normal group showing cylindrical and Columnar Epithelium, sub mucosa and serosa showing normal organization of intestine. Induced group showing disintegration of the cell wall, shrinkage of columnar epithelial cells. Treated group showing less damaged cell wall of the intestine columnar epithelium (EC), Sub mucosa (SM) and Serosa.

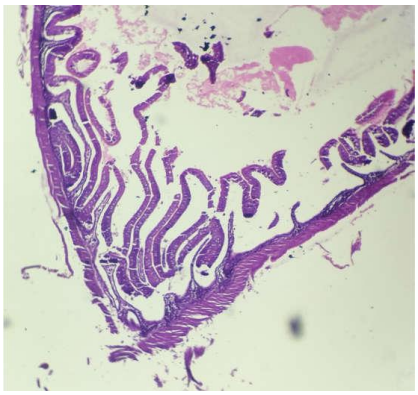

Fig. 2. Histopathology of the intestine (Induced) (X100).

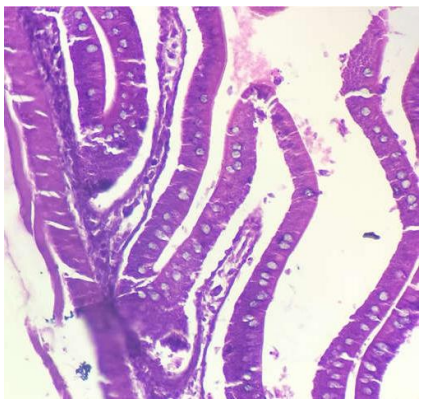

Fig. 3. Histopathology of the intestine (Treated).

4 Conclusions

The fish Rohu (*Labeo rohita*) were collected in a farm located at Chennai and was reared in tanks. About 3 fishes per group namely Normal and Induced (*V. harveyi* infected) were reared in 10 L tanks with continuous aeration. About 75 µL of *V. harveyi* bacterial suspension was injected using 1 mL insulin syringes intramuscularly in separate tanks. About 50 µL of bacterial suspension of *Bacillus subtilis* was injected in the ulcerative fish (Treated group) using 1 mL insulin syringes intramuscularly in separate tanks and analyzed for its antiulcerative property by histopathology. The small intestine of the Normal fish showed cylindrical and columnar epithelium, sub mucosa and serosa showing normal organization of intestine. Histopathology of the Induced group showing disintegration of the cell wall and shrinkage of columnar epithelial cells due to *Vibrio harveyi*. The treated group

showed less damage to the cell wall of the intestine columnar epithelium (EC), sub mucosa (SM) and Serosa.

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