Journal of King Saud University – Science (2016) xxx, xxx–xxx

King Saud University
Journal of King Saud University – Science
www.ksu.edu.sa
www.sciencedirect.com

Growth performance, digestive enzyme activity and immune response of *Macrobrachium rosenbergii* fed with probiotic *Clostridium butyricum* incorporated diets

Mohammad Saifuddin Sumon [a], Fatema Ahmmed [a], Sharmin Sultana Khushi [a], Mirja Kaizer Ahmmed [a,b], Muhammad Abdur Rouf [a], Md. Ayaz Hasan Chisty [a], Md. Golam Sarower [a,*]

[a] *Fisheries and Marine Resource Technology Discipline, Khulna University, Khulna 9208, Bangladesh*
[b] *Department of Fishing and Post Harvest Technology, Faculty of Fisheries, Chittagong Veterinary and Animal Sciences University, Chittagong 4225, Bangladesh*

Received 22 September 2016; accepted 19 November 2016

KEYWORDS

Probiotic;
Clostridium butyricum;
Pathogenic;
Antagonistic effect;
Growth;
Immunity

Abstract To determine antagonistic effect of *Clostridium butyricum* against *Vibrio harveyi* and its probiotic effect on growth performance, digestibility and immune response of fresh water prawn, *Macrobrachium rosenbergii* juveniles were examined following feeding with *C. butyricum* incorporated feed for 60 days. Significant reduction of *V. harveyi* growth was found at 8 hr and onward in *in-vitro* and at 10 days and onward in *in-vivo* challenge test. After rearing prawn with the bacteria in feed treatment for 60 days, body weight and growth rate of prawns was significantly higher ($p < 0.05$) than in control. Digestive protease and amylase activities in the gastrointestinal tract were also significantly ($p < 0.05$) higher than in controls. Immune response was a little high in treatment group, although it was not significant ($p > 0.05$) compared to control group. This study revealed that probiotic, *C. butyricum* incorporated diets were found to be beneficial for *M. rosenbergii* culture in terms of hindering the growth of pathogenic bacteria and increasing the growth, protease and amylase activities of prawn. Results from this study will be helpful to improve fresh water prawn farming.

* Corresponding author. Fax: +880 41 731244.
E-mail address: sarower@yahoo.com (M.G. Sarower).
Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

1. Introduction

Aquaculture has become the fastest-growing food-producing sector and is contributing significantly to global food supply, food security and national economic development over the last

http://dx.doi.org/10.1016/j.jksus.2016.11.003
1018-3647 © 2016 Production and hosting by Elsevier B.V. on behalf of King Saud University.

Please cite this article in press as: Sumon, M.S. et al., Growth performance, digestive enzyme activity and immune response of *Macrobrachium rosenbergii* fed with probiotic *Clostridium butyricum* incorporated diets. Journal of King Saud University – Science (2016), http://dx.doi.org/10.1016/j.jksus.2016.11.003

three decades. *Macrobrachium rosenbergii* is a species of aquaculture importance because of its several biological characteristics such as rapid growth, high fecundity, wide range of salinity and temperature tolerance (Johnson, 1982; New, 1995; Roustaian et al., 2001; Roychoudhury and Mukherjee, 2013). Fresh water prawn is one of the most important cultured species in Bangladesh. In the year of 2014–15, the production of prawn and shrimp was about 44278 MT that comprises 6.2% of the total fish production (National Fish week, 2016). It has a high commercial value in international market due to delicious healthy choice of food for human consumption. This is the second largest export industry after readymade garments, generating $504 million USD annually (National Fish week, 2016). But one of the major constraints behind the development of prawn aquaculture is their high mortality due to the outbreaks of different viral, fungal and bacterial diseases especially vibriosis caused by different *Vibrio* spp. including *V. harveyi*, *V. parahaemolyticus*, *V. alginolyticus*, *V. anguillarum*, *V. vulnificus*, *V. splendidus* that cause a devastating economic loss worldwide (Shruti and Soumya, 2012). Although a number of chemotherapeutic agents including antibiotics have played a major role in combating many diseases of cultured aquatic organisms, their undesirable consequences such as persistence of antibiotic residues in farm and development of antibiotic-resistant bacteria discourage the use of these chemotherapeutic agents. (Karunasagar et al., 1994; Chaithanya et al., 1999; Jernberg et al., 2010). In addition, use of excessive antibiotics in pond has an adverse effect on the aquatic environment. Many drugs used in the culture ponds to control diseases, may be taken up by and accumulated in the polyculture animals after resolving in the water, thus affecting the seafood safety (Biao and Kaijin, 2007).

In recent times, the use of probiotics has become a popular alternative to antibiotics for improving and maintaining a healthy environment that leads to more environment-friendly aquaculture practices (Gatesoupe, 1999). Because prawn possesses a non-specific immune response, probiotic treatments may provide a broader spectrum and greater non-specific disease protection through competitive exclusion and immune modulation (Rengpipat et al., 2000; Nayak, 2010). However in aquaculture, probiotics can be administered either as a food supplement or as an additive to water and have been shown to be effective in a wide range of species for the promotion of growth enhanced nutrition, immunity and survival rate (Moriarty, 1998; Venkat et al., 2004; Wang, 2007).

Clostridium butyricum, a mesophilic endospore-forming gram-positive bacteria is a suitable probiotic supplement in fish feed owing to its capacity to produce butyric acid as well as lactic acid and survive in the media of low pH and relatively high bile concentrations (Kong et al., 2011). It is widely being used as a probiotic for humans and animals in East Asian countries, such as Japan, Korea and China (Kamiya et al., 1997). Although a number of exogenous and commercial probiotics have shown to be effective in prawn and shrimp culture (Rengpipat et al., 2000; Balcazar et al., 2007), no hitherto report has been published yet on the effectiveness of endogenous probiotics in prawn culture. However, relatively less attention has been paid toward the selection and development of probiotic bacteria for the culture of *M. rosenbergii*. In addition to these, expensive commercial probiotics make prawn farmer difficult to its access for application in prawn culture. Therefore, the aim of the present research was to isolate probiotic from the native microflora associated with *M. rosenbergii* and to evaluate its probiotic potential. One bacterial strain such as *C. butyricum* was studied in terms of its antibacterial activity to pathogens in a culture system and ability to enhance the growth, digestibility and immunity of the juveniles of *M. rosenbergii*.

2. Methods and material

2.1. Bacterial species isolation and characterization by PCR

Probiotic bacteria (*C. butyricum*) *and* Pathogenic bacteria (*Vibrio harveyi*), isolated from the intestinal tract of *M. rosenbergii* were subjected to characterize by PCR. Characterized isolates of *C. butyricum* and *V. harveyi* were kept as stocks and served as potential probiotic bacteria and pathogenic bacteria, respectively.

2.1.1. Isolation and characterization of pathogenic bacteria

V. harveyi was isolated from the intestinal tract of *M. rosenbergii*. ISO method was followed in this case. 0.1 mL stock solution of intestinal tract was taken and mixed with 0.9 mL alkaline saline peptone water (ASPW) in sterilized test tube. Then the mixture was incubated at 37 °C for 6 h ± 1. This was the first selective enrichment. After that, whole culture obtained in first selective enrichment was transferred into another test tube containing 10 mL ASPW. Then the solution was incubated at 41.5 °C for 18 h ± 1. This was the second selective enrichment. Serial dilution (tenfold) was done and 0.1 mL suitable dilution of each culture was inoculated in thiosulfate citrate bile and sucrose (TCBS) agar plates. The inoculated TCBS agar plates were incubated at 37 °C. After 24 h ± 3 h of incubation, the colonies of different plates were analyzed by PCR for characterizing *Vibrio harveyi* (ISO/TS 21872-1, 2007). Amplification of 16S rRNA gene specific for *Vibrio* spp. was performed using a pair of primer Vibr-f: 5′-CGGT GAAATGCGTAGAGAT-3′ and Vibr-r: 5′-TTACTAGC GATTCCGAGTTC-3′ (Tarr et al., 2007). A standard PCR was executed with a 50 μL reaction mixture containing 50 ng of template DNA, Top DNA Polymerase (Bioneer Corporation, Daejeon, Korea) at 0.5 U/μL, 2.0 mM $MgCl_2$, 0.4 μM primers, 200 μM dNTP and 1X buffer as recommended by the manufacturer. PCR parameters were carried out in a thermal cycler (C1000TM, BIO-RAD, USA) as follows: predenaturation at 94 °C for 4 min, followed by 35 cycles of denaturation at 94 °C for 1 min, annealing at 60 °C for 1 min, and extension at 72 °C for 1 min. The whole PCR parameters were terminated by a final extension step at 72 °C for 10 min. The PCR products were analyzed by gel electrophoresis in 2% agarose (Bioneer Corporation) containing 0.5 μg/mL ethidiumbromide in TAE buffer. DNA bands were visualized by High Performance UV Transilluminator (UVP, CA, USA) and photographed using the DigiDoc-It 120 gel documentation system (UVP). To compare the size of double stranded DNA from 100 to 2,000 base pairs, 100 bp designed DNA markers were used. The DNA marker consists of 13 double stranded DNA fragments ranging in sizes from 100 to 1,000 (Bioneer, Korea).

Please cite this article in press as: Sumon, M.S. et al., Growth performance, digestive enzyme activity and immune response of *Macrobrachium rosenbergii* fed with probiotic *Clostridium butyricum* incorporated diets. Journal of King Saud University – Science (2016), http://dx.doi.org/10.1016/j.jksus.2016.11.003