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Isolation and Characterization of Coccus Shaped Bacteria Causing Tail Rot Disease in Freshwater Prawn, *Macrobrachium rosenbergii* (de Man) in Haryana, India

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ABSTRACT

The rapid expansion of the prawn farming industry is plagued by diseases affecting shrimp survival and growth. The bacterial pathogens have been found to play an important role in shrimp production. The gram-positive, ovoid bacterial pathogens (Streptococcus grp Q1, Staphylococcus aureus, Micrococcus luteus 1, M. varians and Cellobiosococcus scIuri) were isolated and identified from diseased freshwater prawn/shrimp, Macrobrachium rosenbergii in Ladwa farm, Hisar (Haryana). The diseased prawns displayed rottening of tail and antennular flagellum. The genus and species of the bacterium were characterized and identified by biochemical tests and cofirmed with the help of Computer Programme PIBWin.

Key words: *Physico-chemical parameters, Coccus shaped bacteria, Tail rot, Freshwater prawn/shrimp, Macrobrachium rosenbergii (de Man).*

INTRODUCTION

With the recent progress in aquaculture, intensive systems used for marine organisms create an artificial environment that increases bacterial growth. Bacteria take advantage of ecological changes introduced in the aquaculture practice and cause periodic disease[20]. Most of these bacterial species are part of the autochthonous flora of marine organisms and their ecosystem and therefore, a constant source of possible infection for crustacean, fish and bivalve etc. Different opportunistic bacteria have been reported to cause a serious loss in shrimp production, having specific effects in shrimp motality, tissue lesion, body malformation, slower growth and larval metamorphosis[14]. Bacterial and viral infections are among the most common diseases encountered in shrimp farms, and are usually associated with poor management or environmental conditions[6]. Outbreaks of disease in prawns are often attributed to bacterial infection, as in many cases, bacteria may be readily recovered from diseased prawns. However, bacterial infection of prawns commonly occurs as a sequel to disease due to environmental, nutritional, traumatic or other factors[15].

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The most frequent is damage to the exoskeleton due to fighting or mishandling, sometimes leading to infection. This damage can be repaired when the old exoskeleton is shed during molting. Likewise broken chelae, legs, or antennae will eventually be regenerated at the next molt[11,8]. Varieties of diseases were reported in larval, juvenile and adult freshwater prawn (scampi). This is due to shorter life cycle and faster metabolism of these animals, which results in weaker resistance to pathogens. Prawns are highly sensitive to pathogens during molting period when they cannot feed, have a weak body and poor mobility. The incidence of disease is higher in low quality water, particularly if oxygen levels are low. Prawns need good quality water and enough plant life[1,3,7,12,22,23]. Recently freshwater prawn has been introduced in Haryana, and significant incidences of disease were observed[10]. The present study was carried out from July to December, 2005 to identify and characterizes bacterial pathogens causing tail rot disease in freshwater prawns. The objectives of the present study were: (i) Analysis of pond water for physico -chemical parameters. (ii) Phenotypic and biochemical characterization of bacterial pathogens associated with tail rot and rottening of antennular flagellum of freshwater prawn.

MATERIAL AND METHODS

Collection of material and characterization of disease

Black spot disease was reported during the period of observation in freshwater prawns (*Macrobrachium rosenbergii*), collected from Ladwa fish farm, Hisar (Haryana). These diseased prawns were brought to the Fish Biotechnology Laboratory of the Department of Zoology and Aquaculture, CCS Haryana Agricultural University Hisar, Haryana (India). The outer morphology of these infected prawns was carefully examined and disease characters were noted.

Culture and isolation of pathogenic bacteria

The tissue samples from infected prawns were homogenized in a sterilized macerating tube. The supernatant was poured and spread over the nutrient medium under aseptic conditions for bacterial isolation. The plates were then incubated in B.O.D at 30±2°C for 24 hours. Growth on cultured plate was observed after 24 hours. Pure colonies of bacteria were obtained by further subculturing these on nutrient/ soy/ marine agar. Pure colonies thus obtained were subjected to various biochemical tests for the identification of causative bacteria.

Identification of bacteria

Isotated pure cultures were subjected to a number of primary and secondary biochemical tests[28,27,26]. To diagnose/ identify the disease causing agents each isolate was given an identification ID score with the help of a computer programme, PIBWin[4]. These ID scores were compared with model scores, and the causative bacteria were identified accordingly. These bacteria were confirmed when they showed growth on specific medium (Table 1).

RESULTS AND DISCUSSION

Major objective of this study was to isolate the disease causing pathogens from freshwater prawn, *M. rosenbergii*. In Haryana, traditional aquaculture, in recent years, has entered into commercial activity involving heavy inputs and therefore, diseases of all kinds are now known to occur on an increasingly large scale. However, prawn mortality is not the only criterion to evaluate the effect of shellfish disease. Even the morbidity, which leads to weight losses and poor growth in surviving shellfish, contribute substantial losses to the farms.

Analysis of pond water for physico-chemical parameters:

Poor culture conditions such as overcrowding, poor water exchange, elevated temperature and poor diet were reported in an increase in the incidences of shell disease[2]. With rapid development in hatchery production of juveniles and the number of prawn growout farms, good husbandary and environmental management have often been neglected. Consequently, disease problems developd as

prawn were stressed and weakened under adverse environmental conditions[9,16].

In the present investigations, the level of infection was ranging from 0.9 (July, 05) to 2.5 (October, 05) in freshwater prawns. The physico-chemical parameters are presented in (Table1) and the overall correlation matrix between the level of infection and various parameters are presented in (Table 2). The physico-chemical parameters like pH (r = -0.673), DO (r = -0.308), TDS (r = -0.218) were found negatively correlated with the level of infection and salinity (r = 0.324), temperature (r = 0.427), turbidity (r = 0.779) and EC (r = 0.541) were found positively correlated with the level of infection in freshwater prawn, *M. rosenbergii*. The flucatuations in physico-chemical parameters from optimum range leads the prawn under stress conditions and were probably conducive to disease causing pathogens. Hence, it is clear that the prawn survival in the ponds is dependent on the various physico-chemical parameters.

Identification of disease, Phenotypic and biochemical characterization of bacterial pathogens of freshwater prawn:

The diseased individuals of freshwater prawn, *M. rosenbergii* showed tail rot and rottening of antennular flagellum in postlarval, juvenile and adult stages (Plate 1, Plate 2 and Plate 3).

In the present studies, five bacterial pathogens (*Streptococcus* grp Q1, *Staphylococcus aureus*, *Micrococcus luteus* 1, *M. varians* and *Cellobiosococcus sciur*) were isolated from diseased freshwater prawn, *M. rosenbergii* in Ladwa farm, Hisar. These bacteria were confirmed when they showed growth on specific medium (Table 1). *Micrococcus luteus*1, *M. varians* and *Cellobiosococcus sciuri* were gram-positive, ovoid, aerobic, non fermenters, catalase and oxidase positive. Both *Streptococcus* grp Q1 and *Staphylococcus aureus* were gram-positive, ovoid, aerobic, fermenters, oxidase negative and catalase positive except *Streptococcus* grp Q1. The phenotypic and biochemical tests of these isolates were subjected to a characterization of the pathogenic bacteria with the help of Computer Programme, PIBWin[4] and their probabilistic identification (ID) scores were determined ID modal scores. The ID scores were 0.99966, 0.90954, 0.99620, 0.99404, 0.99995 of *Streptococcus* grp Q1, *Staphylococcus aureus*, *Micrococcus luteus* 1, *M. varians* and *Cellobiosococcus sciur* respectively (Table 1). Thus, five pathogenic bacteria were found to present in the infected tissues of diseased prawns at Hisar.

Gill fouling and tail rot diseases were common in infected prawns[21]. Tail rot in prawns is a condition arose from initial infection and ulceration exacerbated by poor pond conditions or aggressive behaviour[17]. *Epistyles* and *Staphylococcus* caused tail rot in shrimps and *Epistyles* alone caused the rottening in antennular flagellum[18] found associated with diseased freshwater prawns and the commensal microflora of freshwater prawn included opportunistic pathogens such as *Aeromonas* spp. and *Streptococcus* spp[13]. The bacteriological examination of affected post larvae showed the presence of *Staphylococcus* spp. as a predominant organism in diseased freshwater prawn, due to which the incidence of post larval mortalities of 30–100% was reported from commercial freshwater prawn, *M. rosenbergii* (De Man) hatcheries in Andhra Pradesh and Tamil Nadu since 2001[24].

Table 1: Phenotypic and biochemical characterization of coccus shaped (GPACOC) bacteria isolated from diseased freshwater prawn, *M. rosenbergii* during July to December, 2005.

Biochemical tests	Isolate no. 1	Isolate no.2	Isolate no. 3	Isolate no. 4	Isolate no. 5
Primary tests					
Gram reaction	+	+	+	+	+
Shape	Cocci in clusters	Cocci in clusters	Cocci in clusters	Cocci in chains	Cocci in clusters
Colour of colony	Yellow	Yellow-orange	White-cream	White-cream	White-cream
Aerobic	+	+	+	+	+
Anaerobic	-	-	-	+	+

Catalase	+	+	+	-	+
Oxidase	+	+	+	-	-
Glucose Fermentation	-	-	-	+	+
Secondary tests					
Urease	+	+	-	-	+
Simon citrate	+	+	-	-	-
Starch	-	-	-	+	-
Ehrlich indole	-	-	-	-	-
Nitrate-Nitrite	-	+	+	-	+
Adonitol	-	-	-	+	-
Xylose	-	-	-	+	-
Sucrose	-	+	+	-	+
Sorbitol	-	-	+	+	+
Arginine dihydrolase	-	-	-	-	+
Lactose	-	+	+	+	+
Maltose	-	+	+	+	+
Mannitol	-	-	+	+	+
Cellobiose	-	-	+	+	-
Glycreol	-	-	+	+	+
Inositol	-	-	+	-	-
Tryptophan	-	-	-	-	-
Glucose	+	+	+	+	+
Fructose	+	+	+	+	+
Voges-Proskauer 37^0C	+	+	+	+	+
Inuline	-	-	-	-	-
Galactose	-	+	+	+	+
Specific medium					
Hugh Leifson glucose medium and Fermentation medium	+	+	-	-	-
Antibiotic Assay Medium. C	-	-	+	-	-
Azide Blood agar base	-	-	-	+	-
Manitol salt agar	-	-	-	-	+
Bacteria identified	*Micrococcus luteus*1	*M. varians*	*Cellobiosococcus sciuri*	*Streptococcus* grp Q1	*Staphylococcus aureus*
ID Score	0.99620	0.99404	0.99995	0.99966	0.90954
ID Modal Score	1.00000	1.00000	1.00000	1.00000	1.00000

+ growth took place, - growth not observed

Plate 1. Tail rot and rottening of antennular flagellum

Fig.1 Freshwater prawn, *Macrobrachium rosenbergii* (de Man)
Post larval stage

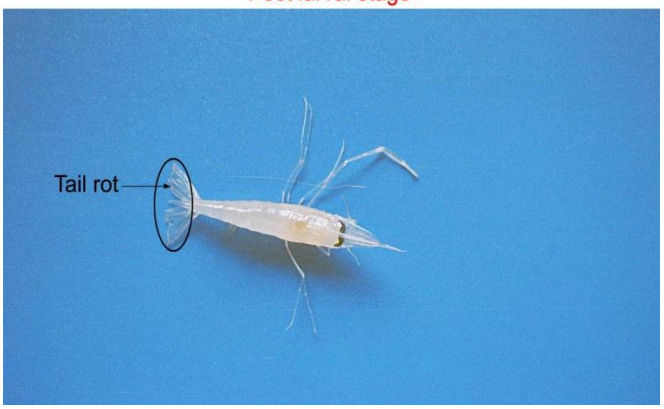

Plate 2. Tail rot and rottening of antennular flagellum

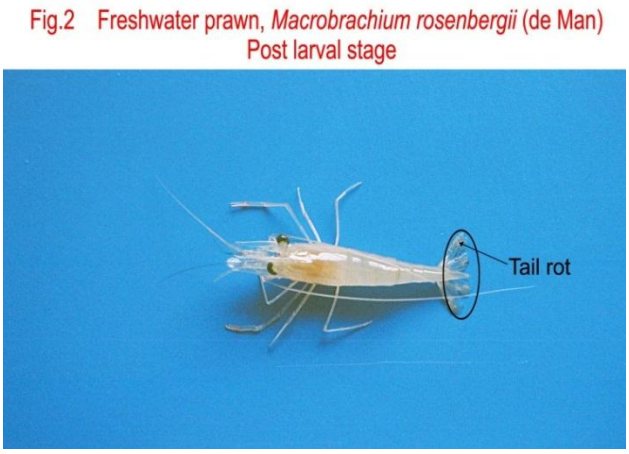

Plate 3. Tail rot in adult prawn

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