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Vibriocin Production by Marine Prawn Associated *Vibrio* spp

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ABSTRACT

*Vibrio*s are the most common genera associated with crustaceans. The genus *Vibrio* were isolated from marine prawn (*Penaeus monodon*). The isolates were screened for vibriocin production. The best producer was identified as *Vibrio parahaemolyticus* .The maximum production of *Vibrio* spp. was manifested at 37ºC at pH 9, after 20h of incubation. The *Vibrio* spp. was confirmed by the presence of biochemical tests methyl red, VP and oxidase test. Its activity was enhanced in the presence of TCBS medium and withstands autoclaving temperature and showed activity even after prolonged chloroform treatment.

Key words: Vibriocin, marine prawn, biochemical key.

INTRODUCTION

Aquaculture is an emerging industrial sector which requires continued research with scientific and technical developments and innovation (Faruque and Nair, 2003). Vibriocin are a group of bacteriocin produced and active against gram-negative bacteria in the genus *Vibrio*. They are curved shaped motile organism with single polar flagella (Thompson at al., 2004). *Vibrios* constitute one of the most common bacteria in surface waters. They are ubiquitous in the aquatic environment and are commonly present or a shellfish and other sea food. *Vibrio* are non-invasive pathogens, they cause some of the most serious cases of diarrhea in humans. These waterborne organisms are transmitted to humans via infected water or through fecal transmission (Chythanya *et al*., 2002).The present study report the production of vibriocin in marine prawn *Penaeus monodon* associated *Vibrio* spp.

MATERIAL AND METHODS

Sample collection

Marine prawn samples *Penaeus monodon* were collected from Nagappatinam District, Tamil Nadu, and East Coast of India. The samples were taken into sterile bags, kept in ice transport to the laboratory.

Identification of *Vibrio* species

Gut region of prawn sample was dissected and the suspended in 100ml of saline. Samples were taken from the suspension were serially diluted and plated on TCBS medium agar to get isolated colonies. From the TCBS medium, the green colonies isolated were selected and identified by gram staining.

Screening for vibriocin production

Vibrio isolates were grown in LB broth and inoculated at 37ºC for 24h. The cells were dissected and the supernatant fluid was overlaid with 3mL soft agar containing $1x10^6$ cells of indicator organisms. Wells (5mm diameter) were cut and 100µL of supernatant fluid of the test organism were poured into each well. Next day zone of inhibition was measured (Godic and Bagovic,2003).

Choice of better producer

The *Vibrio* strains were screened for vibriocin production against the organisms. Since the strain *Vibrio parahaemolyticus* was manifesting the largest zone of inhibition, hence selected for detailed studies.

Biochemical and Physio-chemical characterization

The isolates were identified at the species level with theuse of biochemical tests (Manero and Blanch, 1999). To check the thermal stability, the *V.parahaemolyticus* was exposed to 60ºC (60 min), 100ºC (20 min) and 121ºC (15 min) and activity was checked by agar –well diffusion method. To observe the effect of chloroform on the vibriocin activity 24h old culture were exposed to chloroform vapours for 10-30min, overlaid with sensitive cells and then next day zone size was measured (Ahmed and Rasool,2003).

RESULTS AND DISCUSSION

Vibrio strain growed on TCBS media and give green colour colonies isolated from the gut region of *Penaeus monodon* prawn. In order to identify the producer strain series of morphological and cultural tests were done as given in table-1.

The *Vibrio* strains were screened for vibriocin production by agar-well diffusion method. Carraturo *et al* (2006) reported that out of forty five halophilic *Vibrio* spp. (screened for antimicrobial production) only one strain i.e. *V.mediterranei* showed antimicrobial activity.

Table 1:Phenotypic characters used for the identification of *V.parahaemolyticus*

Tests	Characteristics
Morphological Characterstics	
Gram-reaction	Gram-negative
Shape	Rod or curved-rod shaped
Arrangement	Scattered
Motility	Actively motile
Cultural characterstics	
Growth on TCBS	Green colonies
Biochemical characterstics	
Methyl-red test	+
Urease test	–
VP test	+
Indole test	–
Catalase test	–
Oxidase test	+

Table 2: Phytochemical characterization of *Vibrio* spp

Treatments	Zone of inhibition (mm) Temperature
60ºC	18
121ºC	16
100ºC	15
Chloroform	
Chloroform (10-30min)	17

From the present study, the biochemical characteristics *Vibrio* species was tested and showed in table-1.This confirmed the presence of the *Vibrio* starin. Telesmanich *et al* (2000) reported that vibriocin produced by *V.cholerae* on 01 strain P-11702 was inhibitory to many gram-bacteria including S.flexneri, S.sonnei, *V.cholerae* and *E.coli* strain.

Vibriocin production was subjected to different heat treatments and consequently was found to be thermostable. *V.parahaemolyticus* was found stable at autoclaving temperature (121ºC, 15

psi for 15min) table-2. Earlier study, Parasad *et al* (2005) worked on a novel BLIS from a pathogenic strain of *V.harveyi* and found it stable at 60ºC for 10min.V.parahaemolyticus was found resistant to chloroform. Further there was no increase or decrease in the activity of the vibriocin of the chloroform treatment.

Present study reports the ability of *V.parahaemolyticus* to produce a vibriocin inhibitory against the human pathogens. The properties of resistance to wide range of temperature, stability in the presence of chloroform vapours and production in different physiological conditions suggest, its potential application in food and medical microbiology.

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