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NOTE

Antimicrobial activity of chitosan against vibrios from freshwater prawn *Macrobrachium rosenbergii* larval rearing systems

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ABSTRACT: Chitosan is a biocompatible and biodegradable natural polymer with established antimicrobial properties against specific microorganisms. The present study demonstrates its antibacterial activity against 48 isolates of *Vibrio* species from prawn larval rearing systems. The antibacterial activity had a positive correlation with the concentration of chitosan. This work opens up avenues for using chitosan as a prophylactic biopolymer for protecting prawn larvae from vibriosis.

KEY WORDS: Vibriosis · Chitosan · *Macrobrachium rosenbergii* · Antimicrobial

INTRODUCTION

Chitosan is a cationic polysaccharide derived from chitin, a natural polymer of N-acetyl glucosamine commonly found in crustacean and insect exoskeletons, and in fungal cell walls (Shepherd et al. 1997). It is a biocompatible and biodegradable natural polymers and has interesting biological activity (Akbuga 1995). There are several reports on the anti-microbial activity of chitosan against several species of bacteria, yeasts and fungi (Allan & Hadwiger 1979, Kendra & Hadwiger 1984, Sudarshan et al. 1992, Wang 1992, Roller & Covill 1999, Zheng & Zhu 2003). It has been suggested that its antibacterial effect is based on its ability to increase permeability of the outer membrane of Gram-negative bacteria (Sudarshan et al. 1992, Chirkov 2002). In addition, it has wound healing properties, and has been used in cosmetics, drug delivery, food protection, and as an immunostimulant (Sahoo & Mukergee 1999, Ravi Kumar 2000). However, its application for disease management in aquaculture has not been considered.

The present study describes the antimicrobial activity of chitosan against 48 isolates of *Vibrio* spp. from *Macrobrachium rosenbergii* larval rearing systems. In India, 71 freshwater prawn hatcheries currently under operation have a production capacity of 1.83 billion post larvae (Bojan 2003), but larval production technology has not yet been perfected. One problem is recurrent vibriosis (Sindermann 1977), which reduces larval survival below the level of economic viability. Therefore, management of vibriosis is of great concern in prawn hatchery systems. In general, *Vibrio* spp. are prevalent on eggs, larvae and post larvae of *M. rosenbergii* (Bhat & Singh 1999), and their increase in number during culture operations is a serious problem (Delves-Broughton & Poupard 1976, Takahashi et al. 1985).

The use of antibiotics to control vibriosis in shrimp/prawn hatcheries has been documented (Karunasagar et al. 1994). However, prophylactic use of antibiotics can lead to the emergence of antibiotic-resistant bacteria (Tendencia & de la Pena 2001). Therefore, the industry needs alternative strategies to combat vibrio-

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sis with minimal negative environmental impact. It was in this context that chitosan was considered as a possible prophylactic agent.

MATERIALS AND METHODS

Chitosan. Chitosan used in this study was obtained from M/s South India Sea Foods, Kochi, Kerala, India. It was extracted from crustacean exoskeletons, had an average molecular weight of 180 kDa and was 80% de-acetylated. Different concentrations of chitosan (0.25, 0.5, 0.75 and 1.0%) were prepared by dissolving in 50 ml 5% glacial acetic acid (v/v) (Kubota 1993) up to 100 ml using distilled water. The pH was adjusted to 5.5–6.0 using 1 N NaOH.

Vibrio. The 48 isolates of vibrios used in this study were taken from the culture collection of the Centre for Fish Disease Diagnosis and Management, Cochin University of Science and Technology Kochi, Kerala, India. These isolates were accumulated over time from freshwater prawn larval rearing systems, and characterized phenotypically. All the isolates were grown in ZoBell's Marine Broth (2216E) prepared in 15 ppt salinity seawater for 12 to 15 h on a rotary shaker at 100 rpm. They were harvested at the exponential phase of growth, diluted to 10^{-6}, and used for assaying the antibacterial properties of chitosan.

Antibacterial assay. Antibacterial activity was measured following the method of Zheng & Zhu (2003) with slight modification. Briefly, ZoBell's Marine Agar (2216E) plates were prepared using 15 ppt seawater. Then 100 µl *Vibrio* suspension was spread on the plates followed by 100 µl of chitosan preparation in 5% glacial acetic acid (pH 5.5 to 6.0). Controls were identical except that 100 µl of acetic acid solution (pH 5.5 to 6.0) replaced the chitosan solution. All plates were incubated at 28 ± 1°C for 24 h before the total number of colonies was enumerated. Inhibition rate (η) was calculated using the equation:

$$\eta = \frac{N_1 - N_2}{N_1} \times 100\%$$

where N_1 and N_2 are the number of colonies developed on the control and experimental plates, respectively.

Statistical study. Karl Pearson's coefficient of correlation was used to assess the relationship between the concentration of chitosan and antimicrobial activity. Student's *t*-test was used to assess differences at $p < 0.005$ (Bailey 1995).

RESULTS AND DISCUSSION

Results showed that there was an increase in antimicrobial activity with increasing chitosan concentration ($p < 0.005$) (Table 1). Even though many cells survived at 1% chitosan (Fig. 1), the highest concentration of chitosan used inhibited *Vibrio vulnificus* by 88.8 ± 14.6%, whereas the same concentration inhibited *Vibrio alginolyticus* by only 50.8 ± 19.8%. Major factors believed to contribute to the antimicrobial properties of chitosan are concentration of the chitosan in solution, molecular weight, degree of deacetylation

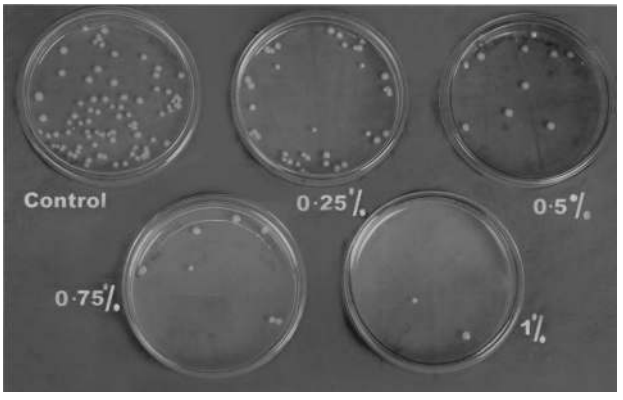

Fig. 1. Effect of different concentrations of chitosan solution on its antimicrobial activity to an isolate of *Vibrio*

Table 1. Inhibition rate (mean ± SD) of *Vibrio* isolates to different concentrations of chitosan(w/v). Correlation coefficient is that between concentration of chitosan and cell count of *Vibrio*

Vibrio isolate	No. of strains	0.25%	0.5%	0.75%	1.0%	Correlation coefficient
V. cholerae	11	55.7 ± 30.8	75.5 ± 23	81.5 ± 19.6	85.4 ± 21.2	0.931
V. parahaemolyticus	5	39.2 ± 26.3	50.2 ± 25.8	57.4 ± 30.8	69.1 ± 22.3	0.996
V. mediterranei	6	51.1 ± 27.2	63.7 ± 26.7	73.9 ± 20.5	80.4 ± 19.7	0.990
V. nereis	11	63.6 ± 20.7	73.1 ± 13.9	81.8 ± 9.6	84.2 ± 10.3	0.973
V. proteolyticus	2	59 ± 36.3	65.2 ± 27.2	72.7 ± 22	88.1 ± 12.4	0.975
V. splendidus	2	26.2 ± 9.8	29.3 ± 2.8	49.7 ± 2.7	68.0 ± 13.7	0.966
V. vulnificus	3	46 ± 41.2	63 ± 29.3	80.1 ± 26.1	88.8 ± 14.6	0.990
V. alginolyticus	8	21.9 ± 19.8	29.2 ± 16.1	41.1 ± 19.9	50.8 ± 19.8	0.996

and the level of protonation of the free groups in the chitosan. Using chitosan at different viscosity average molecular weights ranging from less than 5 to 350 kDa on *Escherichia coli* and *Staphylococcus aureus*, Zheng & Zhu (2003) concluded that its antibacterial properties were directly related to its concentration.

Chitosan in the larval rearing system may function as a *Vibrio* growth depressant. As the risk of infection is directly related to pathogen density, depressed cell counts may help to prevent larval vibriosis. Further, chitosan is recognized as an immunostimulant in fish (Sahoo & Mukergee 1999, Siwicki et al. 1994); thus, it may also be worthwhile to test it as an immunostimulant in prawn larvae .

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