NOTE

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

A. Anas¹, S. Paul¹, N. S. Jayaprakash^{1,3}, R. Philip², I. S. Bright Singh^{1,*}

¹Centre for Fish Disease Diagnosis and Management (CFDDM), and ²Department of Marine Biology Microbiology and Biochemistry, Cochin University of Science and Technology (CUSAT), Kochi 682 016, Kerala, India

³Present address: Centre for Bioseparation Technology, Vellore Institute of Technology, Vellore 632 014, Tamil Nadu, India

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

- Resale or republication not permitted without written consent of the publisher

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-

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 μ l *Vibrio* suspension was spread on the plates followed by 100 μ l of chitosan preparation in 5% glacial acetic acid (pH 5.5 to 6.0). Controls were identical except that 100 μ l 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 $88.8 \pm 14.6\%$, whereas the same concentration inhibited *Vibrio alginolyticus* by only $50.8 \pm 19.8\%$. 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 \pm 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.

Acknowledgements. This work was supported by a grant from the Department of Science and Technology, Government of India (Grant No. SP/SO/C-40/99). We thank Dr. A. Mohandas, Emeritus Professor, CFDDM, CUSAT for editing the manuscript. A.A. and N.S.J. thank DST for fellowship support.

LITERATURE CITED

- Akbuga J (1995) A biopolymer: chitosan. Int J Pharm Adv $1{:}3{-}18$
- Allan CR, Hadwiger LA (1979) The fungicidal effect of chitosan on fungi of varying cell wall composition. Exp Mycol 3:285–287
- Bailey NTJ (1995) Statistical methods in biology. Cambridge University Press, Cambridge
- Bhat SG, Singh ISB (1999) Vibrionaceae associated with larvae and larval rearing systems of *Macrobrachium rosenbergii*: systematics and pathogenicity. In: Flegel TW (ed) Advances in shrimp biotechnology. National Centre for Genetic Engineering and Biotechnology, Bangkok, p 279
- Bojan J (2003) Status of scampi farming in India. Fresh water prawns 2003. College of Fisheries, Kearala Agricultural University, Kochi
- Chirkov SN (2002) The antiviral activity of chitosan. Appl

Editorial responsibility: Timothy Flegel, Bangkok, Thailand

- Biochem Microbiol 38:1-8
- Delves-Broughton J, Poupard CW (1976) Disease problems of prawn in recirculation systems in UK. Aquaculture 7:201–217
- Karunasagar I, Pai R, Malathi GR, Karunasagar I (1994) Mass mortality of *Penaeus monodon* larvae due to antibiotic resistant *Vibrio harveyi* infection. Aquaculture 128: 203–209
- Kendra DF, Hadwiger LA (1984) Characterization of the smallest chitosan oligomer that is maximally antifungal to *Fusarium solani* and elicits pisatin from *Pisum sativum*. Exp Mycol 8:276–281
- Kubota N (1993) Molecular weight dependence of the properties of chitosan and chitosan hydrogel for use in sustained release drug. Bull Chem Soc Jpn 66:1807–1812
- Ravi Kumar MNV (2000) A review of chitin and chitosan applications. React Funct Polym 46:1–27
- Roller S, Covill N (1999) The antifungal properties of chitosan in laboratory media and apple juice. Int J Food Microbiol 47:67–77
- Sahoo PK, Mukergee SC (1999) Influence of the immunostimulant, chiosan on immune responses of healthy and cortesol treated Rohu (*Labeo rohita*). J Aquacult Trop 14:209–215
- Shepherd R, Reader S, Falshaw A (1997) Chitosan functional properties. Glycoconj J 14:535–542
- Sindermann CJ (1977) Disease diagnosis and control in North American marine aquaculture. Elsevier, Amsterdam
- Siwicki AK, Anderson DP, Rumsey GL (1994) Dietary intake of immunostimulants by rainbow trout affects nonspecific immunity and protection against furunculosis. Vet Immunol Immunopathol 41:125–139
- Sudarshan NR, Hoover DG, Knorr D (1992) Antibacterial action of chitosan. Food Biotechnol 6:257–272
- Takahashi Y, Shimoyama Y, Momoyama K (1985) Pathogenicity and characteristics of *Vibrio* sp. isolated from diseased post larvae of Kuruma prawn, *Penaeus japonicus* Beta. J Shimonoseki Univ Fish 32:23–31
- Tendencia EA, de la Pena LD (2001) Antibiotic resistance of bacteria from shrimp ponds. Aquaculture 195:193–204
- Wang GH (1992) Inhibition and inactivation of five species of food borne pathogens by chitosan. J Food Prot 55:916–919
- Zheng LY, Zhu JF (2003) Study on antimicrobial activity of chitosan with different molecular weights. Carbohydr Polym 54:527-530

Submitted: January 12, 2005; Accepted: June 2, 2005 Proofs received from author(s): September 27, 2005