

Antibacterial Activity of Shrimp Chitosan against *Escherichia coli*, *Salmonella*, and *Pseudomonas aeruginosa* Isolated From Seafoods

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Antibacterial activity of shrimp shell based chitosan was determined against three food borne pathogens viz., *Escherichia coli*, *Salmonella*, and *Pseudomonas aeruginosa* isolated from seafoods. Tests were carried out in phosphate buffered saline (PBS) at pH 6.0. Four different concentrations, 0.01%, 0.03%, 0.05% and 0.1% (w/v) of chitosan were used against *E.coli* and *Salmonella*, and 0.01%, 0.05%, 0.5% and 1% were against *Paeruginosa*. Chitosan showed stronger bactericidal activity against *Salmonella* compared to *E.coli* and *Paeruginosa* as evidenced by 3 log cfu.ml⁻¹ reduction in *Salmonella* count with 0.01% chitosan concentration. There was complete elimination of 5 log cfu.ml⁻¹ cell count of *Salmonella* within 24 h at 0.1% chitosan level. There was 3 log reduction in *E.coli* count at 0.01% chitosan, but not completely inhibited even at the maximum used concentration (0.1%). The inhibition concentration against *Paeruginosa* was higher level compared to *Enterobacteriaceae* and it was found that reduction in 5 log cfu.ml⁻¹ was achieved only at 72 h of chitosan exposure. Different chitosan concentrations 0.05%, 0.1% and 1% had almost same inhibition pattern for *Paeruginosa* at 6h, 18h, 36h and 72h of exposure. The effectiveness of chitosan against tested pathogens showed *Salmonella* was most susceptible towards chitosan followed by *E.coli* and *Paeruginosa*.

Key words: Antibacterial activity, chitosan, shrimp, *Paeruginosa*, *Salmonella*, *E.coli*

Chitosan is deacetylated derivative of chitin, a high molecular weight polysaccharide found in abundance in shellfish processing waste. It is considered second most abundant natural biopolymer on earth next to cellulose and major sources are arthropods and crustaceans (Sandford, 1989). It is estimated that several billion tons of chitin are produced annually by crustaceans, which poses a waste disposal problem for shellfish processing industries due to ineffective utilization of resource material. Chitosan has received considerably attention for its commercial applications in biomedical and chemical industries (Knorr, 1994) and is

found to be more economical for removal of colour and organic compounds from wastewater than typical adsorbents like activated carbon (El-Geundi, 1997). Application of chitosan in the environmental industry includes its use as a coagulation agent for the recovery of protein, and polysaccharides from food processing wastewater (Muzzarelli *et al.*, 1990; Jun *et al.*, 1994). Recently, chitosan based gene delivery technology has been developed (Borchard, 2001).

Chitosan exhibits antibacterial activity against various bacteria (Sudershan *et al.*,

1992; Tsai & Su.,1999; Sugumar, *et al.*, 2003). Chitosan exhibit higher antibacterial activity against Gram-positive than Gram-negative bacteria. The antibacterial activity depends on the molecular weight and solvent used for dissolution (No *et al.*, 2002). Since, non-chemically modified chitosan dissolves in solvents at pH less than 6.5, a number of physico chemical properties in the reaction environments, such as pH, ionic strength and temperature affect the antibacterial activity. So far, no attempt has been made to study the antibacterial activity against seafood borne pathogens. The objective of this research work was to examine the antibacterial activity of shrimp chitosan against pathogens isolated from seafood.

Materials and Methods

Chitosan at 85% level of deacetylation was prepared from shrimp shell waste as per method of Madhavan & Nair (1974). All test organisms namely *Escherichia coli*, *Salmonella*, and *Pseudomonas aeruginosa*, were isolated from seafood and identified as per (USFDA, 2001 & Palleroni,1984). 2% (w/v) stock of chitosan solution was prepared in 0.1N HCl. Four different concentrations, 0.01%, 0.03%, 0.05% and 0.1% (w/v) of chitosan prepared in 100ml of phosphate buffered saline (pH 6.0) were used against *E.coli* and *Salmonella*. Similarly, different concentrations 0.01%, 0.05%, 0.1% and 1%(w/v) of chitosan in 100ml of phosphate buffered saline(PBS) at pH 6.0 were used for *Paeruginosa*. Controls PBS without chitosan at pH 6.0 were prepared for each test organisms along with the experimental sets. All flask including controls, containing different chitosan concentration were inoculated with a initial

bacterial cell load of 4-7 log cfu.ml⁻¹for *E.coli*, *Salmonella* and *Paeruginosa*, and incubated at 37°C in a shaking incubator. Cell count was determined on nutrient agar (Difco) by spread plate method at 0h, 3h, 6h, 18h, and 24h of exposure for *E.coli*, and *Salmonella*, whereas, for *Paeruginosa* cell count was determined at 0h, 3h, 6h, 18h, 36h, 54h, and 72h of exposure.

Results

On exposure to chitosan, *E.coli* with initial viable cell count 10⁵ cfu.ml⁻¹ showed sharp reduction in viable cell count after 3 h at different chitosan concentration levels. There was almost 3 log reduction in count at 0.01% chitosan level within 24 h and 4 log reduction in count at 0.1% chitosan (Fig.1). Complete destruction of *E.coli* was not achieved at any of the chitosan concentration used.

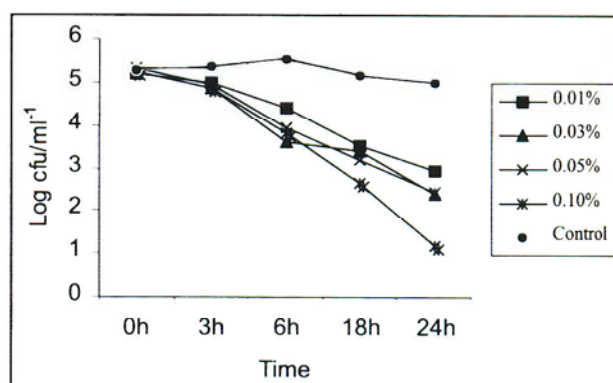


Fig. 1. Antibacterial activity of Chitosan against *E. coli*

The inhibition against *Salmonella* was more effective at different chitosan concentration levels and result showed that viable cell count was reduced very sharply at 0.01% chitosan. A 2 log reduction in viable cell count was observed after 24 h of exposure. There was 5 log cfu.ml⁻¹ reduction in *Salmonella* count at 0.1% chitosan with in

24 h of exposure and total inhibition was recorded (Fig.2).

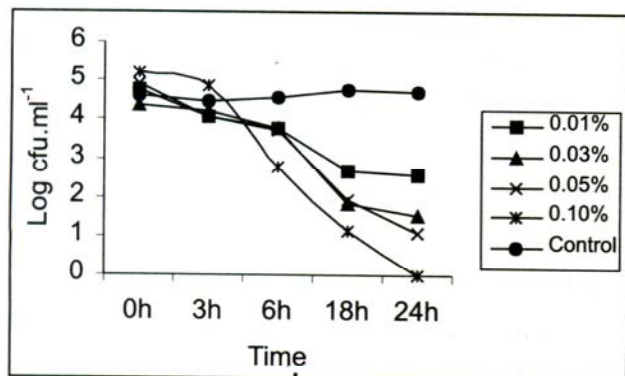


Fig. 2. Antibacterial activity of chitosan against *Salmonella*

The inhibition pattern of *Paeruginosa* viable count have been shown in fig. 3. The inhibition results showed that there was no inhibition of *Paeruginosa* cells at 0.01% chitosan and there was 4 log reduction in viable cells count at 1% chitosan level after 72 h. Complete inhibition did not occur even after 72 h at 1% level chitosan.

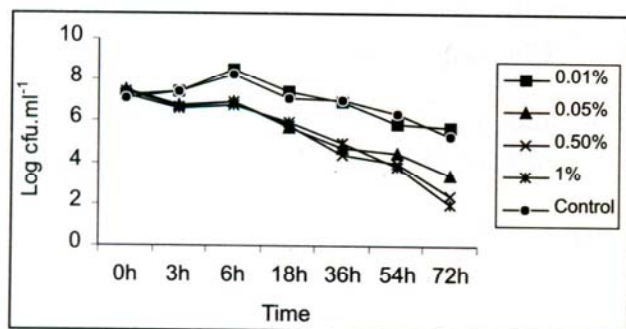


Fig. 3. Antibacterial activity of chitosan against *Paeruginosa*

Discussion

The antibacterial activity of chitosan against bacteria has been studied previously with *E.coli* as a model. The antibacterial activity of chitosan at 150 ppm (0.015%) against *E.coli* showed 5 log cfu.ml⁻¹ reduction in count with in 24h (Tsai & Su, 1999).

Similar result have been reported by several other workers (Wang, 1992; Tsia & Su, 1999; Sugumar *et al.*, 2003).The effect of abiotic factors on the antibacterial activity of waterborne pathogens showed that antibacterial activity of chitosan varied with various factors such as acid solvent, metal ions, pH and ionic strength (Chung *et al.* 2003). The age of a bacterial culture affected its susceptibility to chitosan, cells in the late exponential phase being most sensitive to chitosan, and also demonstrated the leakage of glucose and lactate dehydrogenase from *E.coli* cells after exposure to chitosan (Tsai & Su, 1999). In the present study, we found that 0.01% chitosan inhibited *E.coli* cell growth, Darmadji & Lzumimoto, (1994) reported higher concentration (0.1%) could inhibit *E.coli* growth. Wang (1992) reported that 0.5% chitosan was required for complete inhibition after 2 days of exposure at pH 5.5. These variations were suggested by Chang *et al* (1989) to be due to differences in molecular weight of chitosan used. They demonstrated that with 2800 KD chitosan, 0.2% would be required to inhibit *E.coli*, whereas only 0.01% was sufficient with 35KD chitosan. However, Stossel *et al.* (1984) showed high molecular weight chitosan to be more effective for the controlling growth of soil borne phytopathogenic fungi than those of low molecular weight chitosan.

The inhibition of *Salmonella* with 2.5% of chitosan has been Wang (1992) showed 3 log reduction in *Salmonella* count after 24 h incubation. Contrary to that, present study have shown similar reduction in *Salmonella* viable cell count at 0.01% chitosan concen-

tration within 24 h. *Pseudomonas* forms the dominant spoilage microflora in seafood samples, and it is also considered as a pathogenic bacteria. This organism shows inherent resistance to wide range of macromolecules (Kato *et al.*, 2001). The antibacterial effect of chitosan to some extent has proven this statement. Contrary to the findings of (Sugumar *et al.*, 2003), this investigation showed an initial increase in viable cell count upto 6 h of exposure at 0.01% chitosan, followed by gradual reduction in viable cell counts observed at 18 h, 36h, 54h, and 72h of exposure at 0.05%, 0.1%, and 1% chitosan concentration levels. The *Pseudomonads*, being very resistant to chitosan, required more than 0.01% of chitosan to inactivate it. Simpson *et al* (1997) demonstrated that antibacterial activity of chitosan was as low as 0.0075% towards certain microorganisms and successfully showed the utilization of chitosan for preservation of raw shrimp to improve shelf life.

The inhibitory activity of chitosan towards Gram-negative bacteria is considered to be due to chemical and structural properties of cell membrane. As a polymeric macromolecule, chitosan is unable to pass through outer membrane of Gram-negative bacteria, since this membrane functions as an efficient barrier against macromolecules. The main feature of chitosan is its positive charge at pH 6.3 that creates polycationic structure, which interact with the anionic components i.e. lipopolysaccharide and proteins of the Gram-negative bacteria (Nikiado, 1996). This study showed remarkable bactericidal ability of chitosan against food borne pathogenic bacteria at very low concentration level

(0.01%), but the inhibitory concentration of chitosan differed with individual bacteria. Chitosan has been approved as a food additive in Japan since 1983 and in Korea from 1995, respectively (Weinner, 1992; Anon., 1995). Chitosan has a significant value as a food preservative to prevent hazards associated with consumption of contaminated food with pathogenic bacteria and can also extend the shelf life of perishable food by inhibiting the growth of spoilage bacteria.

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