Journal of Bangladesh Chemical Society, Vol. 24(2), 185-190, 2011.
DOI: 10.3329/jbcs.v24i2.9707

IN VITRO ANTIBACTERIAL ACTIVITY OF SHRIMP CHITOSAN AGAINST *SALMONELLA PARATYPHI AND STAPHYLOCOCCUS AUREUS*

MD. MONARUL ISLAM[1,*], SHAH MD. MASUM[2] AND KHANDAKER RAYHAN MAHBUB[1]

[1]*Bangladesh Council of Scientific and Industrial Research (BCSIR), Dhanmondi, Dhaka-1205, Bangladesh*

[2]*Department of Applied Chemistry &Chemical Engineering, University of Dhaka, Dhaka-1000, Bangladesh*

Abstract

Antimicrobial properties of chitosan extracted from indigenous shrimp processing waste were determined against one gram-negative (*Salmonella Paratyphi*) and one gram-positive bacterium (*Staphylococcus aureus*) *in vitro*. The antimicrobial activities of chitosan were explored by calculation of the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) in media supplemented with 128, 138, 168, 192, 240, 288, 300 and 320 ppm chitosan solution adjusted to pH 6 or 7. The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the prepared chitosan was 288 and 300 ppm for both bacterial strains. These results indicate that chitosan from indigenous shrimp processing waste could be used as an effective antibacterial agent in the food industry.

Introduction

Chitosan, poly-β-(1-4)-2-amino-2-deoxy-D-glucopyranose is a deacetylated product of chitin β-(1-4)-2-acetamido-2-deoxy-D-glucan[1]. It has gained the attention of the scientific community due to its functional properties such as film-forming capabilities, mineral-binding properties, hypolipidemic activity, biodegradability, antimicrobial activity, immunoadjuvant activity, acceleration of wound healing, and eliciting of phytalexins[2]. Recently, research has shifted and focused on the possibility of developing chitosan as a natural disinfectant[3]. It can also be applied to extend the storage life of fresh fruit[4,5]. Much of the interest in the antimicrobial properties of chitosan has focused on the possibility of plant protection[6]. Chen *et al* applied chitosan as a natural disinfectant against waterborne pathogens and proved it to be promising[7]. Numerous studies on bactericidal activity of chitosan have been carried out[8-11] and reviewed[12,13]. It has been reported that antimicrobial properties of chitosan depend on its molecular weight and degree of deacetylation[14]. The antibacterial property of chitosan is particularly useful in the field of medicine where it can be used to make surgical accessories such as, gloves, bandages etc. It has also been used in the removal of waterborne pathogens in waste water and as a food preservative by applying a coat on the exterior of vegetable and fruit

*Corresponding author: Chemical Research Division, BCSIR Laboratories-Dhaka, Dhanmondi, Dhaka-1205, Bangladesh. E-mail: mmipavel@yahoo.com

products[15]. The goal of this study was to evaluate antimicrobial properties of chitosan, extracted from shrimp processing waste. About 30-40% by weight, shrimp raw material is discarded as waste when processed shrimp is headless, shell on products and these are toxic and hazardous for environment[16].

Materials and Methods

Materials

Shrimp shell waste materials were collected from Khulna, Bangladesh. Shrimp shells were scraped free of loose tissue, washed with cold water and dried in sun. All the chemicals purchased from Merck were analytical grade.

Preparation of chitosan and chitosan solution

Chitosan (DD 75%) was prepared from shrimp shell waste via chitin as reported data[1,17]. The typical production of chitosan from crustacean shell generally consists of three basic steps: demineralization, deproteinization and deacetylation. In the preparation of chitosan solutions, 2.4% (w/v) chitosans were dispersed in a 1.0% (v/v) acetic acid. After stirring overnight, the solutions were autoclaved at 120ºC for 15 min (thermostability under these conditions had been previously checked).

Microorganisms

The antibacterial activity of the prepared chitosan from shrimp shell was tested against two bacterial strains. They were *Staphylococcus aureus* (ATCC 25922) and *Salmonella Paratyphi* CRL, (ICDDR.B). *Staphylococcus aureus* is gram positive and *Salmonella Paratyphi* is gram negative bacteria.

Determination of antibacterial activity

Bacterial inoculums were prepared by Clinical and Laboratory Standards Institute (CLSI) guideline. Bacterial cultures were emulsified in normal saline and turbidity was matched with 0.5 McFarland turbidity standards. The agar cup method[18] was followed to investigate the antibacterial activity of the extracts. 0.1 mL of TSB broth culture of the test organisms were firmly seeded over the Mueller-Hinton Agar (MHA) plates. Wells of 6 mm diameter was punched over the agar plates using a sterile cork borer. The bottoms of the wells were sealed by pouring 50 - 100 µL of molten MHA into the scooped out wells. Using a micropipette, solution of chitosan was added to different wells in the plate. These plates were then kept at low temperature (4ºC) for 2-4 hours and incubated at 37 ºC for 24 hours. After the incubation period formation of zones around the wells, confirms the antibacterial activity of the respective extracts. All the results were compared with the standard antibiotic disc of Doxycyclin Hydrochloride 30µg.

Determination of MIC and MBC

Minimal inhibitory concentrations (MIC) were determined as the lowest concentrations of chitosan at which microorganisms cannot grow in Muller–Hinton (M–H) broth (Oxoid): based on the method of Ruparelia *et al*.[19], the strains were inoculated into M–H broth and incubated to the logarithmic growth phase at 37ºC. MIC and MBC values of chitosan against the test pathogens were determined by micro and macrodillution broth technique[20] using Mueller-Hinton medium (Table 2).

Results and Discussion

In this study, antibacterial activity of chitosan prepared from indigenous shrimp processing waste was tested against two strains *Staphylococcus aureus and Salmonella Paratyphi* in Muller–Hinton (M–H) broth. According to literature[21] chitosan possesses antimicrobial activity against a number of gram-negative and gram-positive bacteria. This study has been conducted to assess inhibitory effects of chitosan in terms of MIC and MBC. The Minimum Bactericidal Concentration (MBC) is the lowest concentration of antibiotic required to kill 99% of the germ[22]. Not as commonly seen as the Minimum inhibitory Concentration (MIC). It can be determined from broth dilution MIC tests by sub culturing to agar media without antibiotics. Antimicrobials are usually regarded as bactericidal if the MBC is no more than four times the MIC[23]. The results of antibiotic sensitivity are shown in Table 1.

Table 1. Antimicrobial activity and zone of inhibition of chitosan (mm).

Test organism	Concentration(ppm) growth in peptone broth				Doxycycline HCl (30µg)	R^2 Value
	288	240	192	168		
	Zone of inhibition in diameter(mm)					
Staphylococcus aureus	14	12	10	10	25	1
Salmonella Paratyphi	16	15	14	12	23	0.9643

The highest zone of inhibition against *Salmonella Paratyphi* and *Staphylococcus aureus* were found 16 mm and 14 mm respectively at the dose of 288 µg/ well. The regression (R^2) value of the relationship between dose and zone diameter was found higher for the pathogens tested (Table 1) ranging from 0.9643 and 1. The R^2 values (Fig.**1**.) indicate that there exist a linear relationship between dose used and zone diameter.

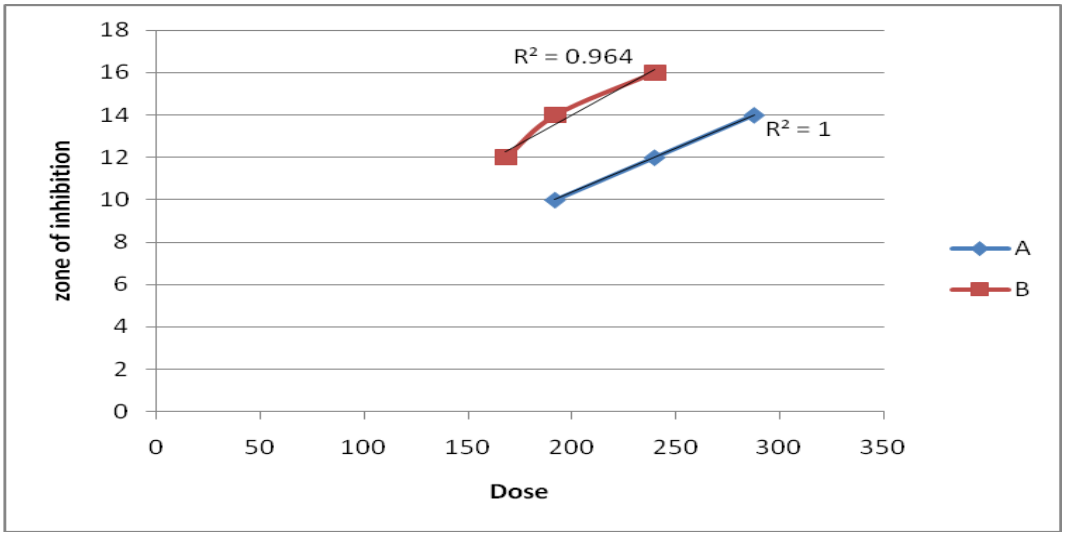

Fig. 1. Effect of different concentration of chitosan on (A) *S. aureus* and (B) *S. Paratyphi.*

Therefore better activity may be obtained by increasing the concentration of chitosan. The MBC and MIC values of the prepared chitosan were measured by macro and micro broth dilution techniques and results are presented in Table 2.

Table 2. MIC and MBC of prepared chitosan against *S. aureus and S. Paratyphi*

Test organism	Concentration(ppm) growth in peptone broth								MIC (ppm)	MBC (ppm)
	320	300	288	240	192	168	138	128		
Staphylococcus aureus	–	–	+	+	+	+	+	+	288	300
Salmonella Paratyphi	–	–	+	+	+	+	+	+	288	300

The MIC and MBC for the both types of strains are same. Many hypotheses have been proposed to explain the mechanism of antimicrobial effects of chitosan. One of hypotheses is that the mechanism involves interactions of chitosan positively charged molecules with negatively charged constituents of microbial cell walls and membranes interrupting normal cell metabolism[24]. Several authors indicated that chitosan may directly affect cell membrane function[25]. They showed that chitosan caused proteinous UV -absorbing materials to leak from cell membranes of *Pythium paroecandrum*, a plant pathogen[25]. The poly cationic chitosan available to bind to a charged bacterial surface causes in leakage of intercellular constituents and tends to form cluster of molecular

aggregation. The more adsorbed chitosan would result greater change in structure and in the permeability of cell membrane.

Conclusion

This study demonstrated that chitosan from indigenous source have excellent antibacterial activity against gram-negative (*Salmonella Paratyphi*) and gram-positive bacterium (*Staphylococcus aureus*). Chitosan could be a good source of drugs that may be used against bacterial infection.

Acknowledgement

Authors are grateful to former chief scientific officer Md. Asaduzzaman and Bazlul Karim Akanda for their worthy help and suggestion.

References

1. M. M. Islam, S. M. Masum, M. M. Rahman, M. A. Islam, A. A. Shaikh and S. K. Roy, *IJBAS-IJENS.,* 2011, **11(1)**,116-130.
2. S. Roller and N. Covill, *Int. J. Food.,* 1999, **47**, 67-77.
3. C. H. Kim, S. Y. Kim, and K. S. Choi, *Polym Adv Technol.,* 1997, **8**, 319-25.
4. A. EI-Ghaouth, R. Ponnampalam, F. Castaigne and J. Arul, *Hortscience*, 1992, **27**, 1016-1018.
5. A. EI-Ghaouth, J. Arul, A. Asselin and N. Benhamou, *Phytooath*, 1992, **82**, 398-402.
6. G. W. Gooday, *Carbohydr Eur.,* 1997, **19**, 18-22.
7. Y. M. Chen, Y. C. Chung, L. W. Wang, K. T. Chen and S. Y. Li, *J. Environ. Sci. Health A*, 2002, **37**, 1379-1390.
8. M. E. I. Badawy, E. I. Rabea, T. M. Rogge, C. V. Stevens, G. Smagghe, W. Steurbaut, et al., *Biomacromolecules.,* 2004, **5**, 589–595.
9. R. Muzzarelli, R. Tarsi, O. Filippini, E. Giovanetti, G. Biagini and P. E. Varaldo, *Antimicrob. Agents. Chemother.,* 1990, **34**, 2019–2023.
10. R. A. A. Muzzarelli, C. Muzzarelli, R. Tarsi, M. Miliani, F. Gabbanelli and Cartolari, *Biomacromolecules*, 2001, **2(1)**,165–169.
11. J. Rhoades and S. Roller, *Appl. Environ. Microbiol.* 2000, **66**, 80–86.
12. E. I. Rabea, M. E. T. Badawy, C. V. Stevens, G. Smagghe and W. Steurbaut, *Biomacromolecules*, 2003, **4(6)**, 1457–1465.
13. S. N. Chirkov, *Appl. Biochem. Microbiol.*, 2002, **38(4)**, 1–8 (review).
14. L. Y. Zheng and J. F. Zhu, *Carbohydr. Polym.*, 2003, **54(4)**, 527–530.
15. R. A. A. Muzzarelli, C. Jeuniaux and G. W. Gooday, *Chitin in Nature and Technology*, Plenum, New York, 1986.
16. S. Begum, K. Ikejima, H. Ara and M Z Islam, *J. Appl. Sci.,* 2006, **6(5)**,1220-1224.

17. R. H. Rodde, A. Enbu and K. M. Varum, *Carbohydr. Polym*., 2008, **71**, 388-393.

18. A. L. Barry, Procedure for testing antimicrobial agents in agar media. In: Antibiotics in laboratory medicine. Lorin V (eds), Williams Wilkins Co. Baltimore: USA, 1980, 1-23.

19. J. P. Ruparelia, A. K.Chatterjee, S. P. Duttagupta and S. Mukherji, *Acta Biomater*., 2008, **4**, 707–716.

20. N. R. Jones, L. A. Barry, L. T. Gavan, and J. A. Washington ii. 1985. Susceptibility tests: Microdilution and macrodilution broth procedures. In *Manual of Clinical Microbiology* (Lennette EH, Bellows A, Hausler WJ Jr & Shadomy HJ eds), 4th edn, 972-976, *American Society of Microbiology*, Washington DC.

21. Y. J. Jeon, P. J. Park and S. K. Kim, *Carbohydr. Polym.*, 2001, **44**, 71-76.

22. Medical microbiology, Mims and Playfair, Mosby Europe, 1993, **35**, 31.

23. G. L. French, *J. Antimicrob. Chemother., 2006,* **58 (6)**, 1107–17. doi:10.1093/jac/dkl393. PMID 17040922.

24. T. Savard, C. Beaulieu, Boucher and C. Champagne, *J. Food. Protect.* 2002, **65**, 828-833.

25. J. L. Leuba and P. Stossel, Chitosan and other polyamines: Antifungal activity and interaction with biological membranes. In: Muzzarelli, R., Jeuniaux, C., Gooday G.W. (Eds.), Chitin in Nature and Technology, Plenum Press, New York, 1986; 215-222.

(Received : 08 October, 2011; Accepted : 10 October, 2011)