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

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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-glucan1. 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 phytalexins2. Recently, research has shifted and focused on the possibility of developing chitosan as a natural disinfectant3. It can also be applied to extend the storage life of fresh fruit4,5. Much of the interest in the antimicrobial properties of chitosan has focused on the possibility of plant protection6. Chen et al applied chitosan as a natural disinfectant against waterborne pathogens and proved it to be promising7. Numerous studies on bactericidal activity of chitosan have been carried out8-11 and reviewed12,13. It has been reported that antimicrobial properties of chitosan depend on its molecular weight and degree of deacetylation14. 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

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products. 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.

**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. 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 120°C 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 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 (4°C) for 2-4 hours and incubated at 37 °C 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.
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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.\textsuperscript{19}, the strains were inoculated into M–H broth and incubated to the logarithmic growth phase at 37°C. MIC and MBC values of chitosan against the test pathogens were determined by micro and macrodilution broth technique\textsuperscript{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 \textit{Staphylococcus aureus} and \textit{Salmonella Paratyphi} in Muller–Hinton (M–H) broth. According to literature\textsuperscript{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\textsuperscript{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\textsuperscript{23}. The results of antibiotic sensitivity are shown in Table 1.

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

<table>
<thead>
<tr>
<th>Test organism</th>
<th>Concentration(ppm) growth in peptone broth</th>
<th>Doxycycline HCl (30µg)</th>
<th>R\textsuperscript{2} Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>288</td>
<td>240</td>
<td>192</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>14</td>
<td>12</td>
<td>10</td>
</tr>
<tr>
<td>Salmonella Paratyphi</td>
<td>16</td>
<td>15</td>
<td>14</td>
</tr>
</tbody>
</table>

The highest zone of inhibition against \textit{Salmonella Paratyphi} and \textit{Staphylococcus aureus} were found 16 mm and 14 mm respectively at the dose of 288 μg/ well. The regression (R\textsuperscript{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\textsuperscript{2} values (Fig.1.) indicate that there exist a linear relationship between dose used and zone diameter.
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*

<table>
<thead>
<tr>
<th>Test organism</th>
<th>Concentration(ppm) growth in peptone broth</th>
<th>MIC (ppm)</th>
<th>MBC (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>320 300 288 240 192 168 138 128</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>-    -     +    +     +    +    +    +</td>
<td>288</td>
<td>300</td>
</tr>
<tr>
<td><em>Salmonella Paratyphi</em></td>
<td>-    -     +    +     +    +    +    +</td>
<td>288</td>
<td>300</td>
</tr>
</tbody>
</table>

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. Several authors indicated that chitosan may directly affect cell membrane function. They showed that chitosan caused proteinous UV-absorbing materials to leak from cell membranes of *Pythium paroecandrum*, a plant pathogen. 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.

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**References**


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