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Short communication

# Study on antimicrobial activity of chitosan with different molecular weights

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**Abstract**

*E. coli* and *Staphylococcus aureus* are used to study the antimicrobial activity of chitosan of different molecular weights (MW). The effect of the concentration and MW of chitosan were investigated, respectively, and the antimicrobial mechanism was discussed. For chitosan with MW below 300 kDa, the antimicrobial effect on *S. aureus* was strengthened as the MW increased. In contrast, the effect on *E. coli* was weakened.


*Keywords:* Chitosan; Antimicrobial activity; Molecular weight; *E. coli*; *Staphylococcus aureus*

## 1. Introduction

Chitosan has strong antimicrobial effects, and is safe for human body. There are lots of reports discussing chitosan's antimicrobial activity in different conditions, with conflicting results. Where *Staphylococcus aureus* used as the microorganism (Guan, Fu, & Zhu, 1997), the results showed that the antimicrobial effect is greater for chitosan with lower molecular weights (MW). Another study (Xia, 1996) demonstrated that the antimicrobial effect on *E. coli* decreases as the MW of chitosan increased. Furthermore, it indicated that the optimum MW of chitosan for antimicrobial activity was 1.5 kDa. Other experiments (Jeon & Kim, 2000) showed that 0.5% oligosaccharides could inhibit the growth of *E. coli* completely. In contrast, it was reported (Ueno et al., 1997) that chitosan with MW less than 2.2 kDa had little effect on microbial growth and the minimal inhibitory concentration of chitosan oligomer was less than 0.004 and 0.032% for MW of 9.3 and 5.5 kDa, respectively. In addition, chitosan with MW of 40 kDa could inhibit 90% of *S. aureus* and *E. coli*'s growth at a concentration of 0.5% and chitosan with MW of 180 kDa could almost completely inhibit the growth of experimental *E. coli* and *S. aureus* at a concentration of 0.05% (Shin et al., 1997). 0.1% chitosan generally showed stronger bactericidal effect on gram-positive bacteria than on gram-negative ones (Hong Kyoon et al., 2002). In another study (Jeon, Park, & Kim, 2001), it was shown that chitooligosaccharides with MW higher than 10 kDa had more effective activity against pathogens than nonpathogens. A study of the antimicrobial activity of a chitooligosaccharides mixture in vitro (Choi et al., 2001) showed that it also could be considered for the treatment of periodontal diseases associated with *Actinobacillus actinomycetemcomitans*. Additionally, it was pointed out that chitosan was a potential useful indirect antimicrobial material in food protection (Helander, Nurmiaho-Lassila, Ahvenainen, Rhoades, & Roller, 2001; Roller & Covill, 1999). Another study (Jumaa, Furkertb, & Mullerb, 2002) on chitosan's antimicrobial activity in lipid emulsions was performed and the same result was achieved. It was also found (Jia, Shen, & Xu, 2001) that the antimicrobial activity of quaternized chitosan against *E. coli* was even stronger than that of chitosan with different MW. Simply put, a detailed study is required to explore the relationship between the antimicrobial activity of chitosan and its MW and concentration.

In this paper, we used *S. aureus* and *E. coli* as experimental microbe to further elucidate this relationship.

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## 2. Materials and method

### 2.1. Materials

Chitosan was prepared by enzymatic degradation method and the viscosity average MW was 5 kDa or less, 48.5, 72.5, 129, 165.7 kDa, respectively. The deacetylation degree was 88.76%.

The culture vessel had a diameter of 950 mm.

*E. coli* and *S. aureus* were provided by Department of Biology in Zhejiang University.

### 2.2. Method

- *The preparation of the microbial suspension*. The bacteria were inoculated into 75-ml peptone liquid culture medium. Having being incubated in air bath shaker (37 °C, 130 rpm) for 12 h, the strain entered the exponential period of growth and the culture broth was diluted. The concentration of *E. coli* was about $1.2 \times 10^3$ cells/ml and the concentration of *S. aureus* was $1.1 \times 10^5$ cells/ml, as determined microscopically.
- *Condition of incubation*. The peptone culture plates were prepared, in which 0.25 ml solution of microbe suspension was first added and then 0.25 ml solution of chitosan (pH 5.5) with different MW and concentrations. Both of them were spread uniformly. A blank without chitosan was prepared for comparison. All the plates were incubated at 37 °C for 20 h. Then the plates were taken out and the inhibition rate was calculated.
- The inhibition rate was defined as

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

Here $N_1$ and $N_2$ mean the number of colony on the plates before and after inhibition, respectively.

## 3. Results and discussion

### 3.1. The effect of chitosan concentration on antimicrobial activity

The antimicrobial effect of chitosan in different concentration and MW was showed in Tables 1 and 2. The results indicated that the antimicrobial effect strengthened as the concentration of chitosan increased. One percent chitosan solution could inhibit both the bacteria completely. It was also observed that the antimicrobial activity to *S. aureus* was much greater than to *E. coli*. 0.5% chitosan solution (MW > 48.5 kDa) could inhibit the growth of *S. aureus* completely. The effects of chitosan solution concentration (MW = 48.5 kDa) on the antimicrobial activity to *E. coli* and *S. aureus* are shown in Figs. 1 and 2, which indicated clearly that increasing the chitosan MW resulted in an enhanced antimicrobial effect.

Table 1
The antimicrobial effect of chitosan on *E. coli*

| MW (kDa) | Inhibition rate $\eta$ (%) | | | |
|---|---|---|---|---|
| | Conc. of chitosan | | | |
| | 0.25% | 0.5% | 0.75% | 1.0% |
| <5 | 50 | 60 | 90 | 100 |
| 48.5 | 30 | 80 | 90 | 100 |
| 72.4 | 5 | 10 | 50 | 100 |
| 129 | 0 | 5 | 90 | 100 |
| 166 | 0 | 40 | 80 | 100 |
| 305 | 0 | 40 | 50 | 100 |

### 3.2. The effect of chitosan MW on the antimicrobial activity

To *E. coli*, a gram-negative bacteria, the antimicrobial activity was enhanced as the MW decreased. It was obvious that 0.25% chitosan solution (MW < 5 kDa) could inhibit the growth of *E. coli*. In contrast, for *S. aureus*, a gram-positive bacteria, the antimicrobial activity increased with increasing MW of chitosan. The inhibiting effect was fairly obvious for higher MW (such as 305 kDa) even if the concentration was quite small. The effect of chitosan MW on the antimicrobial activity to *S. aureus* are shown in Fig. 3, which indicated that increasing chitosan MW resulted in enhanced antimicrobial effect.

### 3.3. The antimicrobial mechanisms

Our results showed that the effects of chitosan were different for the two kinds of bacteria. The possible mechanisms for antimicrobial activity were: (1) the chitosan on the surface of the cell can form a polymer membrane, which prevents nutrients from entering the cell. (2) Chitosan of lower MW entered the cell through pervasion. Since chitosan could adsorb the electronegative substance in

Table 2
The antimicrobial effect of chitosan on *S. aureus*

| MW (kDa) | Inhibition rate $\eta$ (%) | | | |
|---|---|---|---|---|
| | Conc. of chitosan | | | |
| | 0.25% | 0.5% | 0.75% | 1.0% |
| <5 | 0 | 0 | 0 | 0 |
| 48.5 | 0 | 95 | 99 | 100 |
| 72.4 | 0 | 96 | 99 | 100 |
| 129 | 40 | 100 | 99 | 100 |
| 166 | 95 | 100 | 100 | 100 |
| 305 | 99 | 100 | 100 | 100 |

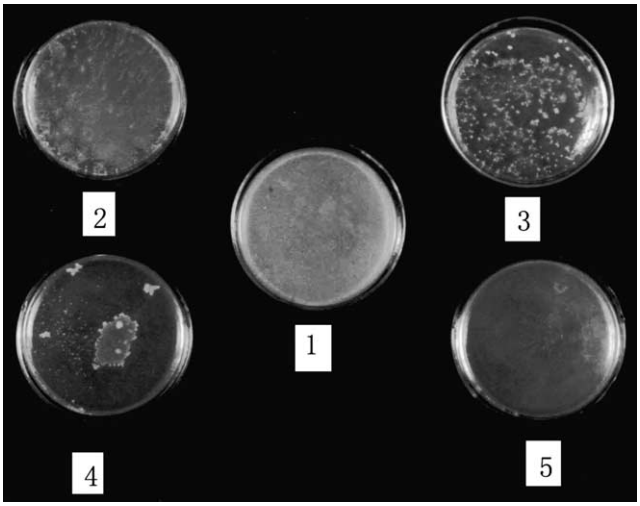


Fig. 1. The effect of chitosan (MW = 48.5 kDa) solution concentration on the antimicrobial activity to *E. coli*; (1) blank, (2) 0.25% chitosan, (3) 0.5% chitosan, (4) 0.75% chitosan, (5) 1.0% chitosan.

the cell and flocculate them, it disturbs the physiological activities of the bacteria and kills them. For *S. aureus*, the dominant mechanism is the former, while for *E. coli* the latter mechanism seems more likely. Electron micrographs for gram-positive and gram-negative bacteria in the presence of chitosan (Jiang et al., 1997) show the cell membrane of *S. aureus* was weakened or even broken, while the cytoplasm of *E. coli* was concentrated and the interstice of the cell were clearly enlarged. This study indicated that the mechanisms of the antimicrobial activity of chitosan were different between gram-positive and negative bacteria. Additionally, the antimicrobial mechanism of chitosan might differ from that of other polysaccharides because there are positive charges on the surface of chitosan.

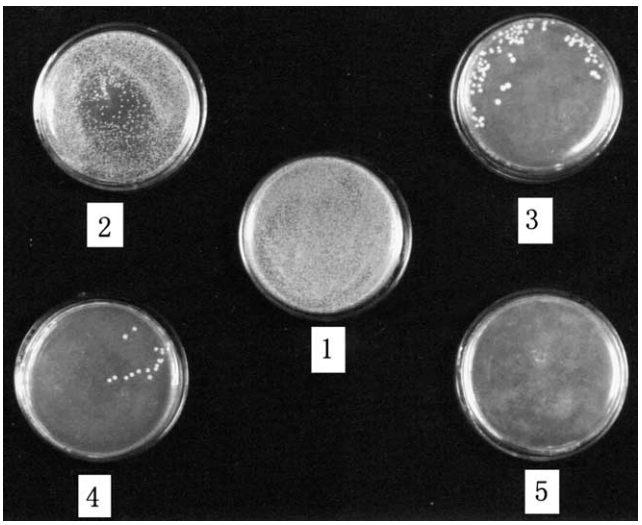


Fig. 2. The effect of chitosan (MW = 48.5 kDa) solution concentration on the antimicrobial activity to *S. aureus*; (1) blank, (2) 0.25% chitosan, (3) 0.5% chitosan, (4) 0.75% chitosan, (5) 1.0% chitosan.

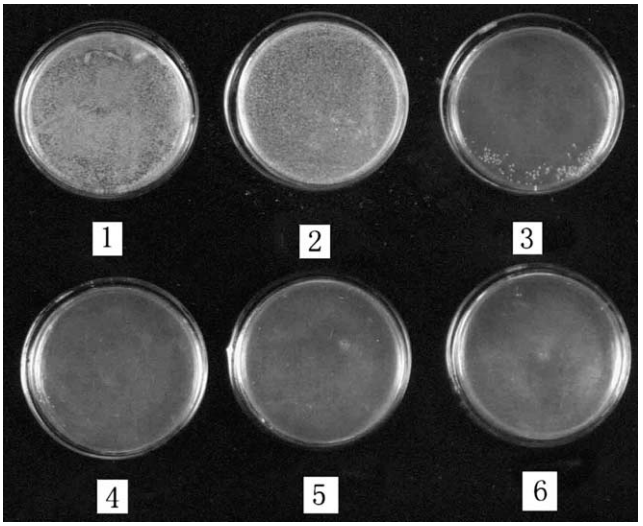


Fig. 3. The effect of chitosan MW (0.75% solution) on the antimicrobial activity to *S. aureus*; (1) blank, (2) MW < 5 kDa, (3) MW = 48.5 kDa, (4) MW = 72.5 kDa, (5) MW = 129.0 kDa, (6) MW = 165.7 kDa.

### 3.4. The effect of MW and concentration

Table 1 shows that some concentrations and MW of chitosan showed much lower antimicrobial activity than others. A possible reason was that the two antimicrobial mechanisms discussed above may coexist.

## 4. Conclusion

- The antimicrobial activity of chitosan with MW below 305 kDa, was studied. As the concentration of chitosan increased, the antimicrobial effect was strengthened. When the concentration reached 1.0%, the inhibition rate reached 100% for both *E. coli* and *S. aureus*.
- For *S. aureus*, a gram-positive bacteria, as the MW of chitosan increased, the antimicrobial effect was enhanced. The main reason might be the chitosan of higher MW forms a film which inhibits nutrient adsorptions.
- For *E. coli*, a gram-negative bacteria, as the MW of chitosan decreased, the antimicrobial effect was enhanced. The main reason might be that the chitosan of lower MW enters the microbial cell more easily, which disturbed the metabolism of the cell.

## Acknowledgements

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