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Short communication

The influence of molecular weight of quaternized chitosan on antifungal activity

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Abstract

Quaternized chitosan derivatives with different molecular weights were synthesized in the laboratory. Subsequent experiments were conducted to test their antifungal activities against *Botrytis cinerea* Pers. (*B. cinerea* pers.) and *Colletotrichum lagenarium* (Pass) Ell.et halst (*C. lagenarium* (Pass) Ell.et halst). Our results indicate that quaternized chitosan derivatives have stronger antifungal activities than chitosan. Furthermore, quaternized chitosan derivatives with high molecular weight are shown to have even stronger antifungal activities than those with low molecular weight.

Keywords: Quaternized chitosan; Molecular weight; Antifungal activity

1. Introduction

Chitosan is a natural cationic aminopolysaccharide copolymer of glucosamine and *N*-acetylglucosamine usually obtained from the exoskeletons of shellfish and insects. With regard to its unique properties such as biocompatibility, biodegradability and non-toxicity, it is widely used in fields like biotechnology, pharmaceutics, cosmetics, textiles and agriculture (Kurita, 2001; Muzzarelli, 1983; Ravi Kumar, 2000), in particular the antifungal activities of chitosan and its derivatives have aroused considerable recent interest (Peng, Han, Liu, & Xu, 2005). EI Ghaouth and co-workers reported that chitosan can inhibit the growth of *Alternaria alternata*, *Botrytis cinerea*, *Colletotrichum gloeosporioides* and *Rhizopus stolonifer* (EI Ghaouth, Arul, Asselin, & Benhamou, 1992). The concentration of chitosan was confirmed to be a key factor of the inhibitory index. The growth of fungi, (*Fusarium oxysporum*, *R. stolonifer*, *Penicillium digitatum* and *C. gloeosporioides*), can be completely inhibited by chitosan at the concentration of 3% (Bautista-Baños, Hernández-López, Bosquez-Molina, & Wilson, 2003; Bautista-Baños et al., 2006). The mechanism for inhibition remains controversial. Two hypotheses are as follows: (1) the polycationic chitosan consumes the electronegative charges on cell surfaces and the cell permeability is changed, thus this interaction results in the leakage of intracellular electrolytes and proteinaceous constituents; (2) chitosan enters fungal cells and then essential nutrients are adsorbed, which inhibit or slow down the synthesis of mRNA and protein (Avadi et al., 2004; Chen, Liau, & Taai, 1998; Chen, Wu, & Zeng, 2005; El Ghaouth, Arul, Wilson, & Benhamou, 1997; Jia, Shen, & Xie, 2001). Whatever mechanism is correct, we believe they should both be affected by the chitosan molecular weight (MW). High MW (H-MW) chitosan should inhibit growth by

Abbreviations: NPDCS, *N*-phenyl-*N*,*N*-dimethyl chitosan; NHPDCS, *N*-(2-hydroxyl-phenyl)-*N*,*N*-dimethyl chitosan; NCHPDCS, *N*-(5-chloro-2-hydroxyl-phenyl)-*N*,*N*-dimethyl chitosan.

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the first mechanism because of its larger size, while low MW (L-MW) chitosan can enter the fungal cell most easily and should sustain the second mechanism.

In order to find the essential mechanism, quaternized chitosan derivatives with different MW were synthesized in our laboratory. Subsequent experiments were conducted to assess their antifungal activities against *Botrytis cinerea* Pers. (*B. cinerea* pers.) and *Colletotrichum lagenarium* (Pass) Ell.et halst (*C. lagenarium* (Pass) Ell.et halst), as well as to verify the impact of MW.

2. Materials and methods

Chitosan was purchased from Qingdao Baicheng Biochemical Corp. (China), with a degree of deacetylation ~97% and an average-molecular weight of 7.0×10^5 (H-MW), and 7.6×10^3 (L-MW). Salicylaldehyde, sodium iodide (anhyd NaI), *N*-methyl-2-pyrrolidone (NMP), sodium borohydride ($NaBH_4$), and iodomethane (CH_3I) were purchased from Sigma–Aldrich Chemical Co. H-MW and L-MW quaternized chitosan derivatives (NPDCS, NHPDCS, NCHPDCS) were synthesized from these two types of chitosan, following methods of Jia et al. (2001), and Guo et al. (Guo, Liu, Chen, Ji, & Li, 2006). Three grams chitosan was dissolved into 1% HAc (100 mL) at room temperature, and various aldehydes were added, respectively, with stirring. After 2 h, 10% $NaBH_4$ (1.5-fold excess to added aldehydes) was added and the reaction was carried out for 2 h. The solution was precipitated in acetone and the precipitants were filtrated. N-substituted chitosan were obtained after drying at 60 °C for 24 h. One gram N-substituted chitosan was dispersed into 50 mL NMP for 12 h at rt. To this mixture, 0.12 mL NaOH (1 M), 1.5 g NaI and 4 mL CH_3I were added, and each reaction was carried out with stirring at 50 °C for 20 h. The solution was precipitated by excess acetone and the precipitates were filtered. Quaternized chitosan were obtained by drying at 60 °C for 24 h.

Antifungal assays were performed based on the method of D. Jasso de Rodríguez and co-workers (Jasso de Rodríguez, Hernández-Castillo, Rodríguez-García, & Angulo-Sánchez, 2005). In test plates, chitosan and quaternized chitosan were, respectively, mixed with sterilized potato dextrose agar (PDA), at different concentrations (500 and 1000 μg/mL). Then the given mycelium of fungi was transferred to these plates and incubated at 27 °C. Under the same condition, the same mycelium was incubated in control plates with non-samples-doped PDA. All incubations were terminated when the mycelium in control plates reached the edge. Finally the growth of the given mycelium in test plates was compared with that of the same mycelium in control plates, and the antifungal index was calculated as follows:

$$\text{Antifungal index } (\%) = (1 - D_a/D_b) \times 100$$

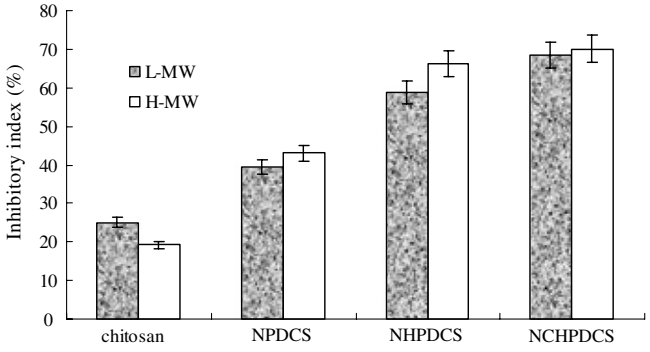
where D_a is the diameter of the growth zone in test plates and D_b is the diameter of growth zone in control plates.

Each experiment was repeated three times and their results were averaged. The Scheme method was adopted to evaluate the differences between antifungal index derived from all assays. Results with $P < 0.05$ were considered to be statistically significant.

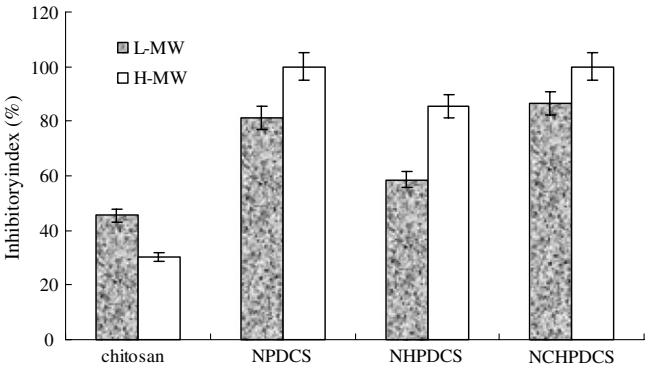
3. Results and discussion

3.1. Antifungal activity of chitosan and quaternized chitosan against B. cinerea Pers.

The antifungal activity of chitosan and quaternized chitosan against *B. cinerea* Pers. experiments were shown in Fig. 1(a) and (b). As show in Fig. 1(a), all tested materials demonstrate inhibitory effect against *B. cinerea* Pers., ranging from 19.6% for H-MW chitosan to 71.2% for H-MW NCHPDCS. For both L-MW or H-MW, the inhibitory activity follows a sequence of Chitosan < NPDCS < NHPDCS < NCHPDCS. Quaternized chitosan derivatives gave stronger antifungal activities than original chitosan, which is as same as found by of Jia et al. (2001). The potential reason is higher charge densities of such derivatives. L-MW chitosan shows stronger inhibitory effect (25%) than H-MW chitosan (19.6%). In contrast, for all the other spe-

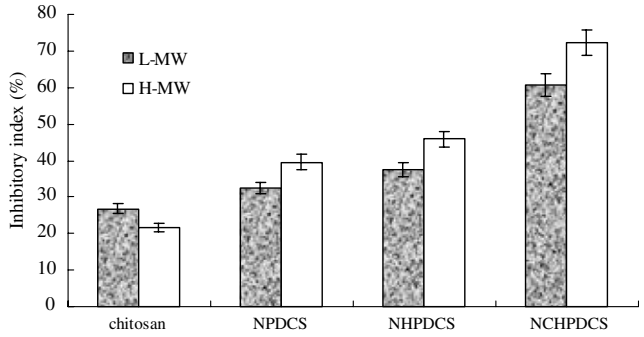


(a) Antifungal activity of chitosan and quaternized chitosan with different molecular weight at the concentration of 500 ppm against *B. cinerea* Pers.

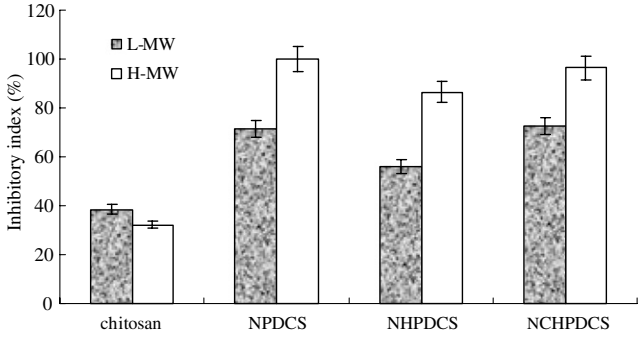


(b) Antifungal activity of chitosanand quaternized chitosan with different molecular weight at the concentration of 1000 ppm against *B.cinerea* Pers.

Fig. 1. Antifungal activity of chitosan and quaternized chitosan against *B. cinerea* pers.



(a) The antifungal activity of chitosan and quaternized chitosan at the concentration of 500 ppm against *C. lagenarium* (Pass) Ell.ethalst.



(b) The antifungal activity of chitosan and quaternized chitosan at the concentration of 1000 ppm against *C. lagenarium* (Pass) Ell.ethalst.

Fig. 2. Antifungal activity of chitosan and quaternized chitosan against *C. lagenarium* (Pass) Ell.et halst.

cies, better inhibitory effects are found in H-MW derivatives. This result is also consistent with the work of Jia et al. (2001), in which H-MW quaternary ammonium salt of chitosan showed stronger antibacterial activity than the L-MW type. Chitosan mainly follow the second mechanism previously discussed. Low MW chitosan can enter the fungal cell easily because of its small size. As for quaternized chitosan derivatives, the first mechanism occurring on cell surface seems to play the major role. This is because of the abundant polycationic charges. However, for any quaternized chitosan derivatives, the difference between L-MW and H-MW is very close to experimental error. The evidence from the 500 μg/mL experiments is the least convincing.

When the concentration of individual target materials was raised to 1000 μg/mL, the differences (as shown in Fig. 1(b)) are clearer. The growth of *B. cinerea* Pers. was completely inhibited by H-MW NPDCS and H-MW NCHPDCS. At 1000 μg/mL, L-MW chitosan still presents stronger inhibitory effects than H-MW chitosan because of its smaller size.

3.2. Antifungal activity of chitosan and quaternized chitosan against C. lagenarium (Pass) Ell.et halst

The inhibitory effects of all target materials against *C. lagenarium* (Pass) Ell.et halst at concentrations of 500 and 1000 μg/mL are shown in Fig. 2(a) and (b). The growth of *C. lagenarium* (Pass) Ell.et halst can also be completely inhibited by H-MW NPDCS and H-MW NCHPDCS when their concentrations in PDA are raised to 1000 μg/mL. Generally, most results and relations are as same as those of experiments on *B. cinerea* Pers.

4. Conclusion

In this article, chitosan and its quaternized derivatives with L-MW and H-MW are tested for inhibitory effects against *B. cinerea* Pers. and *C. lagenarium* (Pass) Ell.et halst. They all demonstrate strong inhibitory effects against two types of fungi, but quaternized chitosan derivatives were found to be much effective because of their high polycationic charge densities. H-MW NPDCS and H-MW NCHPDCS at the concentration of 1000 μg/mL can completely inhibit the growth of tested fungi. Molecular weight is found to have distinct impacts on inhibitory activities of test materials, negative for chitosan but positive for its quaternized derivatives.

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References

Avadi, M. R., Sadeghi, A. M. M., Tahzibi, A., Bayati, K., Pouladzadeh, M., Zohuriaan-Mehr, M. J., et al. (2004). Diethylmethyl chitosan as an antimicrobial agent: Synthesis, characterization and antibacterial effects. *European Polymer Journal, 40*(7), 1355–1361.

Bautista-Baños, S., Hernández-Lauzardo, A. N., Velázquez-del Valle, M. G., Hernández-López, M., Ait Barka, E., Bosquez-Molina, E., et al. (2006). Chitosan as a potential natural compound to control pre and postharvest diseases of horticultural commodities. *Crop Protection, 25*(2), 108–118.

Bautista-Baños, S., Hernández-López, M., Bosquez-Molina, E., & Wilson, C. L. (2003). Effects of chitosan and plant extracts on growth of *Colletotrichum gloeosporioides*, anthracnose levels and quality of papaya fruit. *Crop Protection, 22*(9), 1087–1092.

Chen, C. S., Liau, W. Y., & Taai, G. J. (1998). Antibacterial effects of N-sulfonated and n-sulfobenzoyl chitosan and application to oyster preservation. *Journal of Food Protection, 61*, 1124–1128.

Chen, S. P., Wu, G. Z., & Zeng, H. Y. (2005). Preparation of high antimicrobial activity thiourea chitosan-Ag+ complex. *Carbohydrate Polymers, 60*(1), 33–38.

EI Ghaouth, A., Arul, J., Asselin, A., & Benhamou, N. (1992). Effect of chitosan and other polyions on chitin deacetylase in *Rhizopus stolonifer*. *Experimental Mycology, 16*(3), 173–177.

El Ghaouth, A., Arul, J., Wilson, C., & Benhamou, N. (1997). Biochemical and cytochemical aspects of the interactions of chitosan and *Botrytis cinerea* in bell pepper fruit. *Postharvest Biology Technology, 12*(2), 183–194.

Guo, Z. Y., Liu, H. Y., Chen, X. L., Ji, X., & Li, P. C. (2006). Hydroxyl radicals scavenging activity of N-substituted chitosan and quaternized chitosan. *Bioorganic Medicinal Chemistry Letters, 16*(24), 6348–6450.

Jasso de Rodríguez, D., Hernández-Castillo, D., Rodríguez-García, R., & Angulo-Sánchez, J. L. (2005). Antifungal activity in vitro of *Aloe vera*

pulp and liquid fraction against plant pathogenic fungi. *Industrial Crop Products, 21*(1), 81–87.

Jia, Z. S., Shen, D. F., & Xie, W. L. (2001). Synthesis and antibacterial activities of quaternary ammonium salt of chitosan. *Carbohydrate Research, 333*(1), 1–6.

Kurita, K. (2001). Controlled functionalization of the polysaccharide chitin. *Progress in Polymer Science, 26*(9), 1921–1971.

Muzzarelli, R. A. A. (1983). Chitin and its derivatives: New trend of applied research. *Carbohydrate Polymers, 3*(1), 53–75.

Peng, Y. F., Han, B. Q., Liu, W. S., & Xu, X. J. (2005). Preparation and antimicrobial activity of hydroxypropyl chitosan. *Carbohydrate Research, 340*(11), 1846–1851.

Ravi Kumar, M. N. V. (2000). A review of chitin and chitosan applications. *Reactive and Functional Polymers, 46*(1), 1–27.