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Synthesis, characterization and antifungal properties of N,O-(acyl)-N-(trimethyl) chitosan chloride

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Abstract: Three novel water soluble chitosan derivatives: N,O-(acetyl)-N-(trimethyl) chitosan chloride (ATMCS), N,O-(benzoyl)-N-(trimethyl) chitosan chloride (BTMCS), N,O-(chloracetyl)-N-(trimethyl) chitosan chloride (CATMCS) were successfully synthesized. All the derivatives were soluble in water at pH 7. The antifungal properties of the derivatives against two plant fungus Gloeosporium theae-sinensis (G. theae-sinensis) and Phomopsis asparagi (P. asparagi) were studied. The results indicated that the antifungal activities of ATMCS, BTMCS and CATMCS enhanced compared with that of N-(trimethyl) chitosan chloride (TMCS). Against G. theae-sinensis and P. asparagi, BTMCS and CATMCS exhibited much better activities than ATMCS which was may be due to the phenyl and chlorine respectively. Generally speaking, acyl modification of TMCS was effective.

Introduction

Chitosan, a copolymer of glucosamine and N-acety-glucosamine units linked by 1-4 glucosidic bonds, was obtained by N-deacetylation of chitin, which is the second most naturally occurring biopolymer after cellulose [1]. As a natural renewable resource, chitosan has a number of unique properties such as biocompatibility, biodegradability, non-toxicity, and antimicrobial activity, which have attracted much scientific and industrial interest in such fields as biotechnology, pharmaceutics, wastewater treatment, cosmetics, agriculture, food science, and textiles [2]. However, its poor aqueous solubility above pH 6 is major drawback for its use at physiological conditions [3]. Therefore, special attention has been paid to its chemical modification and depolymerization to obtain derivatives soluble in water over a wider pH range [4].

Furthermore, antimicrobial activities of chitosan derivatives have received considerable attention in recent years due to problems associated with chemical fungicide agents [5]. It has been reported that quaternary ammonium salts of chitosan exhibited good antibacterial activities [6] and the quaternized N-trimethyl chitosan chloride (TMCS) showed higher antibacterial activity than chitosan and good aqueous solubility at appropriate degree of substitution (DS) [7]. In addition, novel N, O-acyl chitosan derivatives were more active against the gray mold fungus *Botrytis cinerea* and the rice leaf blast fungus *Pyricularia oryzae* than chitosan itself [8].

TMCS is a partially quaternized derivative of chitosan with improved solubility and easy preparation [9]. In this paper, we prepared aqueous soluble TMCS according to

the reference [7]. Then, taking TMCS as modification material, we synthesized N,O-(acyl)-N-(trimethyl) chitosan chloride under mild conditions. Their structure was confirmed and the antifungal activities against two plant fungus, *Gloeosporium theae-sinensis* (*G. theae-sinensis*) and *Phomopsis asparagi* (*P. asparagi*) were studied. Both the aqueous solubility and the antimicrobial activity of the derivatives were expected to be satisfactory.

Results and discussion

The yields and the degree of quaternization of TMCS and the DS of the acyl derivatives of TMCS are shown in Table 1, and the FT-IR spectrum of the derivatives are shown in Figure 1.

Tab. 1. The yield, colour, substitution degree and water solubility of CS, TMCS, ATMCS and CATMCS.

Compounds	Yields (%)[a]	Degree of quaternization (%)	Degree of acylation (%)	Colour	[b]Solubility in water at pH 7
CS	—	—	—	Ivory	×
TMCS	95.5	59.0	—	Yellow	√
ATMCS	80.2	59.0	57.2	Yellow	√
BTMCS	77.5	59.0	55.7	Yellow	√
CATMCS	89.6	59.0	60.5	Yellow	√

[a]Yield of TMCS is the ratio of TMCS and added chitosan.
[a]Yield of acylation derivatives of TMCS is the retio of acylation TMCS and added TMCS.
[b]Solubility in water of the derivatives were carried out in 25 °C. The concentration of TMCS, ATMCS, BTMCS and CATMCS in water were all higher than 25 %.

As shown in Figure 1, the IR spectrum of chitosan shows peaks assigned to the saccharine structure at 895 and 1155 cm^{-1}. Characteristic peaks of amine (N-H) vibration deformation appeared at 1600 cm^{-1} for chitosan [10]. After being quaternized, amine vibration deformation peak at 1600 cm^{-1} disappear and new peak appear at 1650 cm^{-1}, which were assigned to the quaternary ammonium salt [6]. There are strong new peak at 1481 cm^{-1}, which were assigned to the characteristic absorbtion of methyl of $(CH_3)_3N^+$–. All the acylation TMCS have the stretching vibration of the carbonyl groups of ester group at 1735, 1708, 1743 cm^{-1} respectively for ATMCS, BTMCS and CATMCS [11,12]. It indicated that the acylate reaction happened between the acyl chloride and the -OH groups of chitosan. Most of the $–NH_2$ of chitosan had been quaternized. The remaining free amino groups were reacted with acyl chloride and the carbonyl groups of acylamide appeared at 1650, 1646, 1662 cm^{-1} respectively for ATMCS, BTMCS and CATMCS which coincided with the quaternary ammonium salt peaks [11, 12]. In the peaks of CATMCS, the C-Cl peaks at 786 cm^{-1} was distinct. Above-mentioned results demonstrated that the acylate TMCS were obtained. The degree of the quaternization and the acyl substitution of the chitosan derivatives were determined by Domard's method [13].

The antifungal activities of TMCS and its acyl derivatives are shown in Figure 2 and 3. As shown in Figure 2, the inhibitory index of TMCS against *G. theae-sinensis* was 16.7 % and 45.2 % at 500 and 1000 μg/mL, respectively. Compared with the activity of TMCS, all the acyl derivatives had better antifungal activities, and the inhibitory

indices of ATMCS, BTMCS and CATMCS were 50.2 %, 79.2 % and 80.1 %, respectively at 1000 μg/mL. Earlier paper reported the antibacterial activities of chitsan derivatives are affected not only by the positive charges but also the amphiphilic nature of the bacterial cell wall is important in enhancing the hydrophobic-hydrophobic interaction between the bacterial cell wall and the chitosan derivative that contained hydrophobic moiety [14].

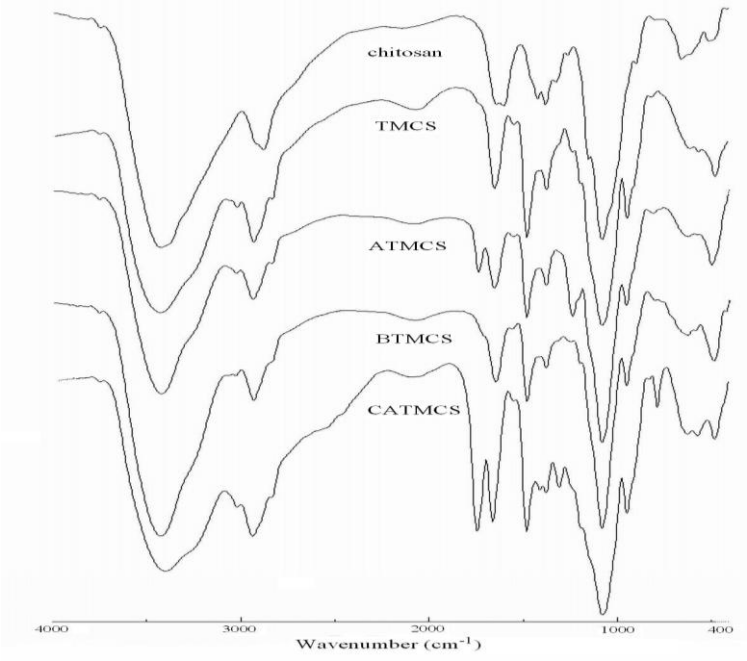


Fig. 1. FT-IR spectra of chitosan, TMCS, ATMCS, BTMCS and CATMCS.

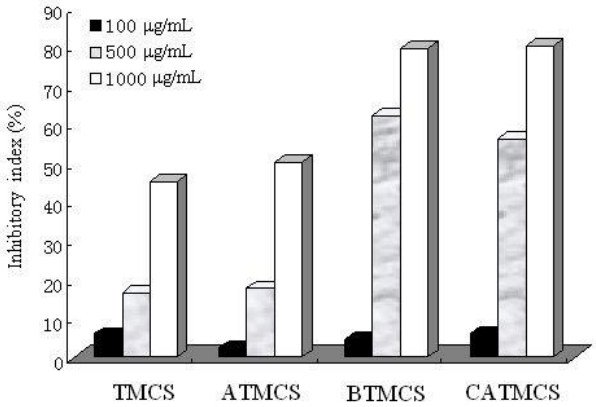


Fig. 2. The antifungal activities of TMCS, ATMCS, BTMCS and CATMCS against *G. theae-sinensis.*

Here, the acetyl, benzoyl and chloracetyl are hydrophobic moiety grafted onto TMCS. So the strengthened antifungal activity of the ATMCS, BTMCS and CATMCS maybe are due to the synergy effect of the N-trimethyl quaternary ammonium salt structure and the hydrophobic acyl moiety. Besides, BTMCS has better activity than ATMCS maybe due to the more hydrophobic property of the aryl substituent [16]. CATMCS exhibited the best antifungal activity in the three TMCS derivatives which probably

due to the chlorine atom on the chloracetyl. The chloro-group was used in many fungicides such as pentachloronitrobenzene, chlorothalonil [15]. In Figure 3, all the acyl derivatives exhibited better antifungal activities against *P. asparagi* than TMCS and the inhibitory indices were 56.5 %, 81.7 % and 86.9 % for ATMCS, BTMCS and CATMCS, respectively at 1000 μg/mL. It should be for the same reason above mentioned for increased activity against *G. theae-sinensis.*

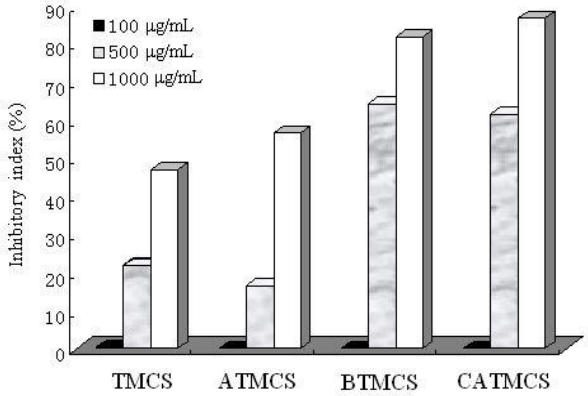


Fig. 3. The antifungal activities of TMCS, ATMCS, BTMCS and CATMCS against *P. asparagi.*

Conclusions

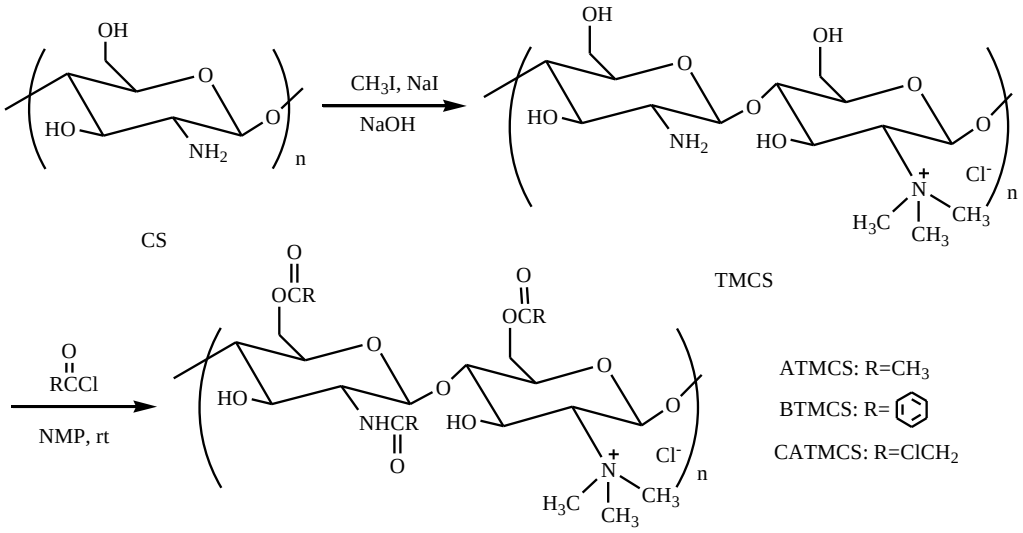
We have successfully synthesized water soluble derivatives of TMCS: ATMCS, BTMCS and CATMCS using a mild and easy method. Their water soluble property was due to the quaternary ammonium salt structure. The results of antifungal activities against two plant fungus, *G. theae-sinensis* and *P. asparagi* suggested ATMCS, BTMCS and CATMCS had improved antifungal property than TMCS. This is consistent with the earlier paper which noted that both the positive charges and the hydrophobic moiety of chitosan derivatives play important role on improved antibacterial activity [14]. Besides, some active groups are necessary, for example chlorine atom. CATMCS exhibited the best antifungal activity in the three TMCS derivatives which probably was due to the chlorine atom on the chloracetyl.

There are increasing environment problems caused by fungicides, especially chemical products. It is necessary to explore new substitutes of chemical fungicides. As a biodegradable polysaccharide, chitosan and derivatives may be further explored in the field of agriculture. This paper work is a successful attempt to graft acyl onto quaternary ammonium chitosan. This kind of chitosan derivatives may be further explored.

Experimental part

Chitosan was purchased from Qingdao Baicheng Biochemical Corp. (China). The degree of deacetylation was 97 % and the viscosity average-molecular weight was 7.0×10^5. Iodomethane (CH_3I), sodium iodide (anhyd NaI), N-methyl-2-pyrrolidone (NMP) were purchased from Sigma-Aldrich Chemical Co. The elemental analyses (C, H, N) were performed on a Carlo-Erba 1106 elemental analyzer. The FT-IR spectra were measured on a JASCO FT-IR-4100 instrument with KBr disks. The other reagents were of analytical grade and used without further purification.

TMCS was synthesized according to the earlier method via steps 1-4[16]. General synthesized method of N,O-(acyl)-N-(trimethyl) chitosan chloride was as follows: 2 g TMCS was dispersed into 80 mL NMP and stirred at room temperature (RT) for 12 h. Then 0.02 mol acyl chloride (acetyl chloride, benzoyl chloride, chloracetyl chloride) was added and keep stirring on for another 24 h at RT. The product was precipitated with excess ethyl ether and the precipitations were filtered. ATMCS, BTMCS, CATMCS were obtained by drying at 60 °C for 24 h (Scheme 1).



Scheme 1. Synthesis pathway of TMCS and its acylation derivatives.

The material chitosan in this paper was not soluble in water at pH 7. So its activity against fungus cannot be compared with that of the derivatives TMCS, ATMCS, BTMCS and CATMCS which were soluble in water. In other words, the improved water solubility just was one of the advantages of the chitosan derivatives.

Antifungal assays of the derivatives were performed based on the method of Guo [10]. Briefly, the compounds were dispersed in distilled water at a concentration of 5 mg/mL. Then, each samples (TMCS, ATMCS, BTMCS and CATMCS) solution was added to sterilized potato dextrose agar to give a final concentration of 100, 500 and 1000 $\mu g/mL^{-1}$. After the mixture was cooled, the mycelium of fungi were transferred to the test plate and incubated at 27 °C for 2 to 3 days. When the mycelium of fungi reached the edges of the control plate (without the presence of samples), the antifungal index was calculated as follows:

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

where D_a is the diameter of the growth zone in the test plates and D_b is the diameter of growth zone in the control plate. Each experiment was performed three times, and the data were averaged. The Scheffe method was used to evaluate the differences in antifungal index in antifungal tests. Results with $P < 0.05$ were considered statistically significant [17].

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References

[1] Bartnicki-Garcia, S. *Ann. Microbiol*. **1968**, *22*, 87.
[2] Kumar, M. N. V. *React. Funct. Polym*. **2000**, *46*, 1.
[3] Kotze, A. F.; Luessen, H. L.; de Boer, A. G.; Verhoef, J. C.; Junginger, H. E. *Eur. J. Pharm. Sci.* **1999**, *7*, 145.
[4] Hirano, S.; Yamaguchi,Y.; Kamiya, M. *Carbohydr. Polym.* **2002**, *48*, 203.
[5] Rabea, E. I.; Badawy, M. T.; Rogge, T. M.; Stevens, C. V.; Smagghe, G.; Hö fte, M; Steurbaut, W. *In Processing of the 9th International Chitin-Chitosan Conference*, Montereal, Quėbec,Canada. **2003**, pp103–104.
[6] Jia, Z.; Shen, D.; Xu, W. *Carbohydr. Res*. **2001**, *333*, 1.
[7] Sieval, A. B.; Thanou, M.; Kotze, A. F.; et al. *Carbohydr. Polym.* **1998**, *36*, 157.
[8] Badawy, M. E. I.; Rabea, E. I.; Rogge, T. M.; Stevens, C. V.; Smagghe, G.; Steurbaut, W.; Ho ¨ fte, M. *Biomacromolecules* **2004**, *5*, 589.
[9] Le Dung, P.; Milas, M.; Rinaudo, M.; Desbrieres, J. *Carbohydr. Polym.* **1994**, *24*, 209.
[10] Guo, Z. Y.; Xing, R.; Liu, S.; Zhong, Z. M.; Ji, X.; Wang, L.; Li, P. C. *Carbohydr. Res.* **2007**, *342*, 1329.
[11] Baumann, H.; Faust, V. *Carbohydrate Research,* **2001**, *331*, 43.
[12] Huang, R.; Du, Y.; Zheng, L.; Liu, H.; Fan, L. *React. Funct. Polym.* **2004**, *59*, 41.
[13] Domard, A.; Rinaudo, M.; Terrassin, C. *Int. J. Biol. Macromol.* **1986**, *8*, 105.
[14] Sajomsang, W. *Carbohydr. Polym.* **2009**, *12*, 037
[15] Nester, E. W.; Anderson, D. G.; Roberts, E.;Pearshall, N. N.; Nester, M. T. *Microbiology*, McGraw-Hill: Boston, MA, **2003**, pp 518–524.
[16] Snyman, D.; Hanmman, J. H.; Kotze, J. S.; et al. *Carbohydr. Polym.* **2002**, *50*, 145.
[17] Jasso de Rodríguez, D.; Hernández-Castillo, D.; Rodríguez-García, R.; Angulo-Sánchez, J. L. *Ind. Crop. Prod.* **2005**, *21*, 81.