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Synthesis and characterization of dithiocarbamate chitosan derivatives with enhanced antifungal activity

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ABSTRACT

In this study, ammonium dithiocarbamate chitosan (ADTCCS) and triethylene diamine dithiocarbamate chitosan (TEDADTCCS) derivatives were obtained respectively by mixing chitosan with carbon disulfide and ammonia (triethylenediamine). Their structures were confirmed by FT-IR, ^{1}H NMR, XRD, DSC, SEM, and elemental analysis. Antifungal properties of them against the plant pathogenic fungi *Fusarium oxysporum* and *Alternaria porri* were investigated at concentrations ranged from 31.25 to 500 mg/L. The dithiocarbamate chitosan derivatives had enhanced antifungal activity compared with chitosan. Particularly, they showed obvious inhibitory effect on *Fusarium oxysporum*. At 500 mg/L, TEDADTCCS inhibited growth of *F. oxysporum* at 60.4%, stronger than polyoxin and triadimefon whose antifungal indexes were found to be 25.3% and 37.7%. The chitosan derivatives described here deserve further study for use in crop protection.

1. Introduction

Chitosan, a kind of natural polymer, has received great attention in the fields of agriculture, food industry, and biomaterial due to its biocompatibility, biodegradability and bioactivity (Kumar, Muzzarelli, Muzzarelli, Sashiwa, & Domb, 2004; Muzzarelli, 1983). Particularly, it was proved to have a broad-spectrum antifungal activity against a variety of fungi (Bautista-Baños et al., 2006). For example, Allan et al. found chitosan had better fungicidal activity than chitin. Of the 46 tested fungi, chitosan had antifungal activity against 32 isolates (Allan & Hadwiger, 1979). Bautista-Baños, Hernández-López, Bosquez-Molina, and Wilson (2003) described the fungicidal effect of chitosan on *Colletotrichum C. gloeosporioides* in vitro. 3% chitosan can completely inhibit growth of the fungi. However, chitosan also presented some drawbacks: it is only soluble in dilute acids, but insoluble in water and most organic solvents. Additionally, it showed relatively low antifungal activity. Hence, many strategies were proposed to overcome these limitations. A widely used approach is the combination of chitosan with other bioactive group: NCB-chitosan were synthesized to overcome the solubility problems and used with 298 microbial strains in the literature (Muzzarelli et al., 1990). N-alkyl chitosan (NAC) derivatives were reported to have good fungicidal activity against *Botrytis cinerea* and *Pyricularia grisea* (Rabea et al., 2006). The most active derivative was N-(2,2-diphenylethyl)chitosan, whose EC_{50} value against *B. cinerea* was 0.031 g/L. Quaternized chitosan derivatives were also proved to have good antifungal activity (Guo et al., 2007). This kind of chitosan derivatives had better antifungal activity against *B. cinerea Pers* and *Colletotrichum lagenarium* (*Pass*) *Ell.et halst* in comparison with chitosan, and the max inhibitory index reached 86.7% in vitro.

Dithiocarbamates are a class of fungicides extensively used in many crops worldwide due to their high efficiency in controlling plant pathogenic fungi and relatively low mammalian acute toxicity (Pease & Holt, 1977). However, more and more studies exhibited that this kind of fungicides were not easily eliminated (Caldas, Conceição, Miranda, de Souza, & Lima, 2001; Gustafsson & Fahlgren, 1983). This may represent a threat to human health and environment. Therefore, how to make better use of the advantages of dithiocarbamate-based fungicides and avoid their weakness is a problem to be solved. Chitosan was easily metabolized to residues which are nontoxic and naturally eliminated (Coutinho et al., 2008). Its derivatives were also proved to possess biodegradable properties of chitosan (Feng & Dong, 2006; Jameela & Jayakrishnan, 1995; Xu, McCarthy, Gross, & Kaplan, 1996). This provided us a method to obtain new chitosan derivatives which may have the biodegradable functions of CS and fungicidal properties of dithiocarbamates.

Dithiocarbamate chitosan is a chitosan derivative in which C2 amino groups are substituted by dithiocarbamate groups.

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Muzzarelli, Tanfani, Mariotti, and Emanuelli (1982) first prepared dithiocarbamate (DTC) chitosan as a metal cheating agent. However, there were no reports on antifungal activity of DTC chitosan derivatives. In addition, no papers described the preparation of triethylene diamine dithiocarbamate chitosan (TEDADTCCS) derivative. Hence, this study was to link dithiocarbamate groups with chitosan, to obtain more potent antifungal chitosan derivatives, and to assay their antifungal activity against some common plant pathogenic fungi.

2. Experiment

2.1. Materials

Chitosan (87% degree of deacetylation) with an average-molecular weight of 230 kDa, was purchased from Qingdao Baicheng Biochemical Corp. Triadimefon (20% emulsifiable concentrates) and polyoxin (10% wettable powders) were purchased from Jinan Wanda Biotechnology Co., Ltd and Kaken Pharmaceutical Co., Ltd, respectively. Ammonia (25.0%), carbon disulfide, triethylene diamine, and 95% ethanol (v/v) were purchased from Sinopharm Chemical Reagent Co., Ltd. All the chemical reagents were analytical grade. Two crop-threatening pathogenic fungi (*F. oxysporum* and *Alternaria porri*) were obtained from Qingdao Academy of Agricultural Sciences.

2.2. Analytical methods

Fourier transform infrared (FT-IR) spectra range in the 4000–400 cm^{-1} regions on a Thermo Scientific Nicolet iS10 FT-IR spectrometer in KBr discs. ^1H NMR spectra were investigated by nuclear magnetic resonance (NMR) with a JEOL JNM-ECP600 spectrometer, using CD_3COOD and D_2O as solvents. The elemental analysis (C, H, N, and S) was performed using a Vario EL-III elemental analyzer. The percentages of carbon, hydrogen, nitrogen, and sulfur were estimated. X-ray diffraction (XRD) measurement of the powder samples were performed with a D8 Advance diffractometer (Bruker) with Cu target ($\lambda = 0.154$ nm) at 40 kV and the scanning scope of 2θ was 5–40°. The surface morphology of the samples was analyzed by scanning electron microscopy by using KYKY-2800B SEM.

2.3. Synthesis of dithiocarbamate chitosan (DTCCS) derivatives

Dithiocarbamate chitosan (DTCCS) derivatives were prepared according to the method described in the literature with minor revision (Muzzarelli et al., 1982). A mixture of chitosan (16 g) and ammonia (0.1 mol) was stirred in 95% ethanol (v/v) for half an hour at room temperature. Then carbon disulfide (8 mL) was slowly dropped into the mixture. After stirring for 2 h, the resulting mixture was filtered through a Büchner funnel and the residue was washed with ethanol and dried at 60 °C. Finally ammonium dithiocarbamate chitosan (ADTCCS) was obtained. Similarly, triethylene diamine dithiocarbamate chitosan (TEDADTCCS) was prepared (Scheme 1).

2.4. Antifungal assay

Antifungal assays were evaluated in vitro according to the literature (Guo et al., 2006). Chitosan and dithiocarbamate chitosan (DTCCS) derivatives were dissolved in 0.5% (v/v) acetic acid at an original concentration of 1% (w/v). The solutions were mixed with sterile molten potato dextrose agar (PDA) to obtain final concentrations of 31.25 mg/L, 62.5 mg/L, 125 mg/L, 250 mg/L, and 500 mg/L, respectively.

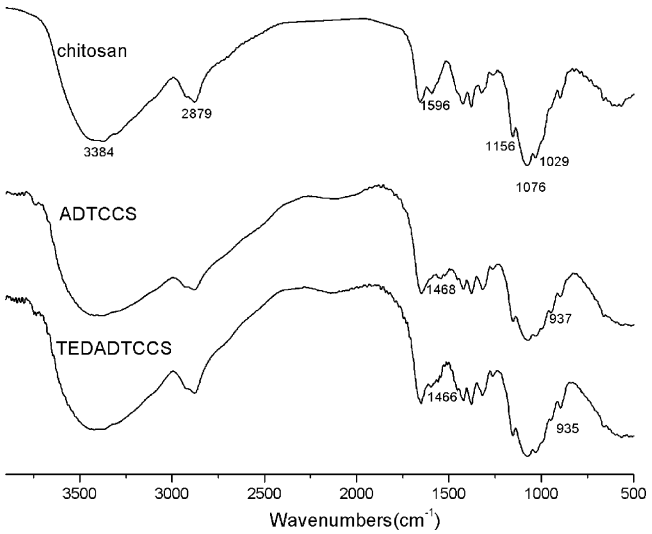

Fig. 1. FT-IR spectra of chitosan and dithiocarbamate chitosan (DTCCS) derivatives.

All tests were repeated three times and the results were averaged. The Scheffe method was used to evaluate the differences in antifungal index in the tests. Results with $P < 0.05$ were considered statistically significant.

3. Results and discussion

3.1. Preparation and characterization of dithiocarbamate chitosan (DTCCS) derivatives

In the present study, chitosan was mixed with carbon disulfide and ammonia (triethylenediamine) to obtain ammonium dithiocarbamate chitosan (ADTCCS) and triethylene diamine dithiocarbamate chitosan (TEDADTCCS). It was interesting that solubility of DTCCS improved visibly in aqueous acetic acid compared with chitosan. The structures of DTCCS were well confirmed by FT-IR, ^1H NMR, elemental analysis, XRD and SEM.

Fig. 1 shows FT-IR spectra of chitosan, ADTCCS and TEDADTCCS. For chitosan, the broad band around 3384 cm^{-1} attributed to —OH and —NH stretching vibration. The weak band at 2879 cm^{-1} was the characteristic absorbance peak of —CH. The absorption peak at 1596 cm^{-1} assigned to NH_2 bending vibration. Additionally, the absorption peaks of symmetric stretching of the C—O—C appeared at 1156 cm^{-1}, 1076 cm^{-1} and 1029 cm^{-1}. Compared with chitosan, new bands at 1468 cm^{-1} (N—C═S) and 937 cm^{-1} were observed for ADTCCS. In addition, the peak at 1596 cm^{-1} of the primary amine became weak which meant amino has been partly substituted. The results coincided with what reported before (Muzzarelli et al., 1982). All of the above results exhibited ADTCCS had been successfully obtained.

Similarly, new bands at 1466 cm^{-1} (N—C═S) and 935 cm^{-1} for TEDADTCCS were also observed, respectively. Hence, the structure of TEDADTCCS was confirmed.

The ^1H NMR spectra of chitosan, ADTCCS and TEDADTCCS are shown in Fig. 2. Spectra of chitosan were in accord with what reported before (Zhang et al., 2010). The signals were well separated and can be easily identified: (a) $\delta = 1.82$ ppm represented the hydrogen of the methyl, meaning the chitosan sample not fully deacetylated; (b) $\delta = 2.88$ ppm attributed to H2; (c) $\delta = 3.36$–3.58 ppm assigned to H5 and H6; (d) $\delta = 3.59$–3.70 ppm attributed to H3 and H4; (e) $\delta = 4.50$ ppm attributed to H1, whose signal was superimposed by the signals of solvent (D_2O). Compared with chitosan, a new weak signal for ADTCCS was observed at 3.34 ppm. It was inferred that it was the signal for substituted H2

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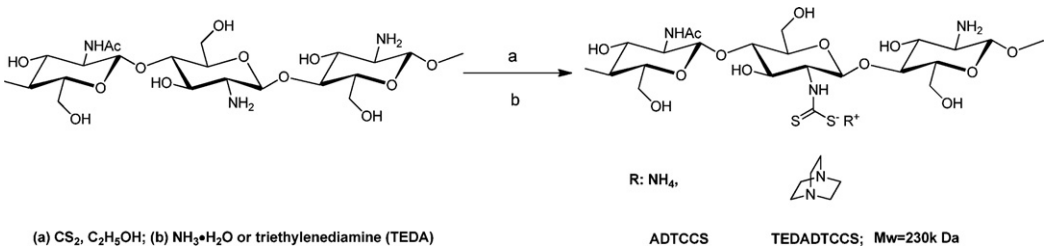

Scheme 1. Synthetic route of dithiocarbamate chitosan (DTCCS) derivatives.

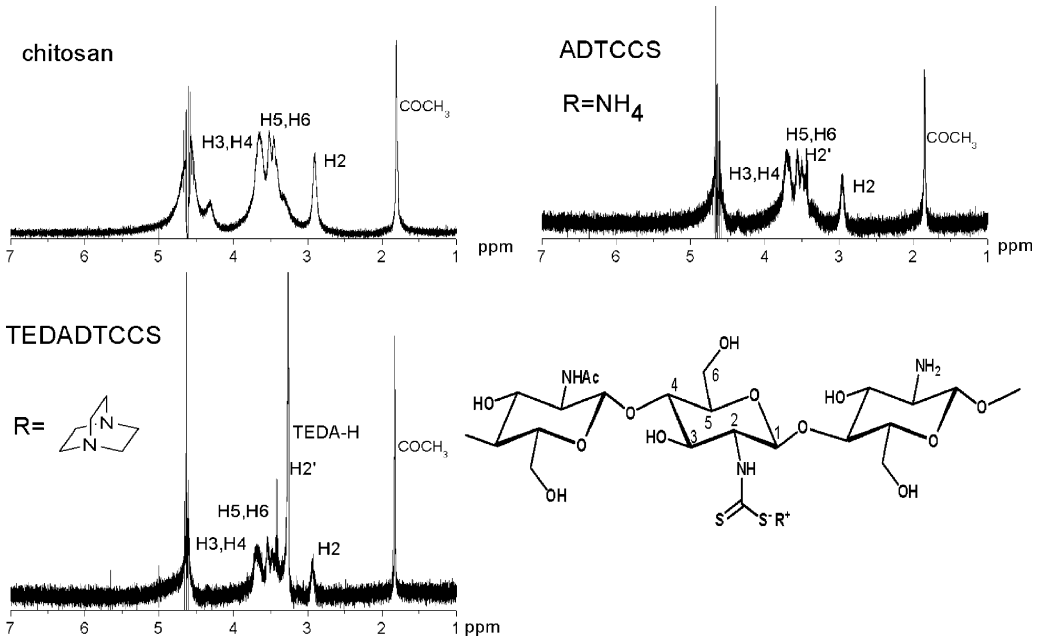

Fig. 2. ^{1}H NMR spectra of chitosan and dithiocarbamate chitosan (DTCCS) derivatives.

(H2′). The chemical shift of H2 changed from 2.88 ppm to 3.34 ppm due to negative conjugation effect of dithiocarbamate group at C2 of chitosan. Additionally, the signal at 2.88 ppm was still observed. It indicated that free amino groups at C2 were partly substituted. This result was also consistent with the FT-IR results we discussed. Hence, the structure of ADTCCS was further confirmed.

For TEDADTCCS, a new strong peak for TEDADTCCS at 3.21–3.34 ppm attributed to H2′ and TEDA-H showed TEDADTCCS had been obtained.

Elemental analysis results of dithiocarbamate chitosan (DTCCS) derivatives are shown in Table 1. The degree of substitution (DS) of DTCCS was calculated on the basis of the percentage. As shown in Table 1, the DS of ADTCCS and TEDADTCCS were 18.0% and 13.5%, which was not so high. It meant that only few free amino groups of chitosan were substituted by dithiocarbamate groups. This result also coincided with the results mentioned above. It was inferred that the lower sulfur content may result from relatively shorter reaction time and heterogeneous reaction conditions.

Table 1
Elemental analysis results and degree of substitution of dithiocarbamate chitosan (DTCCS) derivatives.

Compounds	Elemental analysis (%)				Degree of substitution (%)
	C	N	H	S	
Chitosan	40.05	7.29	6.41	–	–
ADTCCS	38.68	7.49	6.22	4.53	18.0
TEDADTCCS	40.05	7.63	6.31	2.49	13.5

Fig. 3 demonstrates the XRD spectra of chitosan and dithiocarbamate chitosan (DTCCS) derivatives. The XRD patterns of chitosan show three characteristic crystalline reflections at 13.2°, 19.8°, and 29.0° (Klaykruayat, Siralertmukul, & Srikulkit, 2010; Zhang, Zhang, Jiang, & Xia, 2012). The profiles of DTCCS were similar to the pattern of chitosan in terms of the peak position and relative intensity. However, the reflection peak at 13.2° was not observed, it indicated that the crystal structure of chitosan altered in the course of

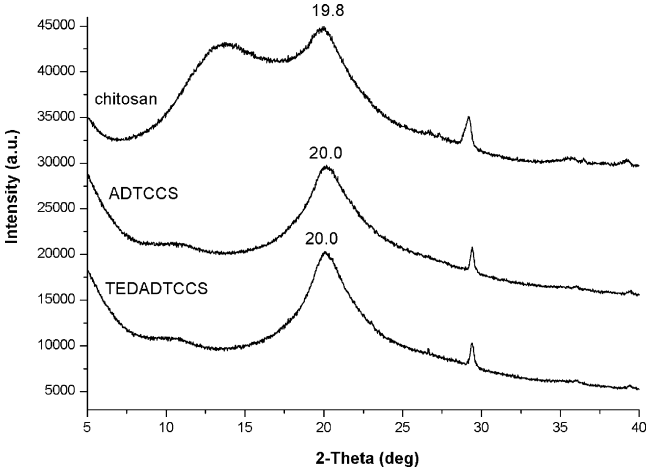

Fig. 3. XRD patterns of chitosan and dithiocarbamate chitosan (DTCCS) derivatives.

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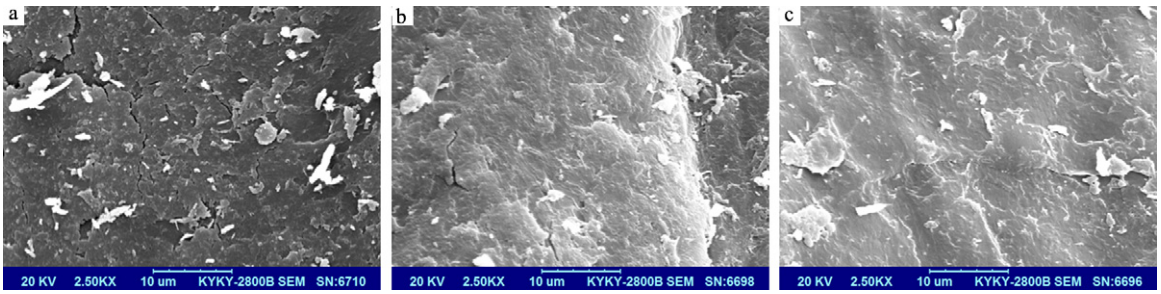

Fig. 4. Surface morphology of chitosan (a), ammonium dithiocarbamate chitosan (ADTCCS) (b) and triethylene diamine dithiocarbamate chitosan (TEDADTCCS) (c).

the reaction. X-ray diffraction exposed the differences among chitosan and DTCCS derivatives. The modification of chitosan changed the elemental composition of the polymer and the macromolecular conformation was partly ruptured, which was consistent with the results mentioned above (He et al., 2011; Klaykruayat et al., 2010).

Fig. 4 displays the surface structure of chitosan, ADTCCS and TEDADTCCS. As shown in Fig. 4, chitosan exhibits a smooth, dense and flat morphology (Trimukhe & Varma, 2008). This may be owing to high inter-molecular hydrogen density of chitosan. While the dithiocarbamate chitosan derivatives, ADTCCS and TEDADTCCS show a rough surface morphology which may be due to the dithiocarbamate groups partly grafted on the chitosan. The insufficient cross-linking of the chitosan derivatives lead to uneven structures. This was in accord with the XRD results. It was deduced that modification of the chitosan deceased its inter-molecular hydrogen bonding and increased the hydrophilicity of the copolymer.

3.2. Antifungal activity of dithiocarbamate chitosan (DTCCS) derivatives

Antifungal properties of dithiocarbamate chitosan (DTCCS) derivatives against the plant pathogenic fungi *F. oxysporum* and *A. porri* were investigated at concentrations ranged from 31.25 to 500 mg/L.

It has been proved that antifungal activity of chitosan and its derivatives are influenced by various factors such as molecular weight, concentration, the pH value and so on (Bautista-Baños et al., 2006). In this study, chitosan and dithiocarbamate chitosan derivatives (DTCCS) were dissolved in 0.5% (v/v) acetic acid at an original concentration of 1% (w/v). Then solutions were diluted with sterile molten potato dextrose agar (PDA). The presence of the acetic acid might contribute to the antifungal action. So the inhibitory effect of different concentrations of acetic acid aqueous solution on *F. oxysporum* and *A. porri* corresponding to the investigated concentrations of samples was evaluated. As shown in Fig. 5(a), the inhibitory effect of acetic acid on the selected fungi was influenced by the concentrations. At low concentrations, no higher than 0.025% (corresponding to 500 mg/L), acetic acid aqueous solution had no inhibitory effect on *F. oxysporum* and *A. porri*. That is to say, at concentrations ranged from 31.25 to 500 mg/L, the diluted acetic acid had no antifungal activity.

While at concentrations, higher than 0.025%, acetic acid would inhibit the growth of the fungi. *F. oxysporum* was more sensitive to acetic acid than *A. porri*. It was shown that 0.05% acetic acid aqueous solution inhibited growth of *F. oxysporum* at 14.9%.

F. oxysporum, is a pathogen which causes fusarium wilt and foot-rot of cucumber, watermelon and so on (Larkin, Hopkins, & Martin, 1993; Woo et al., 1996). As shown in Table 2, both of the derivatives exhibited much better antifungal activity compared with original chitosan and the antifungal activity enhanced with increasing of concentration. Antifungal index of the DTCCS derivatives ranged from 44.1% to 62.0% at 500 mg/L, while the index of chitosan only reached 26.3%. The inhibitory effect followed a sequence of TEDADTCCS > ADTCCS > triadimefon > polyoxin > chitosan. It was interesting that both TEDADTCCS and ADTCCS had stronger inhibitory effect than polyoxin and triadimefon (Fig. 4(b)). At 500 mg/L, TEDADTCCS inhibited *F. oxysporum* at 60.4%, and was stronger than polyoxin and triadimefon whose antifungal indexes were 25.3% and 37.7%, respectively. Thereby, it was concluded that chemical modification was an efficient method to enhance antifungal activity of chitosan. The combination of dithiocarbamate groups with chitosan increased both the antifungal activity and solubility of CS. For TEDADTCCS, although its DS was lower than that of ADTCCS, it had better antifungal activity than ADTCCS. This may due to strong ring tension and alkaline of triethylene diamine. Considering the low DS of DTCCS, it was

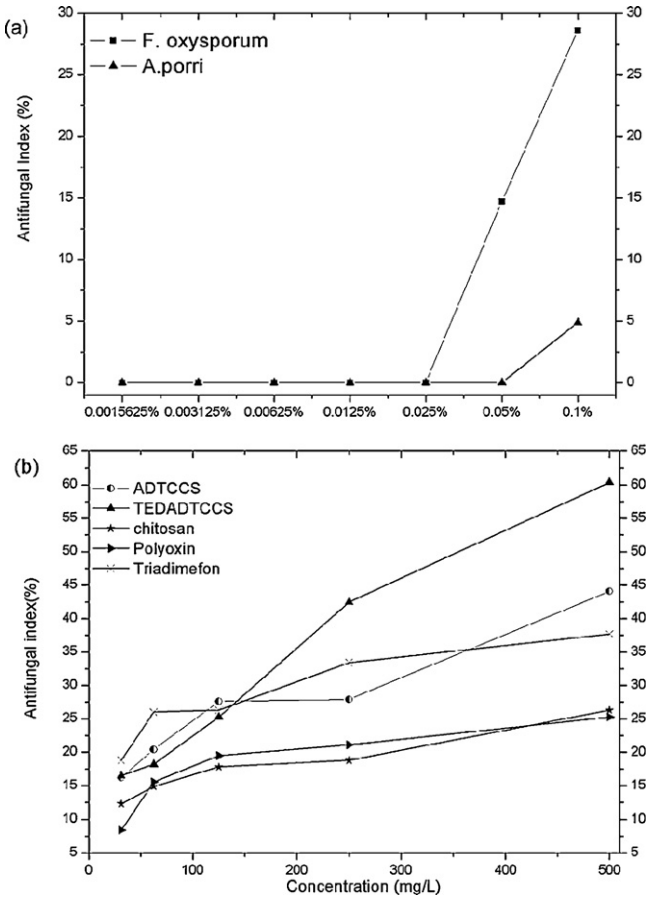

Fig. 5. (a) Inhibitory effect of different concentrations of acetic acid aqueous solution on *F. oxysporum* and *A. porri*. (b) Antifungal activity of chitosan and dithiocarbamate chitosan (DTCCS) derivatives against *F. oxysporum*.

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Table 2
Antifungal activity of dithiocarbamate chitosan (DTCCS) derivatives at different concentrations.

Sample	Concentrations (mg/L)	*F. oxysporum*	*A. porri*
Chitosan	31.25	12.3	13.9
	62.5	14.9	17.1
	125	17.8	23.9
	250	18.8	24.4
	500	26.3	28.8
ADTCCS	31.25	16.2	8.58
	62.5	20.4	10.2
	125	27.6	16.3
	250	27.9	18.0
	500	44.1	34.8
TEDADTCCS	31.25	16.5	5.66
	62.5	18.2	16.3
	125	25.3	16.7
	250	42.5	19.6
	500	60.4	34.5
Polyoxin	31.25	8.44	12.3
	62.5	15.6	44.5
	125	19.5	46.3
	250	21.1	50.6
	500	25.3	80.6
Triadimefon	31.25	18.8	20.6
	62.5	26.0	37.4
	125	26.3	40.1
	250	33.4	45.3
	500	37.7	47.1

necessary to enhance their DS for improving the antifungal properties.

Onion purple blotch disease, caused by *A. porri*, can bring serious losses in onion (Bock, 1964; Meredith, 1966). Table 2 represents the antifungal results of the DTCCS derivatives against *A. porri*. Compared with chitosan, antifungal activity of DTCCS derivatives slightly enhanced. The antifungal index ranged from 8.58% to 34.8%. TEDADTCCS and ADTCCS had no obvious difference on the antifungal activity against *A. porri* and both of them exhibited lower activity than polyoxin and triadimefon. It meant that grafting dithiocarbamate groups into C-2 position of chitosan did not obviously enhance the antifungal activity of CS. *A. porri* was not so sensitive as *F. oxysporum* to DTCCS.

Based on the above bioassay results, it was found that dithiocarbamate chitosan (DTCCS) derivatives had enhanced antifungal activity in comparison with chitosan. The graft of dithiocarbamate groups into C-2 position of chitosan could improve the solubility and antifungal activity of chitosan in general.

4. Conclusion

Ammonium dithiocarbamate chitosan (ADTCCS) and triethylene diamine dithiocarbamate chitosan (TEDADTCCS) were prepared and their structures were well characterized by FT-IR, ^{1}H NMR, elemental analysis, XRD, and SEM. It was shown that both ADTCCS and TEDADTCCS had enhanced inhibitory effects on *F. oxysporum* and *A. porri*. The antifungal activity was affected by concentration of the derivatives and fungal species. *F. oxysporum* was more sensitive than *A. porri* to the DTCCS derivatives. Additionally, the DTCCS derivatives even had stronger inhibitory effect on *F. oxysporum* than polyoxin and triadimefon. Hence, it was inferred that chemical modification of chitosan was an efficient method to improve its antifungal activity. Although the DS of the derivatives was relatively low, their antifungal activity was significantly better than that of chitosan. Thus, further study to enhance DS of the DTCCS derivatives should be carried out and more microbial strains need to be investigated.

Acknowledgments

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References

Allan, C. R., & Hadwiger, L. A. (1979). The fungicidal effect of chitosan on fungi of varying cell wall composition. *Experimental Mycology*, *3*(3), 285–287.
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.
Bock, K. R. (1964). Purple blotch (*Alternaria porri*) of onion in Kenya. *Annals of Applied Biology*, *54*(3), 303–311.
Caldas, E. D., Conceição, M. H., Miranda, M. C. C., de Souza, L. C. K. R., & Lima, J. F. (2001). Determination of dithiocarbamate fungicide residues in food by a spectrophotometric method using a vertical disulfide reaction system. *Journal of Agricultural and Food Chemistry*, *49*(10), 4521–4525.
Coutinho, D. F., Pashkuleva, I. H., Alves, C. M., Marques, A. P., Neves, N. M., & Reis, R. L. (2008). The effect of chitosan on the in vitro biological performance of chitosan-poly(butylene succinate) blends. *Biomacromolecules*, *9*(4), 1139–1145.
Feng, H., & Dong, C.-M. (2006). Preparation, characterization, and self-assembled properties of biodegradable chitosan-poly(L-lactide) hybrid amphiphiles. *Biomacromolecules*, *7*(11), 3069–3075.
Guo, Z., Chen, R., Xing, R., Liu, S., Yu, H., Wang, P., et al. (2006). Novel derivatives of chitosan and their antifungal activities in vitro. *Carbohydrate Research*, *341*(3), 351–354.
Guo, Z., Xing, R., Liu, S., Zhong, Z., Ji, X., Wang, L., et al. (2007). The influence of the cationic of quaternized chitosan on antifungal activity. *International Journal of Food Microbiology*, *118*(2), 214–217.
Gustafsson, K. H., & Fahlgren, C. H. (1983). Determination of dithiocarbamate fungicides in vegetable food stuffs by high-performance liquid chromatography. *Journal of Agricultural and Food Chemistry*, *31*(2), 461–463.
He, G., Chen, X., Yin, Y., Zheng, H., Xiong, X., & Du, Y. (2011). Synthesis, characterization and antibacterial activity of salicyloyl chitosan. *Carbohydrate Polymers*, *83*(3), 1274–1278.
Jameela, S. R., & Jayakrishnan, A. (1995). Glutaraldehyde cross-linked chitosan microspheres as a long acting biodegradable drug delivery vehicle: Studies on the in vitro release of mitoxantrone and in vivo degradation of microspheres in rat muscle. *Biomaterials*, *16*(10), 769–775.
Klaykruayat, B., Siralertmukul, K., & Srikulkit, K. (2010). Chemical modification of chitosan with cationic hyperbranched dendritic polyamidoamine and its antimicrobial activity on cotton fabric. *Carbohydrate Polymers*, *80*(1), 197–207.
Kumar, M. N. V. R., Muzzarelli, R. A. A., Muzzarelli, C., Sashiwa, H., & Domb, A. J. (2004). Chitosan chemistry and pharmaceutical perspectives. *Chemical Reviews*, *104*(12), 6017–6084.
Larkin, R. P., Hopkins, D. L., & Martin, F. N. (1993). Ecology of *Fusarium oxysporum* f. sp. niveum in soils suppressive and conducive to Fusarium wilt of watermelon. *Phytopathology*, *83*(10), 1105–1116.
Meredith, D. S. (1966). Spore dispersal in *Alternaria porri* (Ellis) Neerg. on onions in Nebraska. *Annals of Applied Biology*, *57*(1), 67–73.
Muzzarelli, R. A. A. (1983). Chitin and its derivatives: New trends of applied research. *Carbohydrate Polymers*, *3*(1), 53–75.
Muzzarelli, R. A. A., Tanfani, F., Mariotti, S., & Emanuelli, M. (1982). Preparation and characteristic properties of dithiocarbamate chitosan, a chelating polymer. *Carbohydrate Research*, *104*(2), 235–243.
Muzzarelli, R. A. A., Tarsi, R., Filippini, O., Giovanetti, E., Biagini, G., & Varaldo, P. E. (1990). Antimicrobial properties of N-carboxybutyl chitosan. *Antimicrobial Agents and Chemotherapy*, *34*(10), 2019–2023.
Pease, H. L., & Holt, R. F. (1977). Manganese ethylenebis(dithiocarbamate) (maneb)/ethylenethiourea (ETU) residue studies on five crops treated with ethylenebis(dithiocarbamate) (EBDC) fungicides. *Journal of Agricultural and Food Chemistry*, *25*(3), 561–567.
Rabea, E. I., Badawy, M. E. I., Rogge, T. M., Stevens, C. V., Steurbaut, W., Höfte, M., et al. (2006). Enhancement of fungicidal and insecticidal activity by reductive alkylation of chitosan. *Pest Management Science*, *62*(9), 890–897.

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Trimukhe, K. D., & Varma, A. J. (2008). A morphological study of heavy metal complexes of chitosan and crosslinked chitosans by SEM and WAXRD. *Carbohydrate Polymers*, *71*(4), 698–702.

Woo, S. L., Zoina, A., Del Sorbo, G., Lorito, M., Nanni, B., Scala, F., et al. (1996). Characterization of *Fusarium oxysporum* f.sp. phaseoli by pathogenic races, VCGs, RFLPs, and RAPD. *Phytopathology*, *86*(9), 966–973.

Xu, J., McCarthy, S. P., Gross, R. A., & Kaplan, D. L. (1996). Chitosan film acylation and effects on biodegradability. *Macromolecules*, *29*(10), 3436–3440.

Zhang, K., Helm, J., Peschel, D., Gruner, M., Groth, T., & Fischer, S. (2010). NMR and FT Raman characterisation of regioselectively sulfated chitosan regarding the distribution of sulfate groups and the degree of substitution. *Polymer*, *51*(21), 4698–4705.

Zhang, W., Zhang, J., Jiang, Q., & Xia, W. (2012). Physicochemical and structural characteristics of chitosan nanopowders prepared by ultrafine milling. *Carbohydrate Polymers*, *87*(1), 309–313.

Please cite this article in press as: Qin, Y., et al. Synthesis and characterization of dithiocarbamate chitosan derivatives with enhanced antifungal activity. *Carbohydrate Polymers* (2012), doi:10.1016/j.carbpol.2012.03.018