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Synthesis of chitosan derivative with diethyldithiocarbamate and its antifungal activity

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ABSTRACT

With an aim to discover novel chitosan derivatives with enhanced antifungal properties compared with chitosan. Diethyl dithiocarbamate chitosan (EtDTCCS) was investigated and its structure was well identified. The antifungal activity of EtDTCCS against *Alternaria porri* (*A. porri*), *Gloeosporium theae sinensis* Miyake (*G. theae sinensis*), and *Stemphylium solani* Weber (*S. solani*) was tested at 0.25, 0.5, and 1.0 mg/mL, respectively. Compared with plain chitosan, EtDTCCS shows better inhibitory effect with 93.2% inhibitory index on *G. theae sinensis* at 1.0 mg/mL, even stronger than for polyoxin (82.5%). It was inferred derivatives of this kind may find potential applications for the treatment of various crop-threatening diseases.

1. Introduction

Chitosan, (1–4)-2-amino-2-deoxy-β-D-glucan, the cationic deacetylated derivative of chitin, is a well-affirmed biopolymer owing to biocompatibility, biodegradability and absence of toxicity [1]. Among its widespread biological activities, antimicrobial activity has attracted extensive attention. It is proved that chitosan has a broad-spectrum antimicrobial activity against a variety of bacteria and fungi [2]. However, the weak antimicrobial properties have hampered its widespread application.

Chemical modification of chitosan to obtain new bioactive derivatives is of interest because such procedure may not change the fundamental skeleton of chitosan [3–5]. Additionally, the new generated chitosan derivatives may also possess original properties of the group introduced. In fact, the strategies for introducing functional groups into chitosan are widely used. These grafting techniques involve mainly condensation reactions such as alkylation [6], acylation [7], sulfonation [8], and so on. For example, Muzzarelli et al. reported antimicrobial properties of N-carboxybutyl (NCB) chitosan tested against 298 microbial strains, the data displayed NCB-chitosan may have a potential application as wound dressing [9]. Rabea and his co-authors reported the preparation and antifungal properties of N-alkyl chitosan (NAC) derivatives [10]. The results showed most of the NAC derivatives exerted better antifungal activity than original chitosan. Eweis et al. described antifungal activity of a chitosan thiourea derivative (TUCS) against *Rhizoctonia solani*, *Sclerotium rolfsii*, and *Fusarium solani*. The prepared chitosan derivative had a significant inhibitory effect on the investigated fungi at the concentrations of 5–1000 μg/mL [11].

It is notable that dithiocarbamates modified chitosan and its applications have gained considerable attention in the past few years [12,13]. Chitosan dithiocarbamate is a chitosan derivative in which C2 amino groups are substituted by dithiocarbamate groups. It is usually achieved by adding carbon disulfide (CS_2) and alkaline dissolved in alcohol. Owing to the strong metal binding capacity of dithiocarbamates, various dithiocarbamate chitosan derivatives have been prepared and investigated [14,15]. However, current applications of chitosan dithiocarbamates were mainly focused on metal chelating properties and these chitosan derivatives were usually obtained via insertion of carbon disulfide to form dithiocarbamate directly. There were few articles involved antimicrobial properties or additional synthetic methods of chitosan dithiocarbamate. In fact, dithiocarbamates are a class of fungicides extensively used for the control of a large variety of diseases affecting several types of crops [16–18].

In view of the potential antimicrobial applications of chitosan dithiocarbamates, this study aims to extend synthetic methods and applications of chitosan dithiocarbamates. Hence, diethyl

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dithiocarbamate chitosan was synthesized and its antifungal activity against some common plant pathogenic fungi was investigated.

2. Experiment

2.1. Materials

Chitosan, [weight-average molecular weight (MW) of 230 kDa, with a degree of deacetylation of 0.87], was purchased from Qingdao Baicheng Biochemical Corp., Shandong, China. Polyoxin, 10% wettable powders, was obtained from Kaken Pharmaceutical Co., Ltd. Diethylamine, carbon disulfide, and chloroacetyl chloride were purchased from Sinopharm Group Chemical Reagent Co. Ltd. (China). Other commercial chemical reagents used in the experiment were all analytical grade. Double-distilled water was used throughout the experiment.

2.2. Microbial strains

Three plant pathogenic fungi, *Alternaria porri* (*A. porri*), *Gloeosporium theae sinensis* Miyake (*G. theae sinensis*), and *Stemphylium solani* Weber (*S. solani*), were used as the tested microorganisms. They were obtained from Qingdao Academy of Agricultural Sciences. Fungi for testing were stored on PDA medium at 4 °C.

2.3. Analytical conditions

Fourier transform infrared (FT-IR) spectra of the derivatives were obtained on a Thermo Scientific Nicolet iS10 FT-IR spectrometer between 4000 and 400 cm^{-1} regions using in KBr discs. The ^{13}C NMR (Internal standard: acetone) spectra of the polymers were recorded on a JEOL JNM-ECP600 spectrometer, using DCl and D_2O as solvents. The elemental analysis (C, H, N, and S) was determined according to a Vario EL-III elemental analyzer and the content of carbon, hydrogen, nitrogen, and sulfur were estimated. The degree of substitution (DS) of diethyl dithiocarbamate chitosan (EtDTCCS) was calculated by the percentages of sulfur. DSC temperature scan was performed using The Pyris Diamond DSC (Perkin Elmer). The samples were sealed in aluminum pans and heated from 50 °C to 300 °C under 10 °C/min under the protection of nitrogen purge gas. An empty pan was used as reference in the test. Three tests were performed for each sample. The gross morphology and microstructures of the samples was observed under scanning electron microscopy by using KYKY-2800B SEM.

2.4. Synthesis of diethyl dithiocarbamate chitosan (EtDTCCS)

2.4.1. Synthesis of potassium dimethyldithiocarbamate

A mixture of diethylamine (100 mmol, 7.3 g) and potassium hydroxide (100 mmol, 5.6 g) was stirred in a 250 mL round bottomed flask with 100 mL methanol for an hour at room temperature. Then carbon disulfide (100 mmol, 7.6 g) was added drop-wise over 30 min to the mixture and the solution was stirred for another 10 h. Next, the solvent was removed under vacuum to give a crude product, and then the product was recrystallized two times from ethanol to obtain pure potassium dimethyl dithiocarbamate as a yellow solid, 87.1% yield. ^1H NMR (600 MHz, DMSO-d_6, δ): 3.96 (q, 2H, CH_2), 1.07 (t, 3H, CH_3).

2.4.2. Preparation of diethyl dithiocarbamate chitosan (EtDTCCS)

Chloroacetyl chitosan (CACS) was prepared according to the literatures described by Zhong [19]. Then chloroacetyl chitosan (10 mmol) was mixed with dimethyl dithiocarbamate (10 mmol) in double-distilled water (50 mL). After reacted for 8 h at 80 °C, the resultant was cooled to room temperature and filtered through Filter Funnel Buchner. The precipitate was washed with ethanol and dried to give diethyl dithiocarbamate chitosan (Scheme 1), yield: 56.5%, DS: 0.354.

2.5. Antifungal bioassays

Antifungal assay was evaluated against *Alternaria porri* (*A. porri*), *Gloeosporium theae sinensis* Miyake (*G. theae sinensis*), and *Stemphylium solani* Weber (*S. solani*) in vitro by mycelium growth rate test according to the literatures [20,21]. The tested concentration was 0.25 mg/mL, 0.5 mg/mL, and 1.0 mg/L, respectively.

Each experiment was performed in three replicates, and the data were averaged. Results with $P < 0.05$ were considered statistically significant.

3. Results and discussion

3.1. Preparation and characterization of diethyl dithiocarbamate chitosan (EtDTCCS)

In our attempt to obtain new dithiocarbamate chitosan derivatives with potential antifungal activity, diethyl dithiocarbamate chitosan (EtDTCCS) was synthesized, purified and its structure was confirmed from their analytical and spectral data.

Fig. 1 presents the infrared transmittance spectra for the prepared samples. For chitosan, the broad band around 3400 cm^{-1} attributed to —OH and —NH stretching vibration. The weak peak at 2875 cm^{-1} was the characteristic absorbance of —CH. The absorption peak at 1592 cm^{-1} is associated with NH_2 bending vibration. Additionally, the absorption peaks assigned to symmetric stretching of the C—O—C were observed at 1157 cm^{-1}, 1080 cm^{-1} and 1021 cm^{-1}. Compared with chitosan, new bands at 1643 cm^{-1} (the amide I, C=O), 1533 cm^{-1} (the amide II, NH—C=O), 1380 cm^{-1} (C—N) were observed for chloroacetylated chitosan (CACS). In addition, the peak at 1592 cm^{-1} of the primary amine disappeared which meant amino has been substituted. These results also coincided with that reported before [19]. All of the results exhibited CACS had been successfully obtained.

In the spectrum of diethyl dithiocarbamate chitosan (EtDTCCS), new peaks appear at 2879 cm^{-1} (S—CH_2), 1425 cm^{-1} (NH—C=S), 1261 cm^{-1} (C=S). Additionally, the amide I stretching peak has shifted from 1643 cm^{-1} to 1673 cm^{-1} due to the introduction of dithiocarbamate group. Hence, the FT-IR data are indicative of

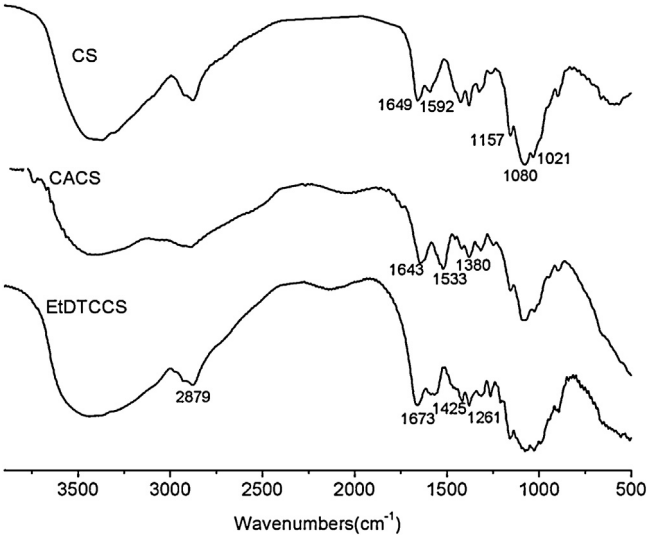

Fig. 1. FT-IR spectra of chitosan, chloroacetylated chitosan (CACS), and diethyl dithiocarbamate chitosan (EtDTCCS).