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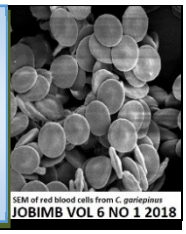

Production of Chitosan from *Aspergillus niger* ATCC 16404 and Application as Antibacterial Activity

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ABSTRACT

Chitosan synthesized from *Aspergillus niger* ATCC 16404, may display potential antibacterial activity. The purpose of this study was to evaluate the *in vitro* antibacterial activity of chitosan against various microorganisms such as *Pseudomonas aeruginosa* ATCC 9027*, Escherichia coli* ATCC 8739 *and Bacillus subtilis* ATCC 6633. The physicochemical properties were analyzed by FTIR. Lower antibacterial activity was exhibited for chitosan concentrations of between10 and 60 µg/ml compared to that of between 70 and 100 µg/ml of chitosan for all bacteria, whilst chitosan concentrations of above 70 µg/ml exhibited strong inhibition for the bacteria *Escherichia coli* ATCC 8739 and *Bacillus subtilis* ATCC 6633 with a slightly lower inhibition was observed for *Pseudomonas aeruginosa* ATCC 9027 indicating the higher antibacterial activity of chitosan for *Escherichia coli* ATCC 8739 and *Bacillus subtilis* ATCC 6633. Results show that chitosan could inhibit the growth of different bacteria tested.

INTRODUCTION

Chitosan is a homopolymer of N-acetyl-D-glucosamine (Glc-NAc) residues linked by β-1-4 bonds, is the most widespread renewable natural resource following cellulose. It is normally produced by the deacetylation of chitin obtained from shrimp waste by strong alkalis at high temperatures for long periods of time [1]. There are problems with the continuing supply of raw materials and high processing costs associated with chemical conversion of the chitin to chitosan [2]. Chitosan (polyglucosamine) is a natural and recyclable biopolymer. Chitosan has been used in several areas, for instance, in cosmetics, pharmaceuticals, food additives and agriculture [3]. Furthermore, the chitosan derived from such a process is heterogeneous with respect to their physio-chemical properties [2]. Current advances in fermentation technology recommend that many of these problems can be overcome by culturing chitosan-producing fungi [4].

The mycelia of various fungi including *Ascomycetes, Zygomycetes, Basidiomycetes* and *Deutermoycetes*, are alternative sources of chitin and chitosan [5]. However, the culture medium is highly cost that an inexpensive procedure of chitosan production would be effectively if chitosan could be created by fungi using raw materials. Chitosan produced using fungi has established much interest due to the need for a different source of chitin to clarify these problems. It is well recognized from studies which definite fungi include chitosan as a cell wall component [6], Fungi with significant quantity of chitin in their cell walls, and upon the extraction of chitosan from these cells [7,8].

The antibacterial activity of chitosan is biased by a quantity of factors that consist of chitosan types, chitosan polymerization levels and additional physicochemical properties. Chitosan capability higher antibacterial activity against Gram-positive bacteria than Gram-negative bacteria. In addition, besides depends on the molecular weight and solvent,5 and differently affected by pH, with higher activity at lower pH values [6].
The aim of this study was to estimate the opportunity to create chitosan from *Aspergillus niger* ATCC 16404 under different conditions. Also, application of chitosan as antimicrobial agent was investigated.

MATERIALS AND METHODS

Strain and culture media

Aspergillus niger ATCC 16404 was obtained from USA. Materials used in this study were obtained from Sigma company.

Preparation of inoculums

Aspergillus niger ATCC 16404 grown on (MGYP) medium plates was containing 0.3 % malt extract, 1 % sucrose, 0.3 % yeast extract, and 0.5 % peptone, incubated 30°C for 5 days. After incubation, plates were observed for the growth of fungi and isolated colonies on the plates were pure cultured and maintained in PDA slants at 4ºC [9].

Culture conditions and fermentation process

One loop of fungal spores from slant tubes of PDA broth medium was inoculated at 30°C for 48 h. Yeast extract and glucose were implemented as nitrogen and carbon sources to effective the growth of fungi. One mL of spore suspension was inoculated into sterile flasks containing (MGYP) medium was containing 0.3 % malt extract, 1 % sucrose, 0.3 % yeast extract, and 0.5 % peptone and shaken to distribute the spores [10]. The flasks incubated at 30°C /96 days [11,12].

Preparation of Biomass

Biomass was washed with distilled water and was separated by centrifugation at 6000 rpm for 30 min. The residue was washed several times with distilled water, recentrifuged until all medium was removed and was dried at 45° C for 24 h [13].

Extraction of Chitosan

The fungal mycelia were extracted by added 50 ml of 1 N NaOH solution (1:30 w/v) gently into the culture medium and the alkaline suspension was homogenized. The content was sterilized at 121°C for 20 mins. The alkali insoluble materials (AIM) were collected by centrifugation at 6000 rpm for 20 mins and then washed several times with distilled water to neutralize them to pH 7.0. AIMs were dried in an oven at 40 °C and treated with acetic acid 2 %(v/v), as a chitosan solvent, under reflux condition for 6 hrs at 95 °C (1:30 w/v).

The acid insoluble fraction was precipitated at 6000 rpm for 15-20 min and the supernatant containing the chitosan was isolated. Chitosan was precipitated with clear yellowish colour, the pH was adjusted with 2N NaOH, and chitosan was centrifuged at 6000 rpm, for 15 min. The isolated chitosan was washed four to five times with distilled water to neutralize and 96% ethanol and acetone were used to rinse chitosan and dried at 60 °C [14,15].

Antimicrobial Activity of chitosan by disk diffusion method

The antimicrobial activity of AgNPs was investigated against *P. aeruginosa ATCC 9027, E. coli ATCC 8739 and B. subtilis ATCC 6633* using disk diffusion method. Each strain was grown into individual plates after incubation at 28 °C for 28 h, the diameter of inhibition zone. The assays were performed in triplicate [16].

Antimicrobial Activity of different concentration of chitosan for some pathogenic bacteria

Test tubes each containing 1mL of LB broth medium were autoclaved for 15min at 121°C. Chitosan powder is only soluble in acetic con. (0.25%) media and adjust pH 5. The first tube, chitosan solution concentration (10-100µg/mL) was added with 50µL of different pathogenic bacteria suspension such as (*Pseudomonas aeruginosa* ATCC 9027*, Escherichia coli* ATCC 8739 *and Bacillus subtilis* ATCC 6633. After that, the tubes were incubated at 37 °C for 48h. The blank control tubes were only contained LB broth medium and 0.25% acetic acid [17].

RESULTS AND DISCUSSION

The isolated chitosan produced by *Aspergillus niger* (**Fig. 1**) was subjected to FTIR analysis from the range of 400 to 4000 cm^{-1}. The FTIR spectrum showed six peaks (**Fig. 2**). The two major peaks (No. 1,3) at ~3455.81 cm^{-1} and ~1638.23cm^{-1} correspond to the hydroxy and amide groups, respectively [12]. The other peaks obtained were at ~2102.99 cm^{-1}, ~1410.67cm^{-1}, ~1083.8$cm^{-1}$and ~ 546.72cm^{-1}. The peak at ~1410.67 cm^{-1} indicated the presence of CH_3 group and the peak at ~1410.67 cm^{-1} corresponded to the N-H deformation of amide group which can be observed clearly in pure chitosan, which decreased dramatically [8].

Fig. 1. Chitosan produced from *Aspergillus niger* ATCC 16404.

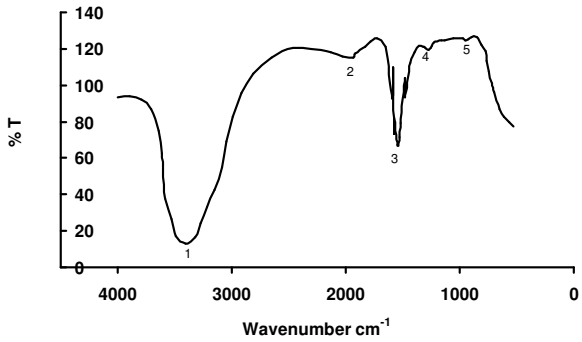

Fig. 2. Characterization of chitosan by Fourier Transform Spectroscopy (FTIR).

Antimicrobial activity

Chitosan is only soluble in acidic media, which could be well dispersed in bacterial suspension after a slight shock for a nice dispersion. Bacteria can hold on surface of chitosan considerably in short time of just 30 min; thus, chitosan and chitosan nanoparticles demonstrate antibacterial activity. According to the literature [4,17-19] chitosan possesses antimicrobial activity against a number of Gram-negative and Gram-positive bacteria. The antimicrobial activity of chitosan, against various microorganism such as *P. aeruginosa* ATCC 9027*, Escherichia coli* ATCC 8739 *and B. subtilis* ATCC 6633 is shown in **Figs. 3** to **5**, respectively.

Lower antibacterial activity was exhibited for chitosan concentrations of between10 and 60 µg/ml compared to that of between 70 and 100 µg/ml of chitosan for all bacteria, whilst chitosan concentrations of above 70 µg/ml exhibited strong inhibition for the bacteria *Escherichia coli* ATCC 8739 and *Bacillus subtilis* ATCC 6633 with a slightly lower inhibition was observed for *Pseudomonas aeruginosa* ATCC 9027 indicating the higher antibacterial activity of chitosan for *Escherichia coli* ATCC 8739 and *Bacillus subtilis* ATCC 6633.

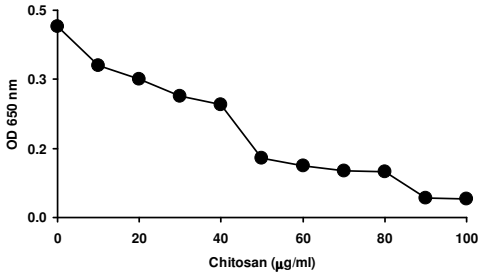

Fig. **3**. The effect of chitosan suspension at pH 6.5 against *Pseudomonas aeruginosa* ATCC 9027.

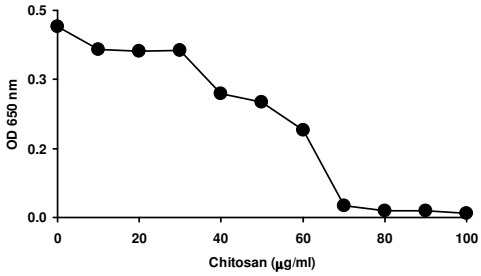

Fig. **4**. The effect of chitosan suspension at pH 6.5 against *Escherichia coli* ATCC 8739.

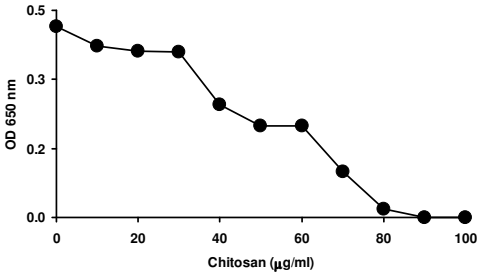

Fig. **5**. The effect of chitosan suspension at pH 6.5 against *Bacillus subtilis* ATCC 6633.

It has been shown that chitosan strong antimicrobial activity against both gram-positive and gram-negative bacteria, such as *P. aeruginosa* ATCC 9027*, Escherichia coli* ATCC 8739 *and B. subtilis* ATCC 6633, which is similar to several reports from the literature [17,18]. Two theories have been considered for chitosan mechanism as antimicrobial agent. First of all, the interaction between the positively charged chitosan molecules and negatively charged microbial cell membranes consequences in the disruption of the cytoplasmic membrane and, ultimately, leakage of intracellular constituents [19]. In additional, chitosan oligosaccharides basically permeate into the nucleus of eukaryotic cell and interfere with the transcription of RNA and the synthesis of proteins. However, chitosan with high molecular weight (above 100 kDa) normally express stronger antibacterial activity than chitosan oligomers [19].

REFERENCES

1. Bartnicki-Garcia S. Cell wall chemistry, morphogenesis, and taxonomy of fungi. Ann Rev Microbiol. 1968;22(1):87–108.
2. Crestini C, Kovac B, Giovannozzi-Sermanni G. Production and isolation of chitosan by submerged and solid-state fermentation from *Lentinus edodes*. Biotechnol Bioeng. 50(2):207–10.
3. Davies DH, Hayes ER. Determination of the degree of acetylation of chitin and chitosan. In: Methods in Enzymology. Academic Press; 1988. p. 442–6.
4. Gil G, del Mónaco S, Cerrutti P, Galvagno M. Selective antimicrobial activity of chitosan on beer spoilage bacteria and brewing yeasts. Biotechnol Lett. 2004;26(7):569–74.
5. Hang YD. Chitosan production from *Rhizopus oryzae* mycelia. Biotechnol Lett. 1990;12(12):911–2.
6. Knorr D (Berlin U of T. Recovery and utilization of chitin and chitosan in food processing waste management. Food technology (USA). 1991. Available from: http://agris.fao.org/agris-search/search.do?recordID=US9128440
7. Kučera J. Fungal mycelium—the source of chitosan for chromatography. J Chrom B. 2004;808(1):69–73.
8. Nwe N, Chandrkrachang S, Stevens WF, Maw T, Tan TK, Khor E, et al. Production of fungal chitosan by solid state and submerged fermentation. Carbohydr Polym. 2002;49(2):235–7.
9. Pochanavanich P, Suntornsuk W. Fungal chitosan production and its characterization. Lett Appl Microbiol. 35(1):17–21.
10. Portero A, Remuñán-López C, Criado MT, Alonso MJ. Reacetylated chitosan microspheres for controlled delivery of anti-microbial agents to the gastric mucosa. J Microencapsul. 2002;19(6):797–809.
11. White SA, Farina PR, Fulton I. Production and isolation of chitosan from *Mucor rouxii*. Appl Environ Microbiol. 1979;38(2):323–8.
12. Kim TH, Park IK, Nah JW, Choi YJ, Cho CS. Galactosylated chitosan/DNA nanoparticles prepared using water-soluble chitosan as a gene carrier. Biomaterials. 2004;25(17):3783–92.
13. Hu Y, Jiang X, Ding Y, Ge H, Yuan Y, Yang C. Synthesis and characterization of chitosan–poly(acrylic acid) nanoparticles. Biomaterials. 2002;23(15):3193–201.
14. Bansal V, Ramanathan R, Bhargava SK. Fungus-mediated biological approaches towards 'green' synthesis of oxide nanomaterials. Aust J Chem. 2011;64(3):279–93.
15. Abdollahi M, Rezaei M, Farzi G. A novel active bionanocomposite film incorporating rosemary essential oil and nanoclay into chitosan. J Food Eng. 2012;111(2):343–50.
16. Gómez-Estaca J, López de Lacey A, López-Caballero ME, Gómez-Guillén MC, Montero P. Biodegradable gelatin–chitosan films incorporated with essential oils as antimicrobial agents for fish preservation. Food Microbiol. 2010;27(7):889–96.
17. Sánchez-González L, Cháfer M, Hernández M, Chiralt A, González-Martínez C. Antimicrobial activity of polysaccharide films containing essential oils. Food Control. 2011;22(8):1302–10.
18. Wu J, Ge S, Liu H, Wang S, Chen S, Wang J, et al. Properties and antimicrobial activity of silver carp (*Hypophthalmichthys molitrix*) skin gelatin-chitosan films incorporated with oregano essential oil for fish preservation. Food Packaging Shelf Life. 2014;2(1):7–16.
19. Remya S, Mohan CO, Bindu J, Sivaraman GK, Venkateshwarlu G, Ravishankar CN. Effect of chitosan based active packaging film on the keeping quality of chilled stored barracuda fish. J Food Sci Technol. 2016;53(1):685–93.