

Studies on the Antimicrobial Activity of Aqueous Acids Solutions of Chitosan

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Abstract

The paper presents the results of the antimicrobial activity of two aqueous acid solutions of chitosan. Chitosan is a copolymer of glucosamine and N-acetylglucosamine prepared from chitin by deacetylation. It is a natural and non toxic product that tends to be widely-used in medicine and food industry.

The antimicrobial effect was tested against the representative Gram-positive bacteria: *Staphylococcus aureus* and *Bacillus cereus* and Gram-negative bacteria: *Escherichia coli* and *Pseudomonas aeruginosa* and one species of yeast: *Candida albicans* using disc diffusion test. Chitosan had a good antimicrobial action of all tested species. The best antibacterial activity was observed for the acetic acid solution. The acetic acid solution chitosan also present an good antifungal activity. The lactic acid solution of chitosan present antibacterial activity.

Key words: chitosan; acid solutions, antimicrobial activity

Introduction

Chitosan was the subject of various researches during last years [1, 4, 5].

This substance is a copolymer of glucosamine and N acetylglucosamine, prepared by deacetylation from chitin. Being a natural product it considered a non toxic substance. It has a high molecular weight, a poor solubility at neutral pH and a high viscosity in solution [6].

Even the chitosan was considered an important source of bioactive material its use in food, cosmetics and health industry is still limited [6, 7].

Taking in consideration that the antimicrobial activities of chitosan are greatly dependent on its physical characteristics it was considered to investigate the antimicrobial activities of chitosan against two aqueous acid solutions of chitosan. Thset were performed using two Gram-positive bacteria (*Staphylococcus. aureus* - ATCC 25923 and *Bacillus cereus* - ATCC 11778) and two Gram-negative bacteria (*Escherichia coli* - ATCC 25922 and *Pseudomonas aeruginosa* - ATCC 27853), and one specie of yeast of *Candida albicans* - ATCC 10231.

Experimental

For these tests, were prepared two acid aqueous solutions from chitosan using acetic and lactic acid. The chitosan solution (acetic and lactic) were prepared in 1 % (v/v) acetic solution acid at a concentration of 1% (w/v) and the lactic solution acid at a concentration of 1% (w/v), respectively. These solutions were maintained at 37°C to maintain theirs fluidity.

The antimicrobial activity of these chitosan acid aqueous solutions was tested on four standard bacteria strains (MediMark Europe Company, France): two Gram-positive bacteria – *S. aureus* (ATCC 25923) and *B. cereus* (ATCC 11778) and two Gram-negative bacteria – *E. coli* (ATCC 25922) and *P. aeruginosa* (ATCC 27853), and on a strain of *C. albicans* (ATCC 10231). Standard microbial cultures were maintained in laboratory conditions, at 4°C, in tubes with Mueller-Hinton broth (Oxoid) for bacteria and Sabouraud dextrose broth for yeasts. For

these tests, active cultures (24 h old for bacteria and, respectively, 48 h for yeast) were prepared and all these cultures were diluted 1: 1000. Both aqueous acids solutions of chitosan were subjected to the disc diffusion test [8].

For this methods were prepared discs from filter paper that were sterilized. These discs were impregnated with aqueous acids solutions of chitosan. For the bacterial strains was also tested the Gentamicine inhibition effect and Nystatin for *C. albicans*. Also, discs impregnated only in acid solutions were tested.

Inhibition zones observed after 24 hours of incubation (48 hours for *C. albicans*) were measured, including the diameter of the disc (6 mm). Tests were repeated three times and the results were statistical analyzed using Microsoft Excel application.

Results and discussion

From both aqueous acids solutions of chitosan that were tested the best results was obtained with the acetic acid solution. The acetic solution of chitosan gave bigger inhibition zones in all bacteria strains tested and also in *C. albicans*. The acetic solution of chitosan were more effective against *Staphylococcus aureus* and *Pseudomonas aeruginosa* (1.56 mm). Against *E. coli* and *B. cereus* the inhibition zone were smaller (1.23 mm) comparatively with the other bacteria strains. It is important to notice the affect of acetic solution of chitosan against *C. albicans* (1.43 mm), that was equal with the same induced by Nystatin.

The lactic acid solution of chitosan inhibit the grow of bacteria strains (0.76 mm for *B. cereus*, 1.06 mm for *Ps. aeruginosa*, 1.13 mm for *S. aureus* and 0.96 mm for *E. coli*) and *C. albicans* (1.23 mm) but much lower than the acetic acid solution.

The Gentamicine inhibition zones were bigger comparatively with those obtained for both aqueous acids solutions of chitosan. The disc impregnated only with the acetic and lactic solution didn't present any antimicrobial activity.

Regarding the antimicrobial action of chitosan there are controversial results. Some authors reported stronger effects of chitosan on Gram-positive bacteria (e.g. *Listeria monocytogenes*, *Bacillus megaterium*, *B. cereus*, *Staphylococcus aureus*, *Lactobacillus plantarum*, *L. brevis*, *L. bulgaris*, etc.) than for Gram-negative bacteria (e.g. *E. coli*, *Pseudomonas fluorescens*, *Salmonella typhimurium*, *Vibrio parahaemolyticus*, etc.) [4].

This aspect was explained by the fact that gram negative bacteria have a higher hydrophilicity than gram- positive bacteria, making them most sensitive to chitosan. This is due to the charge density on the cell surface that is a determinant factor to establish the amount of adsorbed chitosan. More adsorbed chitosan would evidently result in greater changes in the structure and in the permeability of the cell membrane [4]. Allan and Hadwiger, 1974 [2]. have found that 1% solution of chitosan in 1% of acetic acid had completely inhibited growth of *Candida tropicalis*. Quite similar result (the MIC value of 0.6% solution of chitosan in acetic acid) was found also by Balicka – Ramisz and col., 2005 [3].on *Candida albicans*.

Conclusion

Aqueous acids solutions of chitosan had a good antimicrobial action of all tested species.

The best antibacterial activity was observed for the acetic acid solution.

The acetic acid solution chitosan also present an good antifungal activity.

The lactic acid solution of chitosan present antibacterial activity

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