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ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY OF CHITOSAN

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Introduction

In has reported that showed potent immunological activities such as activation of peritoneal macrophages, stimulation of non-specific host resistance against *Escherichia coli* and Sendai virus infection in mice and suppression of growth of Meth-Atumor cells in syngencic mice. (Nishimura et al. 1984, Nishimura et al. 1986, Sawayangi et al. 1982) Chitosan is attractive preparation for wound healing treatment, too (Allan et al. 1984., Fatt 1978) . It forms a tough, water-absorbent, biocompatible film and this film can be also formed directly on a burn by application of an aqueous solution of chitosan acetate. Attention has been paid to evaluation of chitosan for treatment of burns (Burke and Bondoc 1979) open wounds, dermatitis and in ophthalmology (Fatt 1978). Chitosan has been also used for whitlow treatment (Brzeski et al. 1991). However, not too much is known on the mechanisms of non-specific host resistance stimulation and on the antibacteriological and antifungal activities.

The aim of these studies was to establish susceptibility of some common bacteria and fungi to chitosan, applied in a form of its solution in lactic acid.

Material and methods

Chitosan used in this research meets requirements of Polish Standard PN-89/A-86850 (Polish Standard) and, at the same time, the following parameters (Table 1).

Table 1 Parameters of chitosan

Item	Properties	Requirements
1.	Moisture content	Not greater than 5%
2.	Ashes	No more than 1%
3.	Degree of deacetylation	Greater than 80%
4.	Viscosity of standard solution	Not greater than 30mPa*s
5.	Molecular weight*	In the range $4.0*10^5$ to $5.0*10^5$

*According to the method of Roberts and Domszy

Four standard bacteria (*Escherichia coli, Staphylococcus aureus, Salmonella paratyph* and *Pseudomonas aeruginosa*) from collection of Institute Immunobiology and Experimental Therapy, Polish Academy of Science in Wroclaw, and three standard fungi (*Candida*

albicans, Trichophyton mentagrophytes, Microsporum canis) from the collection of the Department of Veterinary Microbiology, Agricultural Academy in Wroclaw, were used in this study. The MIC (minimal inhibitory concentration) method to establish the susceptibility of bacteria and fungi to chitosan was used.

The studies were carried out using the method of serial dilutions on the basis of the Grove and Randall culture and the fluid Sabourand culture. The bacteria strains and *Candida albicans* were inoculated on the Grove, Randall culture and incubated at 37^0 C during 24 hours. *Trichophyton mentagrophytes* and *Microsporum canis* were inoculated on the fluid Sabouraud culture. For the studies, chitosan solution was diluted with sterile physiological saline (pH – 7.2) in relation to the active substance. In order to establish MIC values, chitosan solutions in the ranges from 0.1 mg/cm^3 to 2.5 mg/cm^3 (the concentration increasing by 0.1 mg/cm^3) were prepared.

1 cm^3 of the breeding-ground was poured into the test tubes, and then 0,5 cm^3 of investigated preparation was added. Subsequently, 0,5 cm^3 of given microorganisms' culture was added to the test tubes. In the case of *C. albicans* and all the bacterial strains, cultures were diluted 1: 1000.

Each time, a sample controlling growth of investigated microorganisms was made. Cultures of all the bacteria and *C. albicans* were incubated in 37^0 C for 24 h, while other mycotic strains were incubated in temperature of 25^0 C for 7 days.

Results

The results of antibacterial and fungicidal activities of chitosan are presented in the Tab.2. All bacteria and fungi, which have been used in these studies, were susceptible to chitosan.

Table 2 Antibacterial and fungicidal activity of chitosan.

Microorganism	Chitosan MIC values (mg/cm^3)
Escherichia coli	1.0
Pseudomonas aeruginosa	1.7
Staphylococcus aureus	0.7
Salmonella paratyphi	2.0
Candida albicans	0.6
Trichophyton mentagrophytes	2.2
Microsporum canis	1.1

Discussion

Chemically modified chitins including partially deacetylated and carboxymethlyated chitins were found to have potent immunological and antibacterial activities (Nishimura et al. 1986; Nishimura et al. 1984; Ryan et. al 2001).

The protection of the host against bacterial infection is stimulated by chitosan (Iida et al. 1987). The effectiveness of chitosan bacteriastatic properties were tested against bacterial strains and a common skin fungus. Powered chitin, chitosan or whole crab shells were not affective in any of the tests, but solution of chitosan in acetic acid inhibited the bacterial strains and the fungus (Cheng and Li 2000). The mechanism underlying the inhibition of bacterial growth, is though to be that the cationically charged amino-group may combine with anionic components such as N-acetylmuramic acid, sialic acid and neuraminic acid, on the cell surface, and may suppress bacterial growth by impairing the exchanges with the medium, chelating transition meal ions and inhibiting enzymes.

Allan and Hadwiger (1974) have found that 1% solution of chitosan in 1% of acetic acid had completely inhibited growth of *Candida tropicalis*. That study corresponded to our results- chitosan was highly active against *Candida albicans* as well.

Conclusions

Chitosan is characterized by high antibacterial and fungicidal activities.

References

1. *Allan C.R., Hadwigar L.A.: Studies on the fungistatic activity of chitosan. Exp. Mycology 3, 258,1974.*
2. *Brzeski M.M., Wojtasz-Pajak A., Ramisz A.: Implementation of antarctic krill chitosan In veterinary practice. Proceedings of 5th International Conference on Chitin and Chitosan. Princerton, IMR, Gdynia, 46-53, 1991.*
3. *Burke J.F., Bondoc C.C.: Dermatology in general medicine, eds. T.B. Fitzpatrik et al., McGraw – Hill, Inc., New York, 931, 1979.*
4. *Cheng C., Li Y.: An Aspergillus chitosanase with potential for large-scale preparation of chitosan oligosaccharides. Biotechnol. Apple. Bioch., 32, 197-203, 2000.*
5. *Fatt I.: Soft contact lenses. In: Clinical and applied technology, ed. M. Reben, Willey Medical Publications, New York, 83, 1978.*
6. *Iida J., Une T., Ishihara S., Tokura N., Mitzukoshi N., Azuma I.: Stimulation of non-specyfic host resistence against Sendai virus and Escherichia coli infections by chitin derivatives in mice. 5, 167-171, 1987.*
7. *Nishimura K. Nishimura S., Nishi N., Saiki I., Tokura S., Czuma I., Immunological activity of chitin and its derivatives. Vaccine, 2, 93-99, 1984.*
8. *Nishimura S., Nishi N., Tokura S., Okiei W., Somonin O.: Inhibitory action of chitin heparinoids on the hydrolytic activity of trombin. Carbohydr. Res ., 156, 286, 1986.*
9. *Polish Standard PN-89/A-86850, Raw Materials and Products of Fish and Aquatic Invertebrates, Chitosan.*
10. *Ryan E., Daly L., Mills K. : Iimmunomodulators and delivery systems for vaccination by muscosal routes. Triends Biotech. 19, 293-307, 2001.*
11. *Sawayanagi Y., Nambu N., Nagai T.: Use of chitosan for sustained-relase preparations of water-soluble drugs, Chen. Pharm. Bull. 30, 4213, 1982.*