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Antibacterial Effect of Chitooligosaccharides with Different Molecular Weights Prepared Using Membrane Bioreactor

Se-Kwon Kim, You-Jin Jeon and Pyo-Jam Park
Department of Chemistry, Pukyong National University, Pusan 608-737, Korea

Introduction

Chitosan (deacetylated form of chitin) possesses strong antibacterial activities such as antimicrobial effect, antifungal effect and the induction of plant defense response. Chitosan itself, however, has high molecular weight and viscosity as well as water-insolubility. These natures may restrict applications in various fields, especially in *in vivo* system. While the hydrolysates of chitosan, chitooligosaccharides (COS) are not only lower in the molecular weight and viscosity, but also water-soluble. Thus, they would be expected more efficient absorption *in vivo*. Besides several documents have been reported antibacterial activities of COS against microorganisms (Kendra et al., 1989; Uchida et al.,1989).

In this study, antibacterial effect of the three oligosaccharides was examined against six gram-negative bacteria, nine gram-positive bacteria, two yeasts and three fungi, and the inhibitory effect of COS with different molecular weight on the growth of microorganisms was investigated.

Materials and Methods

COS was prepared by continuous hydrolysis of chitosan in UF membrane reactor system connected with an immobilized enzyme column reactor in which chitosanase from *Bacillus* sp. was adsorbed on chitin as a carrier for immobilization, according to our previous method (Jeon and Kim).

Antimicrobial activity of chitosan and the three oligosaccharides was examined against several types of bacteria which were comprised of six gram-negative bacteria (*Esherichia coli, Escherichia coli* O-157, *Salmonella typhi,*

Pseudomonas aeruginosa, vibrio cholerae and Vibrio parahaemolyticus), nine gram-positive bacteria (*Streptococcus mutans, Micrococcus luteus, Staphylococcus aureus, Staphylococcus epidermidis, Bacillus subtilis, Lactobacillus bulgaricus, Lactobacillus casei, Lactobacillus fermentum* and *Streptococcus faecalis*) and two yeast (*Saccharomyces cerevisiae* and *Cnadida albicans*). The assays were carried out by counting colony grown on agar plate incubated. Minimum inhibitory concentration (MIC) was tested by two-fold serial broth dilution.

Results

COS showed more inhibitory rates in gram-positive than in gram-negative. In particular, the oligosaccharides almost inhibited the growth of *S. mutans, S. aureus* and lactic acid bacteria such as *L. fermentum* and *S. faecalis*. The MIC values of three different COS for the above bacteria were all less than 0.12%.

With regard to the yeast such as *S. cerevisiae* and *C. albicans*, chitosan inhibited the growth of *S. cerevisiae* with 98% of bactericidal activities and 0.06% of MIC. COS also have bactericidal activities of the ranges from 86 to 66%, and low MIC values (0.12%). Chitosan as well as COS, however, showed the worst bactericidal activity for *C. albicans* among the bacteria tested in this study.

The antifungal activity was increased with 0.2% of chitosan concentration and showed 90~100% inhibitory effects by the concentration range of more than 0.4% against *P. chryosogenum* and *A. niger* and more than 0.3% against *A. mali*. In the case of the COS, however, the antifungal activities were extremely low. No activity increase by COS was observed against *P. chryosogenum* and *A. niger*. Of the tested fungi, *A. mali* was inhibited the most sensitively by the oligosaccharide treatments and its growth was blocked up to about 80% by high molecular weight COS concentrations from 0.2 to 0.5%.

References

Kendra D.F., D. Christian and L.A. Hadwiger. 1989. Chitosan oligomers from *Fusarium solani*/pea interactions, chitinase/β -glucanase digestion of sporelings and from fungal wall chitin actively inhibit fungal growth and enhance disease resistance. Physiol. Mol. Plant Path. 35: 215-230.

Uchida Y., M. Izume and A. Ohtakara. 1989. Preparation of chitosan oligomers with purified chitosanase and its application. In G. Skjak-Braek, T. Anthonsen & P. Sandford (Eds.), *Chitin and chitosan*, pp. 373-382. London: Elsevier.

Jeon Y.J. and S.K. Kim. 2000. Continuous production of chitooligosaccharides using a dual reactor system. Proc. Biochem. 35: 623-632.