PD-6

Antimicrobial Susceptibility of Chitooligosaccharides on Pathogenic Halophilic *Vibrio parahaemolyticus*

Pyo-Jam Park[1], Hun-Ku Lee[2] and Se-Kwon Kim[1]

[1]Department of Chemistry and [2]Department of Microbiology, Pukyong National University, Pusan 608-737, Korea

Introduction

Chitosan is a natural biopolymer of N-acetylglucosamine (-1,4 linked 2-acetamido-D-glucose), which is obtained after alkaline deacetylation of the chitin derived from the cell walls of lower plants and in the skeletal tissues of lower animals including arthropods and mollusks. In the recent studies, chitin and chitosan have attracted interest for converting them to oligosaccharides because the oligosaccharides are not only water-soluble but also possess versatile functional properties such as antimicobial activity (Jeon and Kim, 2000; Jeon et al., 2001), antitumor activity, immuno-enhancing effects, protective effects against infection with some pathogens in mice, and cholesterol-reducing effect. With respect to antimicrobial activity, chitosan is superior to chitin since chitosan possesses a lot of polycationic amines which intereact with the negatively charged residues of macromolecules at the cell surface of bacteria, and subsequently inhibit the growth of bacteria. The antibacterial effect of chitooligosaccharides has been shown to be greatly dependent on their degree of polymerization or molecular weight. In addition, the water-soluble chitooligosaccharides may be advantageous as antibacterial agents in *in vivo* system compared to water-insoluble chitosan.

Vibrio parahaemolyticus is a prevalent food-borne pathogen in many Asian countries where marine foods are frequently consumed, and also reported to be an important agent of travelers diarrhea. In addition, *V. parahaemolyticus* is recovered from a high percentage of fresh and refrigerated seafood, and even from frozen items. The strains of *V. parahaemolyticus* are typically not human pathogens, but these strains cause disease in shrimps, oysters, mussels and other marine invertebrates and in fishes.

In this study, the antimicrobial activity of chitooligosaccharides with different molecular weights was investigated against *V. parahaemolyticus* isolated various sources.

Materials and Methods

Materials. Chitosan (degree of deacetylation: 93%, average molecular weight: 280,000 Da, viscosity13 cps) was donated by Kitto Life Co. (Seoul, Korea), and prepared from crab shell. Chitosanase (35,000 units/g protein) for the preparation of chitosan oligosaccharides was from *Bacillus sp.* (Amicogen Co., Jinju, Korea). The ultrafiltration membrane (UF) reactor system for the production and the fractionation of chitosan oligosaccharides, based on molecular weight, was from Millipore Co. (Bedford, USA). The microorganisms tested for antimicrobial activity were purchased from ATCC (Americal Type Culture Collection) and isolated in our laboratory.

Assay for antimicrobial activity. Antimicrobial activity of chitosan and four chitooligosaccharides was examined against 32 strains of *V. parahaemolyticus.* The viable bacteria counting was executed by a microdilution method, and inoculations of bacteria/plate were made with a Steers type multiple inoculator on Mueller-Hinton agar (MHA, Difco Lab., Detroit, MI, USA). The plate was incubated at 37℃ for 17 h.

Results

Four kinds of chitooligosaccharides were prepared from chitosan and fractionated with the molecular weight of 10-5 kDa, 5-3 kDa, 3-1 kDa and below 1 kDa, respectively, using ultramembrane system. A viable bacteria counting is executed by a microdilution method and is subcultured with a Steers-type multiple inoculator on Mueller-Hinton agar. Chitosan and chitooligosaccharides have a dose dependent antimicrobial activity which is maximum at 4 mg/ml.

References

Jeon, Y. J. and S. K. Kim. 2000. Production of chitooligosaccharides using an ultrafiltration membrane reactor and their antibacterial activity. *Carbohyd. Polym.* **41:** 133-141.

Jeon, Y. J., P. J. Park and S. K. Kim. 2001. Antimicrobial effect of chitooligosaccharides produced by bioreactor. *Carbohyd. Polym.* **44:** 71-76.