

**Antimicrobial effect of chitosan oligosaccharides,  
prepared under ultrafiltration membrane bioreac-  
tor, against *Vibrio* spp. causing fish diseases**

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**INTRODUCTION**

Chitin, a polymer of N-acetylglucosamine ( $\beta$ -1,4 linked 2-acetamido-D-glucose), is a cellulose-like biopolymer present richly in the exoskeleton of crustaceans and in cell walls of fungi, insects and yeast. Chitosan is derived from chitin by deacetylation, to different degrees, in the presence of alkali. [1]. Recent studies for chitin and chitosan have been concentrated in bioactivities such as antitumor activity, immuno-enhancing effects, enhancing protective effects against infection with some pathogens in mice, and antimicrobial activity [2].

At our previous studies, an higher antimicrobial activity of chitosan or chitosan oligosaccharide (COS) against *vibrio cholerae* and *vibrio parahaemolyticus* [3] was observed. *Vibrio* spp. are well-known as representative marine bacteria occurring fish and human diseases. Every year many aquacultural farms around the world have severe problems due to fish disease caused by some *Vibrio* spp., in particular *V. salmonicida*, *V. mimicus*, *V. anguillarum*, *V. alginolyticus*, and *V. harveyii*. In this study, the antimicrobial effect of chitosan as well as COS with a variety of molecular weights, which are produced under the ultrafiltration membrane bioreactor according to the early work [4], against those vibrio bacteria was investigated in *in vitro*.

**MATERIALS AND METHODS**

The chitosan (degree of deacetylation, 89%; viscosity 20 cps), used as a starting material for the preparation of COS, was donated from Kitto Life Co. (Korea). The chitosanase (694 units per 1g protein, derived from the *Bacillus Pumilus* BN-262 strain; molecular weight, approximately 30,000 Da; optimal pH and temperature,

5.5-6.5 and 30-50°C, respectively) was purchased from Wako Pure Chemical Co. (Japan). The ultrafiltration membrane reactor system for the production of COS was from Millipore Co. (USA).

Preparation of COS : The COS was prepared by the enzymatic hydrolysis of chitosan and separated into three fractions with respective molecular weight ranges in the ultrafiltration membrane bioreactor system according to the previous report [4]. When fractionated into the three kinds of COS, the molecular weight cut-off of the membrane used was 1 kDa, 5 kDa, and 10 kDa, respectively. The molecular weight ranges of the resultant three fractions obtained are as the follow: a high molecular weight COS (HMWCOS) ranging 7.0 to 24.0 kDa; a medium molecular weight COS (MMWCOS) ranging 1.5 to 6.0 kDa; a low molecular weight COS (LMWCOS) ranging 1.0 to 1.5 kDa.

Antimicrobial assay : The antimicrobial activity of chitosan and the COS was examined for the growth of those five vibrio bacteria. Respective bacteria was mixed with a special concentration of sample during 1 hr inoculation time and grew on an agar plat having Tryptic soy broth (TSB) as a medium for 24 hrs. Antimicrobial activity was expressed as bactericidal activity according to our previous calculation method [5]. Minimum inhibitory concentration (MIC) was tested by two-fold serial broth dilution and the antimicrobial effects depending on inoculation time and incubation time were also observed.

## RESULTS AND SUMMARY

Chitosan effectively inhibited the growth of most of vibrio bacteria tested in the study, especially *V. salmonicida*, *V. anguillarum* and *V. alginolyticus*. Most COS samples have less effective inhibition rates as compared to that of chitosan but showed the effective suppression for *V. anguillarum* growth. The antimicrobial activity increased with the molecular weights of COS. MICs of chitosan were about 1000 ppm or less for all the bacteria and those of HMWCOS less than 2000 ppm except for *V. harveyi*. It was proved that chitosan and HMWCOS can be used for natural antimicrobial agent for those bacteria causing fish diseases.

## REFERENCE

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