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Antimicrobial effect of chitosan oligosaccharides, prepared under ultrafiltration membrane bio-reactor, against hygienic bacteria of *Vibrio* spp.

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INTRODUCTION

Many people living in Asia countries, particularly Korea, Japan and China, have consumed very widely fresh seafood products, such as shrimps, oysters, mussels and other marine invertebrates and fishes, without any heating or cooking. A variety of *Vibrio* spp., including *V. parahaemolyticus, V. cholera, V. vulnificus,* and *V. fluvialis,* lives in these seafoods and cause great problems associated with human disease. A strong antimicrobial agent to effectively inhibit the growth of these pathogenic bacteria in *in vivo* or *in vitro* is urgently need for preventing fish and human diseases.

At our previous study, it was proved that chitosan oligosaccharides (COS), produced under ultrafiltration membrane bioreactor system, with relatively higher molecular weights as well as chitosan could significantly suppress the growth of *V. parahaemolyticus* and *V. cholera* [1]. In the present work, thus, we tried to examine the antimicrobial activity of chitosan and COS against different *Vibrio* spp. involving important pathogenic bacteria such as *V. parahaemolyticus, V. vulnificus* and *V. fluvialis.*

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℃, 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 under the ultrafiltration membrane bioreactor system according to the previous report [2]. The molecuar weight ranges of the respective oligosaccharides 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 three bacteria. Respective bacteria was mixed with a special concentration of sample during 1 hr innoculation time and growed on an agar plat having Tryptic soy broth (TSB) as a medium for 24 hrs. Antimicrbial activity was expressed as bactericida activity according to our previous calculation method [3]. Minimum inhibitory concentration (MIC) was tested by two-fold serial broth dilution and the antimicrobial effects depending on innoculation time and incubation time were also observed.

RESULTS AND SUMMARY

Chitosan showed higher microbial activities for most *Vibrio* spp. tested except for only *V. logei*. In particular it effectively inhibited the growth of *V. furnissii, V. cincinniensis, V. adaptatus, V. parahaemolyticus, V. vulnificus, V. mediterianei,* and *V. cyclosites* regardless of the concentration added to the bacteria. HMWCOS among the COS samples indicated the most antimicrobial activity against, especially *V. furnissii, V. cincinniensis, V. adaptatus, and V. mediterianei* as well as *V. parahaemolyticus* at 1000 ppm. It was revealed that MMWCOS and LMWCOS possessed inhibition effect only for the growth of *V. furnissii* at 1000 ppm. No activity was observed in the inhibitory effect of *V. logei* by all COS samples. *V. parahaemolyticus* of the most important pathogenic *Vibrios* associated with human disease was the most susceptibility to chitosan and HMWCOS.

REFERENCE

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