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Antimicrobial effect of chitosan oligosaccharides, prepared under ultrafiltration membrane bioreactor, against pathogenic bacteria causing flounder fish diseases in aquacultural farm

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INTRODUCTION

Despite a variety of development in fish farming during the last decades, fish diseases by bacteria, virus, and parasites are still major problems in aquaculture. Aquaculture of flounder fish is widely performed around Korea as well as Jeju island, due to relatively stable seed production, short farming period, and a higher value in market. However, intensive feeding and environmental pollution in aquacutural farm act as a great limiting factor in economic aspect. In particular pathogenic bacteria such as vibrio, edwardsiella, and streptococcus were closely associated with major bacterial diseases for aquacutred flounder fish.

Many results has been revealed during recent years that chitosan possesses a very excellent antimicrobial activity against quite different species of microorganisms [1, 2, 3]. At our previous studies, antimicrobial activity of chitosan oligosaccharides (COS) as well as chitosan showed, especially against bacteria causing pathogen [2, 3].

In the present work, chitosan and COS, which was produced under ultrafiltration membrane bioreactor system, was investigated for their antimicrobial effect against *Vibrio* spp., *Edwardsiella tarda*, and *Streptococcus* spp., representative bacteria causing severe disease of flounder fish.

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 [4]. 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 three bacteria used, *Vibrio* ssp., *E. tarda*, and *Streptococcus* spp., were wild types and directly isolated from the intestine of flounder fish living at a aquacultural farm located in Jeju island of Korea. The antimicrobial activity of chitosan and the COS was examined for the 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 very effectively inhibited for the growth of *Vibrio* spp. and *Streptococcus* spp., but was less effective for *E. tarda*. HMWCOS showed effective suppression, regardless of sample concentration, for the growth of *Streptococcus* spp. and about 80% inhibition rate at 500 ppm concentration or more. MMWCOS and LMWCOS showed the lowest antimicrobial activities against all bacteria tested and possessed 20~50% inhibition rates for *Vibrio* spp. and *E. tarda*, and almost no inhibitory rate for *Streptococcus* spp.

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