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Antimicrobial Property of Chitosan and Its Oligosaccharides for Development of Functional Fish Feed to Inhibit Growth of *Vibrio* Species Causing Fish Diseases

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Every year many aquacultural farms around the world have suffered severe problems due to fish disease caused by some *Vibrio* species, in particular *V. salmonicida, V. mimicus, V. anguillarum, V. alginolyticus*, and V. *harveyi* These vibrio species have been well-known as representative marine bacteria occurring fish or human diseases Therefore, the development of functional fish feeds involving effective antimicrobial materials which effectively inhibit the growths of these Vibrio species is required. For this purpose antimicrobial activities of chitosan or its enzymatic hydrolysates, chitosan oligosaccharides (COS), against some vibrio species were investigated in *in vitro* Chitosan was prepared from crab shell through some chemical processing including a strong sodium hydroxide and a hydrochloric acid. For the preparation of COS, chitosan washydrolysed by chitosanase action and its hydroysates were separated into three kinds of fractions with different molecular weights using ultrafiltration membranes with different molecular weight cut-offs, such as 10, 5, and 1 kDa, respectively. 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. From the results, it was found that chitosan effectively inhibited the growth of most vibrio bacteria tested in the study, especially *V. anguillarum, V. alginolyticus* and *V. salmonicida.* Most COS samples exhibited less effective inhibition rates as compared to that of chitosan but showed the effective suppression for *V. anguillarum* growth. The antimicrobial activity increased with increasing molecular weight of the COS. MICs of chitosan were about 1000 ppm or lower for all the bacteria and those of HMWCOS less than 2000 ppm, except for *V. harveyi.* It was proved that chitosan and HMWCOS wouldbe used as natural antimicrobial agents against pathogenic vibrio species causing fish diseases.

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Reactive Oxygen Radical Species Scavenging Effects of Brown Seaweed Enzymatic Extracts

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Enzymatic extracts prepared from seven brown seaweeds (*Ecklonia cava, Ishige okamurae, Sargassum fulvellum, Sargassum honeri, Sargassum ringgoldianum, Sargassum thunbergii* and *Scytosiphon lomentaria*) using five carbohydrases (Viscozyme L, Celluclast 1.5 L FG, AMG 300L, Termamyl 120L, Ultraflo L) and five proteases (Protamex, Kojizyme 500 MG, Neutrase 0.8 L, Flavourzyme 500 MG, Alcalase 2.4 L FG) were investigated on four different reactive oxygen species (ROS) effects (superoxide anion scavenging activity, hydroxyl radical scavenging activity, hydrogen peroxide scavenging activity and free radical scavenging activity). Superoxide anion scavenging activity was measured using pyrogallol auto-oxidation system. *E. cava* Termamyl extact indicated the highest activity (68%) over other seaweed enzymatic extracts. The scavenging activity against hydroxyl radical was investigated by using hydroxyl radicals produced in the Fenton reaction ($Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + {}^{\cdot}OH$). *S. fullvelum* alcalase extract showed the highest hydroxyl radical scavenging activity (about 47%) and its flavourzyme extract also indicated a relatively higher scavenging activity as 39%. The extracts prepared from *I. okamurae* by the four proteases, except for Neutrase, indicated comparatively higher hydrogen peroxide scavenging activities, reaching around 95% Of the enzymatic extracts of the seven seaweeds, only the *E. cava* extracts could effectively scavenge free radicals released from DPPH (around 70%). In comparison of total phenolic contents and the activity levels of ROS scavenging, the phenolic content indicated a marked correlation with DPPH activity only. This fact implicates that a variety of antioxidant compounds, other then phenolic compounds, in the enzymatic extracts might influence on scavenging ROS. According to the results, enzymatic extracts of seaweeds appear to possess potential antioxidant activities but no study have tried in preparing enzymatic extracts from seaweeds and observing their ROS scavenging effect so far. Therefore further works involving identification of antioxidant compounds from the extracts would be required.