

Protease and Virulence of the Extracellular Products Produced by *Vibrio carchariae* after Growth on Various Media

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Protease and virulence of the extracellular products (ECP) of *Vibrio carchariae* strain EmI82KL, a causative agent of gastroenteritis in *Epinephelus coioides*, cultured on different media were studied. The bacteria grown on peptone agar, nutrient agar or brain heart infusion agar produced higher protease activities than that grown on tryptic soy agar (TSA) in terms of protein content. The addition of ethylenediamine di(o-hydroxyphenylacetic acid) or horse serum in TSA enhanced the ECP protease production while the addition of grouper serum apparently reduced the enzyme activity indicating the presence of protease inhibitor(s) in the fish serum. Furthermore, the use of grouper meat or peptone as a single nutrient source remarkably enhanced the production of ECP protease. Adding proteinaceous materials from animal sources (horse serum, grouper meat or peptone) on agar manifested higher ECP protease activity than that from plant source (TSA), indicating the intestine of carnivorous groupers might favour the existence, survival or infection of the bacterium. The protease was a metal-chelator-sensitive serine protease since it was inhibited by 3,4-dichloroisocoumarin and phenylmethanesulfonyl fluoride while about 80% of its activity inhibited by chelating agents (ethylene-diaminetetraacetic acid and ethylene glycol-bis(β -amino-ethylether) N,N,N',N'-tetraacetic acid). The ECP obtained from each medium was not lethal to the groupers suggesting that the bacterium is low virulent. As grouper is carnivorous, therefore, the role of the protease played in the fish intestine probably is competing for nutrients and/or associated with the cause of edema leading to gastroenteritis.

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