Molecular Characterization of *Vibrio vulnificus* Isolated from Cockle and Shrimp

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Introduction

Vibrio vulnificus has emerged as an important pathogen responsible for septicemia and severe wound infections in immunocompromised patients. *V. vulnificus* is an estuarine bacterium commonly found in coastal waters and has been detected in shellfish and intestinal contents of fish (Oliver, 1989; Amaro et al., 1995). Molecular typing is being used for epidemiological study of this potential pathogen in the environment. Loi et al. (1997) showed that it is necessary to characterize several isolates from the same source because it could harbor different clones of *V. vulnificus*. Thus, the aim of this study was to characterize and compare the strains of *V. vulnificus* isolated from cockles and shrimps. The characterization was done by randomly amplified polymorphic DNA (RAPD) analysis.

Materials and Methods

10 g of the shrimp or cockles samples were stomached in 90 ml of peptone water, and the samples were serially diluted in 10-fold increments of 100 ml of 0.5% peptone water. One ml of serially diluted sample was transferred into 9 ml alkaline peptone broth, incubated overnight at 37°C and were streaked onto thiosulfate-citrate-bile salt-sucrose agar. Colonies were inoculated into API 20E biochemical strips for identification. Prior to amplification, chromosomal DNA of the *V. vulnificus* was isolated as described by Ausubel et al. (1987). Reactions mixtures of 25 µl were made consisting 2.5 µl 10x reaction buffer, 0.5 µl of 10 mM dNTP mix, 2 mM of primer, 2 µl of 25 mM $MgCl_2$, 0.5 µl *Taq* polymerase, 20-30 ng DNA and made up to 25 µl with sterile distilled water. A thermal cycler (Perkin Elmer model 2400) was used for amplification of 45 cycles at 94°C for 2 min, 36°C for 1 min and 72°C for 2 min. A final elongation step of 72°C for 5 min was included.

Results and Discussion

The initial experiments were performed with a subset of *Vibrio vulnificus* strains to identify primers that provide polymorphic band patterns. RAPD-PCR using primers GEN15001 and GEN15008 resulted in amplification of genomic DNA ranging in sizes from 0.25 to 10.0 kilobases pairs (kb). Of the 50 strains examined, 46 and 47 different RAPD patterns were generated using primer GEN15001 and GEN15008, respectively. Four and three strains were untypable using these two primers respectively. From the dendrogram generated, it was observed that some strains of *Vibrio vulnificus* from cockles and shrimps samples were grouped into the same clusters, indicating their possible genetic relatedness. Interest in the microbiological relationship of *V. vulnificus* from seafoods has been driven by the recognition that strains of these species are associated with human infections. Despite the increased interest in the *Vibrio* and the rapidly expanding body of knowledge concerning the various associations of these genera with seafoods, limited studies have reported the presence of *V. vulnificus* in seafoods in Malaysia (Son et al., 1998). Biochemical characterization revealed that some of the *Vibrio vulnificus* strains isolated from cockles and shrimps in this study were biotype 1, which is known as an opportunistic human pathogen with infection resulting from consumption of contaminated seafood or exposure to marine environment in the case of wound infections. Thus it would be of great benefit to apply rapid screening method such as RAPD-PCR to screen for presence of clonal strains of *V. vulnificus* that are potentially pathogenic in humans from seafood samples. This PCR sreening method is simple and rapid and may be useful in epidemiological analysis, particularly in large studies and in urgent situations. This report suggested that *V. vulnificus* biotype 1 is present in seafood samples marketed in Malaysia and thus emphasize the need for caution when dealing with seafoods contaminated with this organism.

Conclusions

The presence of *V. vulnificus* biotype 1 may be a health hazard to consumers of raw shellfish in the study area as cockles and shrimps are popular ingredient in many types of local foods.

Benefits from the study

The information on the isolation of *V. vulnificus* biotype 1 from cockles and shrimps marketed locally would be useful for the public health agency to take the necessary measures to reduce the *V. vulnificus* contamination of these products, thereby decreasing the risk of human infection. This research has provided training for postgraduate student on molecular tools used in molecular fingerprinting of human pathogen isolated from food sources.

Literature cited in the text

Amaro, C., Biosca, E.G., Fonz, B., Alcaide, E. and Esteve, C. 1995. Evidence that water transmit *Vibrio vulnificus* biotype 2 infections to eels. *Applied and Environmental Microbiology.* 61: 1133-1137.

Ausubel, F.M., Brent, R. and Kingston, R.E. 1987. Current protocols in molecular biology. John Wiley, New York.

Loi, L., Dalsgaard, A., Larsen, J.L., Warner, J.M. and Oliver, J.D. 1997. Comparison of ribotyping and randomly amplified polymorphic DNA CR fr characterization of *Vibrio vulnificus. Applied and Environmental Microbiology.* 63: 1674-1678.

Son R, Nasreldin EH, Zaiton H, Samuel L, Rusul G, Nimita F, Yuherman and Endang P. 1998. Characterization of *Vibrio vulnificus* isolated from cockles (*Anadara granosa*): antimicrobial resistance, plasmid profiles and random amplification of polymorphic DNA analysis. FEMS Microbiology Letters. 165: 139-143.

Project Publications in Refereed Journals

Ahbrizal T, Son R, Reezal A, Mutalib AR, Rusul G, Nasreldin EH and Yuherman. 2000. Random amplified polymorphic DNA analysis to differentiate strains of *Vibrio vulnificus* isolated from cockles and shrimps. *Malaysian Journal of Medical Science*. 7: 41-46.

Son R, Nasreldin EH, Zaiton H, Samuel L, Rusul G, Nimita F, Yuherman and Endang P. 1998. Characterization of *Vibrio vulnificus* isolated from cockles (*Anadara granosa*): antimicrobial resistance, plasmid profiles and random amplification of polymorphic DNA analysis. FEMS *Microbiology Letters*. 165: 139-143.

Son R, Yuherman, Rusul, G. Lum KY and Nishibuchi M. 2000. Detection and molecular characterization of *Vibrio vulnificus* from coastal waters of Malaysia. *Southeast Asian Journal of Tropical Medicine and Public Health*. 31: 668-673.

Project Publications in Conference Proceedings

Nasreldin EH, Son R, Rusul G, Khair J, Hassan Z, Reezal A, Nimita F and Sahilah AM. 2000. Prevalence *of Vibrio* spp. in shrimp (*Peneus indicus*) marketed in Malaysia. 3rd National Symposium on Health Sciences. 29-30 April 2000. Putra World Trade Center, Kuala Lumpur, Malaysia.

Nasreldin EH, Zaiton H, Son R, Rusul G and Nimita F. 1998. Prevalence studies of *Vibrio vulnificus* isolated from cockles (*Anadara granosa*) in Malaysia. Fith Symposium of Applied Biology, 5-6 May 1998. Universiti Putra Malaysia.

Yuherman, Son R and Rusul, G. 1999. Characterization of *Vibrio vulnificus* isolated from sea water by plasmid profiling, antibiotic resistance pattern and random amplified polymorphic DNA (RAPD). 35th Annual Scientific Seminar, Malaysian Society of Parasitology and Tropical Medicine. 17-18 March 1999. Institute for Medical Research, Malaysia.

Graduate Research

Tg Ahbrizal. 2000. Molecular Biology [MS]. Universiti Putra Malaysia.

Yuherman. 2000. Molecular Biology [Ph.D]. Universiti Putra Malaysia.

Nasreldin Elhadi Hussein. 1999. Molecular Biology [MS]. Universiti Putra Malaysia.

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