# Recovery of pathogenic bacteria from aquatic environment, fish and cockles

Son Radu

Department of Biotechnology
Faculty of Food Science and Biotechnology
Universiti Putra Malaysia
43400 UPM, Serdang, Selangor
Malaysia

*Telephone Number of Corresponding Author: 03-89468361*
*E-mail of Corresponding Authorson@fsb.upm.edu.my*

***Key words:*** Prevalence, *Aeromonas*, *Listeria*, *Vibrio*, *Salmonella*

## Introduction

The true incidences of diseases transmitted by seafood (or from aquatic environment) is not known, due to lack of awareness of the etiological role of foods. In addition, most countries does not have the regulation to enforce the reporting of foodborne diseases to the public health authorities. According to Bryan (1980), "fish" has been most frequently involved, followed by bivalve mollusks and crustacea. Bacterial pathogens in the aquatic environment could be categorized into three general groups: the normal components of the aquatic environment (*Vibrio* spp., *Listeria monocytogenes*, *Aeromonas hydrophila*, etc..), the enteric bacteria which are present due to faecal contamination, and bacterial contamination during processing. Various researchers have reported on that these pathogens have been been isolated from fish and shellfish, and the danger of infections is higher because these pathogens are resistant to various antibiotics. This study reports on the isolation of *Aeromonas* species from raw fish, *Listeria monocytogenes* from fermented fish, *Salmonella* Enteritidis from raw fish, and *Vibrio vulnificus* from cockles, shrimp and seawater.

## Materials and Methods

Raw fish, cockles, shrimp and fermented fish samples were purchased from the wet market in Selangor, whereas the seawater samples were collected from the coastal area around Pulau Kapas, Terengganu. The general procedure used in isolating these pathogens from fish, cockles and shrimp samples were as follows: Twenty-five grams of the samples were homogenized with 225 ml of the appropriate enrichment buffer (alkaline peptone water for *Aeromonas* spp., *Salmonella* Enteritidis and *Vibrio vulnificus*; Listeria enrichment broth for *Listeria monocytogenes*). After incubation at 37°C for 6-18 hours, the samples were serially diluted in peptone water and 0.1 ml of the appropriate dilutions were spread plated onto glutamate starch agar (*Aeromonas* species), thiosulfate citrate bile salts agar (*Vibrio vulnificus*), Palcam agar (*Listeria monocytogenes*) and Salmonella-Shigella agar (*Salmonella* Enteritidis). For the seawater samples, direct dilution were made and spread plated in the same manner. After an overnight incubation at 37°C, suspected colonies for the various pathogens from the selected plates were picked and subjected to standard biochemical tests for identification to species level.

## Results and Discussion

A total of 87 market fish samples representing five types of fish were evaluated for the presence of Aeromonas spp. Of the samples examined, 69%, 55%, 11.5% and 2.3% harbored *Aeromonas* species, *A. veronii* biovar *sobria*, *A. hydrophila* and *A. caviae*, respectively. More specifically, they were isolated from 72%, 60%, 67%, 50% and 50% of 32 ikan tilapia, 20 ikan keli, 15 ikan terubuk, 10 ikan merah and 20 ikan puyu samples, respectively. Though the occurrence of foodborne infections due to *Aeromonas* has not been recognized in Malaysia, it has been suggested in many countries in association with consumption of various foods. In Malaysia, fish is usually eaten after being cooked, and therefore, fish may be a low risk food, despite the presence of *Aeromonas* species.

Forty of the 148 cockle samples, 30 of the 433 shrimp samples and all the 57 samples of seawater were positive for *Vibrio vulnificus*. *V. vulnificus* biotype 1 and 2 were isolated from all three types of samples. Biotype 1 has been associated with human infection while biotype 2 was identified as a pathogen for eel or fish. The high percentage of cockles positive for *V. vulnificus* is not surprising as filter feeding mollusks such as oysters, clams, mussels and scallop have high concentration of the bacteria. In Malaysia, the consumption of half-cooked cockles is very popular, and this will increase the risk of infection. I addition, the isolation of *V. vulnificus* from seawater from a recreational resort area can pose a health hazard to visitors who used the beach for recreational purposes.

In general, *Listeria* species were detected in 14 (56%) of the 25 samples of fermented fish from the wet market. However, *Listeria monocytogenes* were isolated from 3 (12%) of the 25 samples examined. The major outbreaks of listeriosis has been associated with consumption of foods of animals and fish origin, with case-fatality rates of about 20-30%. Thus the detection of all the *Listeria* species and especially the *Listeria monocytogenes* in the fish samples examined is of great concern since fish have been reported as possible vector of *Listeria monocytogenes*.

*Salmonella* Enteritidis is one of the most common causes of nontyphoidal salmonellosis after *S.* Typhimurium, thus it is of importance to determine the prevalence of this pathogen in popular foods, such as fish. In this study, *S.* Enteritidis has been isolated from 10 of 23 samples of tilapia (*Tilapia mossambica*) fish obtained from the wet market. Since tilapia is a popular fish served in restaurants as well as at food stalls in Malaysia, there is a need for consumer protection against infection by

potential pathogenic strains of the bacteria from fish. Though foods are normally prepared thoroughly by cooking, there is always the possibility of cross-contamination at the processing, preparation and service steps. In addition, the consumption of ready-to-eat raw fish is getting popular in Malaysia.

## Conclusions

This study confirms the present of the various species of human bacterial pathogens from the different types of samples examined, and reinforce the notion that foodborne pathogen can be a potential health hazard. There is no proper surveillance system for these pathogenic bacteria in Malaysia. The ingrained tradition of not taking infections seriously such as having a bout of stomach-ache after consumption of foods could contribute to the under reporting of foodborne diseases in Malaysia.

## Benefits from the study

This study provide an insight into the prevalence of these human bacterial pathogens in the foods and environmental samples examined. In addition, several postgraduate students have been trained in the isolation, identification and molecular characterization of these pathogens.

## Patent(s), if applicable:

Nil

## Stage of Commercialization, if applicable:

Nil

## Project Publications in Refereed Journals

1. Son Radu, Noorlis A., Foo HL and Reezal A. 2003. Prevalence and characterization of *Aeromonas* species from retail fish in Malaysia. *International Journal of Food Microbiology* 81: 261-266

2. Lesley MB, Son Radu, Laurence J, Vickneswaran V, Cheah YK, Wong CMVL and Nishibuchi M. 2002. *Vibrio parahaemolyticus* from local cockles (*Anadara granosa*). *The Medical Journal of Malaysia* 57 (Suppl. D): 137.

3. Son Radu, Tg Ahbrizal TA and Sahilah AM. 2002.
Characterization of *Vibrio vulnificus* isolated from retail cockles and shrimps by plasmid profiling and antibiotic susceptibility. *Journal of Tropical Agriculture and Food Science* 30: 31-37.

4. Zaiton H, Endang P, Son Radu, Raha AR and Rusul G. 2001. Prevalence of *Listeria* spp. and *Listeria monocytogenes* in meat and fermented fish in Malaysia. *Southeast Asian Journal of Tropical Medicine and Public Health* 32: 402-407

5. Endang P, Son Radu, Zaiton H, Ooi WL and Raha AR. 2000. Plasmid-mediated streptomycin resistance of *Listeria monocytogenes*. *Malaysian Journal of Medical Sciences* 7: 48-51

6. Tg. Ahbrizal, Son Radu, Abdul Reezal AL, Rahim AM, Rusul G, Nasreldin E and Yuherman. 2000.
Random amplified polymorphic DNA analysis of *Vibrio vulnificus* strains isolated from cockles and shrimps. *Malaysian Journal of Medical Sciences* 7: 41-46

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

8. Son Radu, Sahilah AM, Rusul G, Samuel L, Zuraini MI and Nasreldin E. 2000. Comparison of arbitrarily primed PCR, antibiotic resistance and plasmid profiling for differentiating *Salmonella enteritidis* isolated from fish. *Asian Fisheries Science* 13: 13-20

## Project Publications in Conference Proceedings

1. Son Radu, Cheah YK, Lesley M, Vickneswaran V, Gwendolyne BT, Nasreldin EH, Yuherman, Chien HC and Nishibuchi M. 2002. Detection of *Vibrio cholerae* and other *Vibrio* spp. from environmental sources in Malaysia. In: Proceedings of the US-JAPAN Cooperation Medical Science Program Conference. 17-19 December. Laguna Garden Hotel, Naha, Okinawa, Japan

2. Son Radu and Nishibuchi M. 2002. Detection of toxigenic *Vibrio cholerae* O139 and O1 in the seafood marketed in Malaysia. In: Proceedings of the US-JAPAN Cooperation Medical Science Program Asian Region Collaboration Research Project Meeting. 21-23 January 2002. International Medical Center, Tokyo, Japan.

3. Lesley MB, Son Radu, John L, Cheah YK, Belinda E and Nishibuchi M. 2003. Improved method and rapid identification of *Vibrio parahaemolyticus* strains from local cockles (*Anadara granosa*) using CHROMAgar™ Vibrio and PCR targeted the *toxR* gene. In: Proceedings of the 13th Scientific Meeting of the Malaysian Society for Molecular Biology and Biotechnology. 19-21 May 2003. Putra Jaya, Malaysia. p44.

4. Lesley MB, Son Radu, Raha AR, John L, Cheah YK, Belinda E and Nishibuchi M. 2003.
Antibiotic susceptibility of *Vibrio parahaemolyticus* isolated from cockles (*Anadara granosa*). In: Proceedings of the 7th Symposium of Applied Biology. 3-4 June 2003. Selangor, Malaysia. p64.

5. Gwendolyne BT, Son Radu and Nishibuchi M. 2002. Identification of *Vibrio parahaemolyticus* strains by CHROMagar™ Vibrio and detection of thermostable direct hemolysin gene (*tdh*), thermostable direct hemolysin-related (*trh*) and *toxR* gene using PCR based method. In: Proceedings of the Malaysia Science and Technology Congress 2002. 12-14 December 2002. Kuching, Sarawak, Malaysia.

6. Nasreldin H, Son Radu, Rusul G, Zaiton H, Yousr A, Reezal A, Lum K, Nishibuchi M. 2001. Prevalence of pathogenic *Vibrio* species in seafood. In: Proceedings of the 24th Symposium of the Malaysian Society for Microbiology. September 9-12, 2001. Cherating, Pahang. p23-27
7. Noorlis A, Son Radu, Foo HL, Reezal A, Yuherman and Sahilah AM. 2001. Characterisation of strains of *Aeromonas* spp. by whole cell protein fingerprints. In: Proceedings of the 24th Symposium of the Malaysian Society for Microbiology. September 9-12, 2001. Cherating, Pahang. p43-44
8. Yousr A, Zaiton H, Son Radu, Rusul G, Nasreldin EH, Samuel L and Apinya V. 2001. Incidence of *Listeria* spp. in shrimp available in Selangor wet markets. In: Proceedings of the 24th Symposium of the Malaysian Society for Microbiology. September 9-12, 2001. Cherating, Pahang. p164-167
9. Nasreldin, E. H., Son Radu, Rusul, G., Hassan, Z., Reezal, A. and Lum, K. Y. 2000. Application of polymerase chain reaction (PCR) and DNA colony hybridization in detection of *Vibrio cholerae* genes isolated from seafood marketed in Malaysia. In: Proceedings of the 23rd Symposium of the Malaysian Society for Microbiology. November 19-21, 2000. Langkawi, Malaysia. p29-32
10. Hassan, Z., Purwati, E., Son Radu, Raha, A. R. and Rusul, G. 2000. Incidence of *Listeria* species from food sources in Malaysia. In: Proceedings of the 23rd Symposium of the Malaysian Society for Microbiology. November 19-21, 2000. Langkawi, Malaysia. p33-36
11. Tg Ahbrizal, Son Radu, Reezal A, Mutalib AR, Nasreldin E and Yuherman. 2000. SDS-PAGE analysis of whole cell proteins for analyzing the clonal lineage among *Vibrio vulnificus* strains isolated from cockles (*Anadara granosa*) and shrimps (*Paneus indicus*). In: Proceedings of the 23rd Symposium of the Malaysian Society for Microbiology. November 19-21, 2000. Langkawi, Malaysia. p160-162
12. Tg Ahbrizal TA, Son Radu, Reezal A, Mutalib AR, Rusul G, Nasreldin E and Yuherman. 2000. Strain typing of *Vibrio vulnificus* biotype 1 in cockles and shrimps by random amplified polymorphic DNA assay. In: Proceedings of the 4th UNESCO National Workshop on Promotion of Microbiology in Malaysia, 26 August, 2000, University Malaya. p32.
13. Noorlis A, Son Radu, Ling FH, Latif ARA, Haryanti T and Ooi WL. 2000. Random amplified polymorphic DNA (RAPD) analysis of *Aeromonas* spp. from fish. In: Proceedings of the 6th National Conference on Medical Science, 10-11 May 2000, Universiti Sains Malaysia, Kota Bharu, Kelantan, Malaysia. p11
14. Nasreldin EH, Son Radu, Rusul G, Khair J, Hassan Z, Latif ARA, Nimita F and Sahilah AM. 2000. Prevalence of *Vibrio* spp. in shrimp (*Penaeus indicus*) marketed in Malaysia. In: Proceedings og the 3rd National Symposium on Health Sciences, 29-30 April 2000. Putra World Trade Center, Kuala Lumpur. p144-148
15. Endang P, Hassan Z, Son Radu, Raha AR, Rusul G, Salasiah K, Sahilah AM, Samuel L and Nasreldin E. 2000. Studies on the multiple antibiotic resistance of *Listeria monocytogenes*. 6th National Conference on Medical Science, 10-11 May 2000, Universiti Sains Malaysia, Kota Bharu, Kelantan, Malaysia. p88

*Graduate Research*

| Name of Graduate | Research Topic | Field of Expertise | Degree Awarded | Graduation Year |
|---|---|---|---|---|
| **Yuherman** | **Molecular characterization of *V. cholerae*, *V. parahaemolyticus* and *V. vulnificus* isolated from marine sources** | Molecular Biology | **Ph.D.** | **2001** |
| **Nasreldin Elhadi Hussein** | **Molecular characterization of *Vibrio cholerae* and other *Vibrio* spp. from seafoods and clinical sources.** | **Molecular Biology** | **Ph.D.** | **2001** |
| **Endang Purwati** | **Prevalence and molecular characterization of *Listeria* spp. isolated from food sources.** | **Molecular Biology** | **Ph.D.** | **2003** |

| | | | | |
|---|---|---|---|---|
| **Tg Ahbrizal Farizal Tg Ahmad** | **Isolation and molecular characterization of *Vibrio vulnificus* from shrimps.** | **Molecular Biology** | **MS** | **2001** |
| Noorlis Ahmad | **Isolation and molecular characterization of *Aeromonas* spp. from seafoods.** | Molecular Biology | **MS** | **2001** |
| Lesley Maurice | **Isolation, identification and molecular characterization of *Listeria* species.** | Molecular Biology | **MS** | **2002** |

**IRPA Project number**09-02-04-0172
**UPM Research Cluster:AFF**