Internet Journal of Food Safety, Vol.10, 2008, p.58-64

Isolation and Enumeration of Coliform Bacteria and *Salmonella* spp. from Short Necked Clam *Orbicularia orbiculata* at East Coast, Malaysia

Ruhil Hayati Hamdan[1], Najiah Musa[1, 2*], Nadirah Musa[1, 2], Lee Seong Wei[1], and Aliuddin Sarman[1]

[1]*Department of Fisheries Science and Aquaculture, Faculty of Agrotechnology and Food Science,Universiti Malaysia Terengganu (UMT).*
[2]*Institute of Tropical Aquaculture, Universiti Malaysia Terengganu (UMT).*

Abstract: Shellfish including short necked clam *Orbicularia orbiculata* are filter feeders. The present study were carried out to estimate the total coliform and to identify bacteria species present in short-necked clams. The short necked clam locally known as lala were sold at wet markets at East Coast, Malaysia. They were screened for coliform bacteria and *Salmonella* spp. via Most Probable Number (MPN) method. Bacterial identification to species level was carried out using morphological, biochemical and physiological tests. Different selective media like Eosine Methylene Blue (EMB), MacConkey and Xylose Lysine Deoxycholate (XLD) were used to isolate bacteria. *Escherichia coli, Klebsiella* spp. and *Salmonella* spp. were successfully identified from *Orbicularia orbiculata* meat. The present study showed high index of coliform bacteria in raw meat of short necked clams. Bacterial identification indicated the presence of *E.coli*, *Klebsiella* spp. and *Salmonella* spp. In this respect, the consumption of raw or semi-cooked short necked clam could cause food borne diseases in humans.

Key word: Coliform bacteria, *Salmonella* spp., short necked clam

[*] Corresponding author. mailing address: Mengabang Telipot, 21030, Terengganu, Malaysia.Tel: +609-6683636, Fax: +609-6683434, E-mail: najiah@umt.edu.my

Introduction

Shellfish are important seafood for human consumption. Microorganisms like bacteria are ubiquitously found in the water environment. Most of bacterial diseases associated with shellfish are opportunistic infections due to stressors such as trauma-induced lesions or poor environmental conditions (Whitman and MacNair 2004). Sewage contamination of filter-feeding bivalve caused a well documented human health risk. This is due to microorganisms transmitted by the fecal-oral route, particularly when the shellfish are consumed raw or lightly cooked. Surveys on microbial pathogens and toxins transmitted in foods have been compiled by World Health Organization (WHO) in 1997 (Bryan et al. 1982 and Lund et al. 2000). Overall, most of the publications concluded that bacterial pathogens such as *Salmonella* spp. and enteropathogenic *E.coli* are responsible for the majority of 80% disease outbreaks including death.

Coliform is a group of Gram-negative, facultative anaerobic rod-shaped bacteria that ferments lactose to produce acid and gas within 48 hours at 35℃ incubation (Feng *et al*. 2002). Coliforms are abundantly found in the feces of warm-blooded animals, as well as in the aquatic environment, soil and on vegetation. Enumeration of coliforms has been adopted as a more convenient standard of sanitary significance by U.S. Public Health Service in 1914 (Anon 2004).

Seafood contamination by *E. coli* and *Salmonella* spp. is of great concern to human public health (Hatha and Lakshmanaperumalsamy 1997). A total of 604, 000 deaths were reported from diarrheal diseases at South East Asia in 2002 (Anon 2004). In Malaysia, incidence rate of typhoid and paratyphoid in 2006 is 0.77 per 100,000 population. Meanwhile, dysentery incidence rate is 0.39 per 100,000 population (Anon 2007). In the United States of America, Salmonellosis accounts for about 60% of all bacterial disease outbreaks (Bean and Griffin 1990). Seafood such as fresh and shellfish were involved in 26% of the disease outbreaks caused by microorganisms. Food service and commercial establishment accounted for three fourths of the reported outbreaks (Bean and Griffin 1990).

In the present study, Most Probable Number (MPN) method was used to determine the coliform bacteria in short necked clam, *Orbicularia orbiculata*. The Most Probable Number (MPN) method is a statistical, multi-step assay consisting of presumptive, confirmation and completed phases or tests (Feng *et al*. 2002). In the assay, serial dilutions of a sample were inoculated into broth media as lactose broth. Enumeration methods that are based on lactose fermentation are frequently used to detect *E. coli* and total coliform of fecal coliform. The 3-tube MPN test was used for testing most foods.

Materials and Methods

Samples. The raw short necked clams, *Orbicularia orbiculata* were obtained from three biggest stalls (A, B and C) at East Coast Malaysia. 300 grams of raw short necked clams were obtained from three different stalls (in triplicate). Aseptically, the shell was cut opened and the meat was put into sterile plastic bags and weighed approximately 100 g each. The samples were then homogenized using a stomacher

Presumptive Test / Most Probable Number (MPN). Nine sets of lactose broth containing Durham's tube were divided into three parts and each was inoculated with 10ml, 1ml and 0.1ml of aliquots sample. Turbidity and production of gas bubbles were observed after incubation at 37℃ for 24 to 48h. The number of organisms in the original culture was estimated from a MPN Determination Chart to determine the MPN index per gram (Benson 2002).

Confirmed Test. The positive tubes from presumptive test were streaked onto selective agars such as Eosine Methylene Blue agar (EMB), Xylose Lysine Deoxycholate agar (XLD) and Mac Conkey agar (Merck, Germany). Typical colonies in each agar were selected for further studies (Benson 2002).

Completed Test. Selected typical colonies were inoculated into lactose broth containing Durham's tube once again and incubated at 37ºC for 24h. Each positive tube was streaked on Trypticase Soy agar (TSA) (Merck, Germany) plates for identification test (Benson 2002).

Bacterial identification. 24-hours cultures were used for conventional identification including Gram's staining, motility, colony formation on different selective agars (XLD, EMB, and MacConkey), a series of biochemical test such as lysine and ornithine decarboxylase, acid production, indole production, citrate utilization, gelatin liquefaction, oxidative and fermentative, hydrogen sulfide production, methyl red, ONPG, oxidase, catalase and urease test were done. For physiological test, the isolates were tested for tolerance to different NaCl concentrations and temperatures (Holt *et al*. 1994).

Results

The presence of coliform bacteria in the short necked clams were more than 2400 MPN/g. Colonies of *Escherichia coli* appeared are circular, flat and green metallic sheen on EMB agar. While, *Salmonella* spp. appeared as yellow colonies on XLD agar. *Klebsiella* spp. on MacConkey agar produces smooth, circular mucoid pink colonies with spreading growth pattern.

All isolates were Gram negative rod shaped bacteria and motile. The results of biochemical and physiological test were summarized in Tables 1, 2 and 3. All the isolates showed typical biochemical characteristics of *Salmonella* spp., *E. coli* and *Klebsiella* spp. In terms of temperature tolerance test, all isolates could grow at 27℃ and 37℃ but failed to grow at 0℃ and 55℃. All isolates also could grow without NaCl.

Discussion

Detection of coliform is used as an indicator of water sanitation or as a general indicator of sanitary condition in the food-processing environment (Feng *et al.* 2002). The MPN/g of coliform bacteria like *E. coli* was very high in short necked clam samples. Jeyasekaran *et al.* (1990) and Karunasagar *et al.* (1992) have reported fecal coliforms contents in shrimps and other food vary depending on the sanitary and hygienic condition of the landing centers. Besides that, Jay (1978) and Ekanem and Adegoke (1995) reported that the shellfish contamination level depends on the extent of pollution in the growing waters. According to Malaysia Food Act and Regulations, coliform count should not exceed 5×10^1 per gram in fish and fish product (MDC 2000).

Garcia-Lopez *et al.* (1998) reported positive lactose tubes and the presence of gram negative coccobacilli in gram stain for further confirmation of the presence of *E.coli*. Study of cultural characteristics on selective media such as Eosine Methylene Blue (EMB), Xylose-Lysine-Deoxycholate (XLD), and MacConkey could be used in preliminary identification purposes.

The isolates of *Escherichia coli* and *Klebsiella* spp. from stall B showed positive results for lysine and ornithine tests. These results were similar with MacFaddin (1999) who stated *Escherichia coli* and *Klebsiella* spp. are usually positive for lysine and ornithine decarboxylase tests. ONPG disc was used for determine enzyme β-Galactosidase. Table 1, 2 and 3 showed variable results of isolates. Some isolates were positive, which indicated the presence of the enzyme β-Galactosidase by using of the organic compound *o-nitrophenyl-β-galactopyranoside* (ONPG), and negative result, indicated absence of this enzyme.

E. coli isolated from all stalls showed positive result in methyl red test. The principle of Methyl Red test is to test the ability of an organism to produce and maintain stable acid end products from glucose fermentation and results are valuable characteristics for the identification of bacterial species showed that strong acids was produced from glucose (MacFaddin 1999). The utilization glucose of *E.coli* for this study was in agreement with Whitman and MacNair (2004).

All *E. coli*, *Klebsiella* spp. and *Salmonella* spp. isolates showed negative tolerance for temperature at both 4°C and 55°C but positive for 28˚C and 37˚C. These results were in agreement with the finding Naemura and Seidler (1978).

Based on present study, it can be concluded that raw short necked clam meat at East Coast Malaysia is unsafe for consumer consumption particularly if being eaten raw or semi-cooked. Bacteria like *E. coli*, *Salmonella* spp. and *Klebsiella* spp. were isolated and identified from the raw meat of short necked clams. Hence, the presence of these foodborne bacteria in short necked clam indicated possible contaminations to the food source. The present findings suggest that the person who involved in short necked clam production should practice a proper and good hygiene management either at collection centers or cold storage centers in order to keep the quality of short necked clam as well as to avoid high growth of pathogens such as *E. coli*, *Klebsiella* spp. and *Salmonella* spp. which are hazardous to human.

References

Anon, 2004. World Health Organization. www.who.org. Accessed on 31st March 2008.

Anon, 2007. Malaysia Ministry of Health. www.moh.gov.my/mohPortal/health_fact.jsp. Accessed on 31st March 2008.

Bean, NH and Griffin, PM. 1990. Foodborne Disease Outbreaks In The United States. 1973-1987: Pathogens,Vehicles, and Trends. J Food Prot. 53: 804-817.

Benson, HJ. 2002. Microbiological applications: Laboratory manual in general microbiology. New York: McGraw-Hill. 478p

Bryan, FL, Guzewich, JJ and Todd, ECC. 1997. Surveillance of Foodborne Disease: Summary and Presentation of Descriptive Data and Epidemiologic Patterns; Their Value and Limitation. J Food Prot. 60:567-578.

Ekanem, EO and Adegoke, GO. 1995. Bacteriological Study of West African Clam (*Egeria radiata* Lamarch) and

Their Overlying Waters. J Food Microbiol. 12:381-385.

Feng, P, Weagent, SD and Grant, MA 2002. Bacteriological Analytical Manual Online**.**www.lib.ncsu.**ed**u/**p**ubweb/www/ETDdb/web_root/collection/available/etd-04102005-213953/ unrestrict**ed**/etd.**p**df

Garcia-Lopez, ML, Prieto, M and Otero, A. 1998. The Physiology Attributes of Gram-Negative Bacteria Associated With Spoilage of Meat and Meat product. *In The Microbiology of Meat and Poultry*, ed. A. Advies and R. Board, p. 1-28. Blackie Academic and Professional, London.

Hatha, MA.A and Lakshmanaperumalsamy, P. 1997. Prevalence of Salmonella in Fish and Crusteceans From Markets in Coimbatore, South India. J Food Microbiol. 14: 111-116

Jay J.M. 2000. Modern Food Microbiology.6th ed. Gaithersburg, Maryland: Aspen Publishers. 679p.

Jeyasekaran, G., Karunasagar and I. Karunasagar. 1990. Validity of Faecal Coliform Test in Tropical Fishery Products, In. Proceedings of Second Indian Fishery Forum, Indian Branch of Asian Fishery Society, Mangalore, India. p.273-275, College of Fisheries, Mangalore.

Karunasagar, I., Ismail, S.M., Amarnath, H.V. and Karunasagar, I. 1992. Bacteriology of Tropical Shrimp and Marine Sediments. *FAO Fisheries Report*. 470 (Suppl.). pp.1-8.`

Lund, B.M., Baird-Parker, T.C and Gould, G.M. 2000. The Microbiological Safety and Quality of Food. Aspen Publisher, Gaithersbourg,Maryland, 2 volumes.

MacFaddin, J.F. 1999. Biochemical Test for Identification of Medical Bacteria. 3rd ed. Lippincott Williams & Wilkins.

MDC. 2000. Food Act 1983 (act 281) & regulations (as at 25th February 2000). Kuala Lumpur: International Law Books. 287p.

Naemura, L.G. and Seidler, R.J. 1978. Significance of Low Temperature Growth Associated with The Faecal Coliform Response, Indole Production, and Pectin Liquecfaction in *Klebsiella*. Appl Environ Microbiol. 35:392-396.

Whitman, K.A and MacNair, N.G. 2004. Finfish and Shellfish Bacteriology: Manual Techniques and Procedures

Table 1: Morphological, biochemical and physiological tests of *Escherichia coli* and *Salmonella spp.* from Stall A.

Test	Isolates											
	E1	**E2**	**E3**	**E4**	**E5**	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**
1.Gram staining	-/s	-/s	-/s	-/s	-/s	-/s	-/s	-/s	-/s	-/s	-/s	-/s
2.Blood test	Γ	Γ	Γ	β	A	γ	α	γ	β	γ	γ	β
3.Motility	+	+	+	+	+	+	+	+	+	+	+	+
4.Indole production	+	+	+	+	+	-	-	-	+	-	-	+
5.Oxidase	+	+	-	+	+	+	+	+	+	-	+	+
6.Catalase	+	+	-	+	-	-	-	+	-	+	+	-
7.ONPG	-	+	-	-	-	+	-	-	-	+	-	-
8. H_2S formation	-	-	-	-	-	+	-	-	-	-	-	-
9.Methyl-Red	+	+	+	+	-	-	-	-	+	+	+	-
10.Urease test	+	+	+	+	-	-	-	-	-	-	-	-
11.Oxidative	+	+	-	+	+	+	+	+	+	+	+	+
12.Fermentative	+	+	+	+	+	+	+	+	+	+	+	+
13.Gelatin liquefaction	+	+	+	+	-	-	-	+	-	-	-	+
14.Fermentation to acid												
14.1 Glucose	+	+	+	+	+	+	+	+	+	+	+	+
14.2 Sucrose	+	+	+	+	+	-	-	+	-	-	+	+
14.3 Lactose	+	+	+	+	+	-	-	+	-	-	+	+
15. Utilization of												
15.1 citrate	-	-	-	-	-	+	-	+	+	-	+	+
15.2 lysine	-	-	-	-	+	+	+	+	+	+	+	+
15.3 ornithine	+	+	-	+	+	+	+	+	+	+	+	+
16. Temperature tolerance												
4 ^oC	-	-	-	-	-	-	-	-	-	-	-	-
28 ^oC	+	+	+	+	+	+	+	+	+	+	+	+
37 ^oC	+	+	+	+	+	+	+	+	+	+	+	+
55 ^oC	-	-	-	-	-	-	-	-	-	-	-	-
17. NaCl tolerance												
0%	-	-	-	-	-	-	-	-	-	-	-	-
2%	+	+	+	+	+	+	+	+	+	+	+	+
6%	+	+	+	+	+	+	+	+	+	+	+	+
8%	-	-	-	-	-	-	-	-	-	-	-	-

Key word: E=Escherichia coli,S=Salmonella spp.,+=positive,=negative,S=shortrod

Table 2: Morphological, biochemical and physiological tests of *Escherichia coli* and *Klebsiella* from Stall B

Test	Isolates											
	E1	**E2**	**E3**	**E4**	**K1**	**K2**	**K3**	**K4**	**K5**	**K6**	**K7**	**K8**
1.Gram staining	-/s	-/s	-/s	-/s	-/s	-/s	-/s	-/s	-/s	-/s	-/s	-/s
2.Blood test	Γ	Γ	β	γ	Γ	γ	γ	γ	α	α	α	α
3.Motility	+	-	-	-	+	-	-	-	-	-	+	-
4.Indole production	+	-	-	-	+	-	-	-	-	-	+	-
5.Oxidase	+	+	+	+	+	+	+	+	+	+	+	+
6.Catalase	+	+	+	+	+	+	+	+	+	+	+	+
7.ONPG	-	-	-	-	-	-	-	-	-	-	+	-
8. H_2S formation	-	-	-	-	-	-	+	+	-	-	-	-
9.Methyl-Red	+	+	+	+	-	-	+	-	-	+	-	+
10.Urease test	-	-	-	-	-	-	-	-	-	-	-	-
11.Oxidative	+	+	+	+	+	+	+	+	+	+	+	+
12.Fermentative	+	+	+	+	+	+	+	+	+	+	+	+
13.Gelatin liquefaction	+	+	+	-	+	+	+	+	+	+	+	+
14.Fermentation to acid												
14.1 Glucose	+	+	+	+	+	+	+	+	+	+	+	+
14.2 Sucrose	+	+	+	+	+	+	+	+	+	+	+	+
14.3 Lactose	+	+	+	+	+	+	+	+	+	+	+	+
15.Utilization of												
15.1 citrate	+	+	+	+	+	+	+	+	+	+	+	+
15.2 lysine	+	+	+	+	+	+	+	+	+	+	+	+
15.3 ornithine	+	+	+	+	+	+	+	+	+	+	+	+
16.Temperature tolerance												
4 ^oC	-	-	-	-	-	-	-	-	-	-	-	-
28 ^oC	+	+	+	+	+	+	+	+	+	+	+	+
37 ^oC	+	+	+	+	+	+	+	+	+	+	+	+
55 ^oC	-	-	-	-	-	-	-	-	-	-	-	-
17. NaCl tolerance												
0%	-	-	-	-	-	-	-	-	-	-	-	-
2%	+	+	+	+	+	+	+	+	+	+	+	+
6%	+	+	+	+	+	+	+	+	+	+	+	+
8%	-	-	-	-	-	-	-	-	-	-	-	-

Key word: K=Klebsiella spp, S=Salmonella spp.,+=positive, -=negative, S=shortrod

Table 3: Morphological, biochemical and physiological tests of *Escherichia coli* and *Salmonella spp.* from Stall C.

Test	Isolates											
	E1	**E2**	**E3**	**E4**	**E5**	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**
1.Gram staining	-/s	-/s	-/s	-/s	-/s	-/s	-/s	-/s	-/s	-/s	-/s	-/s
2.Blood test	A	A	A	α	A	α	α	α	γ	γ	α	α
3.Motility	+	+	+	+	+	+	+	+	+	-	-	+
4.Indole production	+	+	+	+	+	-	-	-	-	+	-	+
5.Oxidase	+	-	-	+	+	+	+	+	-	+	+	-
6.Catalase	+	+	+	+	+	+	+	+	+	+	+	+
7.ONPG	-	+	+	-	-	+	-	-	-	+	-	-
8. H_2S formation	-	-	-	-	-	-	-	-	-	-	-	-
9.Methyl-Red	+	+	+	+	+	+	+	-	+	-	-	-
10.Urease test	-	-	-	-	-	-	-	-	-	-	-	-
11.Oxidative	+	+	-	+	+	+	+	+	+	+	-	+
12.Fermentative	+	+	+	+	+	+	+	+	+	+	+	+
13.Gelatin liquefaction	-	-	-	-	+	-	-	+	+	+	+	-
14.Fermentation to acid												
14.1 Glucose	+	+	+	+	+	+	-	+	+	+	-	-
14.2 Sucrose	+	+	+	+	+	+	-	+	+	+	-	-
14.3 Lactose	+	+	+	+	+	+	-	+	+	+	-	-
15.Utilization of												
15.1 citrate	+	+	+	+	+	-	+	-	-	+	+	+
15.2 lysine	+	+	+	+	+	+	+	+	+	+	+	+
15.3 ornithine	+	+	+	+	+	+	+	+	+	+	-	+
16. Temperature tolerance												
4 ^oC	-	-	-	-	-	-	-	-	-	-	-	-
28 ^oC	+	+	+	+	+	+	+	+	+	+	+	+
37 ^oC	+	+	+	+	+	+	+	+	+	+	+	+
55 ^oC	-	-	-	-	-	-	-	-	-	-	-	-
17. NaCl tolerance												
0%	-	-	-	-	-	-	-	-	-	-	-	-
2%	+	+	+	+	+	+	+	+	+	+	+	+
6%	+	+	+	+	+	+	+	+	+	+	+	+
8%	-	-	-	-	-	-	-	-	-	-	-	-

Key word: E=Escherichia coli,S=Salmonella spp.,+=positive,-=negative,S=shortrod