Bacteriology of the fresh water bivalve clam *Batissa violacea* (Kai) sold in the Suva market

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ABSTRACT

Forty samples of freshwater clam (Batissa violacea), popularly known as Kai, collected from the Suva market were analysed for total aerobic plate count and total coliforms. The heterotrophic bacteria isolated by plate count were also characterised up to genera. The results indicated higher than acceptable level of plate count and coliform bacteria. While total plate count of aerobic heterotrophic bacteria ranged from 5.5 x 10^5 colony forming units (cfu) per gram to more than 10^7 cfu/ gram, total coliform load varied between 1.1 x 10^4 to 1.1 x 10^5 per 100 gm of Kai flesh. The characterisation of the heterotrophic bacteria revealed the predominance of the genera Micrococcus (34%) and Bacillus (24%). Other genera encountered included Acinetobacter, Vibrio, Aeromonas, Alcaligenes, Pseudomonas, Streptococcus and members of the family Enterobacteriaceae. The results revealed the need for depuration of the Kai in clean running water to reduce the bacterial load to acceptable levels. Temperature control soon after harvesting by proper icing and thorough cooking of the Kai before consumption is also recommended.

Keywords: *Batissa violacea, heterotrophic bacteria, coliforms.*

1 INTRODUCTION

Molluscan shellfish, especially the bivalve clams, are considered as potentially hazardous food because of their inherent tendency to bioaccumulate pathogenic bacteria and toxic metals through filter feeding. It was understood that the inappropriate disposal of raw and partially treated sewage was a principal reason for increasing incidence of shellfish-borne illness. Hence strict guidelines are issued by the government regulatory authorities of the developed countries regarding bacteriological quality of the harvesting waters of the wild caught shellfish as well as the water bodies used for cultivation of shellfish (Leonard *et al.* 1990). It is assumed that shellfish should be cultivated and harvested from approved waters considered as safe for rearing shellfish. However, because of the strong economic incentive from popular demand for shellfish, people tend to harvest shellfish from productive natural growing areas where contaminant load exceeds generally accepted safe level resulting in the entry of sewage contaminated shellfish to market place.

The freshwater clam, Kai (*B. violacea*) is a popular and widely consumed food item in Fiji. It is being harvested from most of the natural growing beds, irrespective of the level of pollution in those waters. The depuration, a process to reduce the high load of bacteria and toxic metals in the shellfish, is not followed and the catch is being sold in the market at ambient temperatures, conducive for the proliferation of mesophilic bacteria that includes coliforms and most human pathogens. The present investigation has been taken up to study the bacteriology of the Kai sold in the Suva market.

2 MATERIALS AND METHODS

B. violacea (Kai) samples were collected from the Suva market during the period 2004 February-May. Samples were collected from different shellfish vendors in sterile polythene bags. Soon after collection the samples were placed in an icebox, carried to the laboratory and analysed within 2-4 hours.

The shells of the Kai were opened aseptically using a sterile scalpel. One of the retractor muscles was aseptically cut which lead to the opening of the whole shell. Ten grams of muscle was aseptically transferred to the sterile homogenizing bag and homogenized with 90ml of sterile distilled water using a stomacher (IUL Instruments, Spain). The homogenate was serially diluted up to 10^{-4} using 9 ml sterile dilution blanks. Using pour plate method 1 ml of the 10^{-3} and 10^{-4} dilutions were transferred to sterile petri dishes and plated in duplicate in standard plate count agar. The plates were incubated at 37^{o}C for 48 hours. After incubation, the plates with 30-300 colonies were chosen for counting and the total plate count bacteria is expressed as number of colony forming units (cfu) per gram of shellfish.

After counting and estimation of total bacterial load, morphologically different colonies were picked up using a sterile inoculation needle and aseptically transferred to sterile nutrient agar slants for further characterisation. A total of 67 isolates were selected for characterisation. The isolates were checked for purity and characterised up to genera following a standard characterisation key (Buchanan and Gibbons 1979) based on Gram staining, spore staining, motility, Kovac's oxidase, oxidation/fermentation (O/F) test and catalase tests.

Sample homogenate was prepared in the same way as described for total plate count bacteria and 10^{-2} dilutions were used for estimating the coliform bacteria. A standard 3-tube dilution most-probable number (MPN) method (West 1989) procedure was used to enumerate the coliform load in the shellfish samples. Using a sterile pipette 10 ml sample each were inoculated into three McCartney bottles containing 10ml sterile double strength EC broth. Similarly, three 1ml and 0.1ml volumes of the samples were inoculated into 10ml sterile single strength EC broth. After inoculation, the McCartney bottles were incubated at 37^{o}C for 24 hours and checked for gas production in the inverted Durham's tubes. The tubes with gas production were recorded and referred to the MPN table to find out the MPN index for coliforms.

3 RESULTS AND DISCUSSION

Total aerobic plate count of heterotrophic bacteria and total coliform load is presented in Table 1. It was found that 15% of the shellfish samples had bacterial load in extremely high numbers (>10^7 cfu/gm) while 47.5% of the samples had TPC values ranging from 2.46 x 10^6 to 2.75 x 10^6 cfu/gm. Only less than 5% of the samples had acceptable level of TPC (≤5 x 10^5 cfu/gm) as per the guidelines of centre for food safety and applied nutrition (CFSAN 2003) of US Food and Drug Administration, the food standards of which are accepted world-wide. The total coliform load of all the samples was also very high. While the bacteriological quality based on TPC and coliforms were comparable to the findings of Fernandez and Ryan (1983) from Costa Rica, it was much inferior when compared to the findings of Villalobos de Bastardo and Elguezabal Aristizabal (2001) from Venezuela and Legnani *et al.* (2002) from Spain.

The very high TPC and total coliforms in the Kai samples collected from Suva market is a direct reflection of the quality of the shellfish harvesting waters coupled with inappropriate temperature and time of storage in the markets. The shellfish is kept at ambient temperatures for long duration, which results in the multiplication of mesophilic bacteria such as coliforms. Most of the total plate count bacteria in the tropical latitude are also mesophiles that can also proliferate in the clam meat to dangerous levels. Our previous studies (Nazeem Beena *et al.* 2002; Hatha *et al.* 2003) clearly proved the significance of time/temperature control to maintain the bacteriological quality of highly perishable food stuff such as seafood.

We have further characterised the plate count bacteria into various genera (Table 2). Results indicated predominance of bacteria belonging to the genera *Micrococcus* and *Bacillus*. Other genera encountered included *Vibrio*, *Alcaligenes*, *Acinetobacter*, *Aeromonas*, *Pseudomonas*, *Streptococcus* and members of the family Enterobacteriaceae. Occurrence of *Aeromonas* (Rodriguez and Antillon 1989), *Vibrio* spp. (Garcia Cortes and Antillon 1990; Pujalte *et al.* 1999), *Acinetobacter* (Kueh and Chan 1985) and *Pseudomonas* (Cheng *et al.* 1995) were reported earlier.

The genus *Vibrio* includes potential pathogenic species such as *V. parahaemolyticus, V. cholerae* and *V. vulnificus.* Similarly *Aeromonas hydrophila* is fast emerging as a pathogen of significance in seafoods (Hatha *et al.* 2005; Vivekanandan *et al.* 2005). *Aeromonas* and *Vibrio* are ubiquitous to aquatic bodies (Montfort and Baleux 1990) and are likely to be concentrated in the bodies of bivalve shellfish during filter feeding. In our ongoing studies on shellfish bacteriology along the coast of Fiji, we have encountered considerable prevalence of *V. parahaemolyticus* (results unpublished). Lack of depuration process and temperature abuse of the Kai in the market can lead to the proliferation of these potentially pathogenic genera to hazardous levels, especially to those consumers who take it raw or after marinating with lemon juice, a popular dish in Fiji.

Packing of fresh shellfish with good quality ice can control the temperature abuse to a great extent and prevent the multiplication of mesophilic bacteria. More importantly the consumers should be made aware of the consequences of consumption of raw shellfish and may be encouraged to thoroughly cook the shellfish before eating.

Table 1. Total plate count (TPC) and coliform load of the Kai (*B. violacea*) samples from Suva market.

Sample No.	TPC (cfu/ gm)	Coliforms (MPN index/ 100g)
1	2.77 x 10^6	1.1 x 10^4
2	1 x 10^7	1.1 x 10^4
3	2.75 x 10^6	1.1 x 10^4
4	1.13 x 10^6	1.1 x 10^4
5	1 x 10^6	1.1 x 10^4
6	1.32 x 10^6	1.1 x 10^4
7	9.6 x 10^5	1.1 x 10^4
8	2.46 x 10^6	1.1 x 10^4
9	2.66 x 10^6	1.1 x 10^4
10	1 x 10^7	1.1 x 10^4
11	2.16 x 10^6	1.1 x 10^4
12	2.9 x 10^6	1.1 x 10^4
13	2.99 x 10^6	1.1 x 10^4
14	2.57 x 10^6	1.1 x 10^4
15	2.59 x 10^6	1.1 x 10^4
16	9.7 x 10^5	1.1 x 10^4
17	1 x 10^7	1.1 x 10^4
18	1.34 x 10^6	1.1 x 10^4
19	2.7 x 10^6	1.1 x 10^4
20	2.08 x 10^6	1.1 x 10^5
21	1.54 x 10^6	1.1 x 10^5
22	2.63 x 10^6	1.1 x 10^5
23	1 x 10^7	1.1 x 10^5
24	2.99 x 10^6	1.1 x 10^5
25	1 x 10^7	1.1 x 10^5
26	2.85 x 10^6	1.1 x 10^5
27	1 x 10^7	1.1 x 10^5
28	5.5 x 10^5	1.1 x 10^5
29	2.97 x 10^6	1.1 x 10^5
30	2.4 x 10^6	1.1 x 10^5
31	2.63 x 10^6	1.1 x 10^5
32	1.62 x 10^6	1.1 x 10^5
33	2.17 x 10^6	1.1 x 10^5
34	1.18 x 10^6	1.1 x 10^5
35	1 x 10^7	1.1 x 10^5
36	7.4 x 10^5	1.1 x 10^5
37	2.84 x 10^6	1.1 x 10^5
38	1.66 x 10^6	1.1 x 10^5
39	1.52 x 10^6	1.1 x 10^5
40	2.58 x 10^6	1.1 x 10^5

Table 2. Percentage distribution of various genera of heterotrophic bacteria in Kai (*B. violacea*) samples from Suva market.

Name of Genera	Percentage of incidence
Micrococcus	34
Bacillus	24
Acinetobacter	13
Vibrio	7
Aeromonas	6
Alcaligenes	4
Pseudomonas	1
Streptococcus	1
Enterobacteriaceae	10

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