PHYSICOCHEMICAL PARAMETERS AND OCCURRENCE OF
Vibrio parahaemolyticus AND
Escherichia coli IN BIVALVES

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DECLARATION

No portion of the work referred to in this dissertation has been submitted in support of an application for another degree of qualification of this or any other university or institution of higher learning.

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<th>Description</th>
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<tr>
<td>APW</td>
<td>Alkaline Peptone Water</td>
</tr>
<tr>
<td>CFU</td>
<td>Colony-forming units</td>
</tr>
<tr>
<td>EMB</td>
<td>Eosin-methylene blue</td>
</tr>
<tr>
<td>TCBS</td>
<td>Thiosulfate citrate bile sucrose</td>
</tr>
<tr>
<td>PCR</td>
<td>Polymerase Chain Reaction</td>
</tr>
<tr>
<td>STEC</td>
<td>Shiga-like toxin producing <em>Escherichia coli</em></td>
</tr>
<tr>
<td>EHEC</td>
<td>Enterohemorrhagic <em>E. coli</em></td>
</tr>
<tr>
<td>FAO</td>
<td>Food and Agriculture Organization</td>
</tr>
<tr>
<td>ICMSF</td>
<td>Internal Commission on Microbiological Specification for Food</td>
</tr>
<tr>
<td>SLT</td>
<td>Shiga-like toxin</td>
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<td>texR</td>
<td>toxin operon gene</td>
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Physical Parameters and Occurrence of *Vibrio parahaemolyticus* and *Escherichia coli* in Bivalves

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**ABSTRACT**

Food-borne diseases caused by consumption of shellfish are becoming more common among human. Therefore, this situation makes people become more aware of their daily food consumption. The accumulation of bacteria such as *Vibrio parahaemolyticus* and *Escherichia coli* in bivalve can be harmful to human. Those bacteria are usually found in the water and infect human from traditional consumption of undercooked bivalve. The occurrence of *Vibrio parahaemolyticus* and *Escherichia coli* can be influenced by the physicochemical parameters such as pH and salinity of water. This study was focusing at Sg Melaban and Kg Melaban. The aim of this study is to identify the occurrence of *Vibrio parahaemolyticus* and *Escherichia coli* in bivalves with the association of physicochemical parameters. PCR was carried out to target toxR gene which is regulatory gene for *Vibrio parahaemolyticus* and *Escherichia coli* and *Slt1, Slt1l, rfbE, and fliC* genes are the virulence factor for *Escherichia coli*. It can determine the fitness of bivalves for human consumption. The result from this study could give a huge impact towards food safety can reduce the risk of food-borne diseases among human.

Keywords: physicochemical parameters, *Vibrio parahaemolyticus, Escherichia coli*.

**ABSTRAK**


**Kata kunci:** parameter fizikokimia, *Vibrio parahaemolyticus, Escherichia coli*.
Bivalves such as clams, mussels, scallops, cockles and oyster are marine and freshwater organisms. They have flattened shell with two valves and most of them are filter feeders. Marsh clam (Polymesoda sp.) is a type of bivalves which are commonly found in many part of the world such as marine, brackish, fresh and terrestrial areas (Hamli et al., 2012). As filter feeders, they consume all tiny organisms and accumulate bacteria from water (Bennani et al., 2010). Consumption of raw or uncooked bivalve in some dishes tends to make the consumers suffer from food poisoning. Usually, the bivalves are cooked briefly in order to avoid the meat clam hard. The microorganisms which commonly presence in marsh clam are *Vibrio parahaemolyticus*, *Escherichia coli*, *Salmonella* spp, *Aeromonas hydrophila*, *Listeria monocytogenes* and *Shigella*.

*Escherichia coli* are also a gram negative bacterium. It has a rod shape and facultative aerobic bacteria which are commonly found in intestinal microflora of warm blooded animal. They are well distributed at higher temperature area compare to lower temperature, thus they are easily found on the edge of hot springs. Most of *E.coli* is harmless inhabitant of the intestinal tract. Only small percentage of strain is considered pathogenic. *E. coli* is a typical faecal indicator bacterium in natural waters. Shiga toxin *E.coli* (STEC) is commonly found in shellfish.

*Vibrio* species commonly isolated from fresh, brackish and marine waters. Water temperature, salinity, nutrient availability, and association with marine organisms can influence the bacterial loads in water. *Vibrio parahaemolyticus* which are halophilic enteropathogen grows at a minimum temperature of 15°C and a maximum temperature of 44°C. In addition, 5-30% of water salinity favours growth and its survival. *Vibrio*
*V. parahaemolyticus* can cause disease at dose of about $2 \times 10^5 - 3 \times 10^7$ cfu. About 15 hours required for incubation and the disease may last for 2 – 3 days (Bhunia, 2008).

The physicochemical parameter such as pH and salinity plays a crucial role in presence of *V. parahaemolyticus* and *E. coli* in water and bivalves. As marsh clam (*Polymesoda sp.*) is common for human consumption, the count of these indicator microorganisms in those bivalves need to be well studied.

The aims of this research are to determine the occurrence of *V. parahaemolyticus* and *E. coli* with the association of physicochemical parameters. Nowadays, people are more concern about food safety as the outbreaks of bivalve-associated infections are more common. Through this study, the fitness of marsh clam (*Polymesoda sp.*) has been evaluated for human consumption. Thus, it can reduce the shellfish-associated infection among human.

### 1.1 Objectives

The objectives for this study are to:

1) Determine the association between physicochemical parameters (pH and salinity) and the occurrence of *V. parahaemolyticus* and *E. coli* in bivalve from Sg. Melaban and Kg. Melaban.

2) Evaluate the fitness and safety of bivalve for human consumption.

3) Detect the presence of virulence genes in *V. parahaemolyticus* and *E. coli* by PCR method.
2.0 LITERATURE REVIEWS

2.1 Bivalves

Organism which can be characterized by two shell valves gaping at both ends but connected by hinges, two siphons and one strong foot are commonly called shellfish or bivalves. They can provide human another important source of protein besides fish. As cited in Hamli et al., (2012), in Southeast Asia, about 1211 species of bivalves was reported, and it is the highest diversity for bivalves compared to 29 regions around the world, while Vermeij (1990) believed that seasonal pattern in the Southeast Asia such as monsoonal rainfall provide nutrients enriched environment for these filter feeder organism which eventually help to increase the number of mollusc diversity in this area. According to Potasman et al., (2002), the shellfish are common in areas where nutrient level are high and water are sheltered. He also stated that, when the pathogenic microorganisms contaminate the harvesting sites, they are filtered by the gills and become highly concentrated in liverlike digestive glands. Hassan et al., (2013) mention that bivalves play a significant role in the complex coastal food web as well as actively involved in ecological services as they are filter feeder. Thus, it can commonly use in environmental monitoring studies because they can provide accurate information on the impacts and bioavailability of pollutants.
2.2 Bivalve-associated Infectious Outbreaks

As a filter-feeder, bivalves are the most prevalent organisms in transmitting microorganisms to human. Potasman et al., (2002) mention that the largest – ever recorded outbreak was occur in Shanghai in 1988 which involved almost 300 000 people infected with hepatitis A after eating clams. He also stated that large outbreaks of infection involving more than 800 patients also occurred in Australia in 1979, United States in 1986, and Japan in 1991. From the history of infections, Vibrio species are most common cause of bivalves-associated infection. This infection commonly occurs by eating undercooked shellfish. Dileep et al., (2003) stated that as only a small proportion of the environmental strains are virulent, to determine seafood safety, it would be important to specifically detect these strains in the seafood.

2.3 Escherichia coli

E. coli is Gram-negative bacteria, short rods (1-2μm in length) that live in the intestines of healthy humans and most mammals. This bacterium plays importance role in maintaining the balance of normal intestinal flora in fighting harmful bacteria and synthesize some vitamins. E. coli is a typical faecal indicator bacterium in natural waters. Since the isolation of pathogens is a difficult, expensive and time consuming process, indicator microorganisms are used as an index of human-specific faecal pollution (Jozic et al., 2012). Major source of infection can be from ingestion of contaminated food and water. Raw or undercooked shellfish also can be the way to E. coli to spread in human consumption. Recent study has shown that Shiga toxin-producing E. coli (STEC) was detected in shellfish. Enterohemorrhagic E. coli (EHEC) is also classified as subset of stx producing E. coli (STEC) that is pathogenic to human.
2.3.1 Infections or occurrence of *E.coli* in seafood

According to Costa (2013), occurrence of *E.coli* in seafood is directly related to fecal contamination. This also related to water contamination and unhygienic conditions during the handling process. In Brazil, only one strain of STEC from shellfish tested was isolated (Costa 2013). As cited by Kumar *et al.* (2001), the occurrence of Shiga-toxigenic *E.coli* was detected in fish and clams marketed in Mangalore, India. He stated that Shiga-toxin producing *E.coli* (STEC) is dominant in seafood in India and non-O157 serotype is more common. Based on study which was conducted by Hamdan *et al.* (2008), the presence of *E.coli* also was detected in short-necked clams at East Coast Malaysia and the consumption of raw or semi-cooked short-necked clam could cause food borne disease in human.

2.4 *Vibrio parahaemolyticus*

*Vibrio parahaemolyticus* is a Gram-negative bacterium, have curved shaped, with size ranging from 1.4 to 2.6μm long and halophilic estuarine organism. *V.parahaemolyticus* infection is usually associated with eating raw or undercooked shellfish, and in Asia, particularly in Japan and Taiwan, this infections are commonly reported (Ray *et al.*, 2000). Daniels *et al.*, (2000) stated that *V.parahaemolyticus* is a halophilic gram negative bacterium that is widely distributed in coastal waters of virtually all temperate region-sand infection occur primarily during the warmer month of the year. Marano *et al.*, (2000) mentioned *V.parahaemolyticus* infections are frequently reported in coastal areas, because of the high consumption of sea products and direct contact with estuarine waters.
2.4.1 Infections or occurrence of \textit{V.parahaemolyticus} in seafood

Over the last 25 years, the US has experienced several shellfish-related disease outbreaks, with only 4% being due to fecal contaminants (coliforms, \textit{Salmonella} \textit{sp.}, Norwalk virus, Hepatitis A), whereas 20% of the disease and 99% of the death were link to \textit{Vibrio spp} present in seafood (Bej, 2010). \textit{V.parahaemolyticus} is common cause of foodborne disease in Asia. Although the outbreaks are very small scale but it occur frequently. Between 1986 and 1995, 197 outbreaks of foodborne disease were caused by \textit{V.parahaemolyticus} in Taiwan and 47% of the residents interviewed in Hat Yi city in Thailand reported experiencing diarrhoea after consumption of bloody clam (FAO, 2011). Based on study carried out by Zulkifli et al., (2009), there was occurrence of \textit{V.parahaemolyticus} in cockle samples from Padang, Indonesia. At Tanjong Karang Selangor, 62% from sample tested was positive \textit{V.parahaemolyticus} (Bilung et al., 2005).

2.5 Association between Physicochemical Parameters and \textit{V.parahaemolyticus} and \textit{E.coli}

According to the Jozic et al., (2012), the concentration of \textit{E.coli} in bivalves was controlled by temperature and salinity. Some strain of pathogenic \textit{E.coli} can grow at temperature as low as 7°C and as high as 46°C, the optimum range being 35-40°C. There have been study reveal that these bacteria can grow in 6% NaCl and more tolerant to sodium chloride than typical strain of \textit{Salmonella} spp. (ICMSF, 1996). The interaction between temperature and salinity was statistically significant and suggested that their simultaneous effects on \textit{E.coli} concentration were higher than these factors acted independently. \textit{V.parahaemolyticus} grows optimally in warmer water and recent study shows that \textit{Vibrio} grows to high level during the summer months. All \textit{V.parahaemolyticus} strains inhabit
marine, brackish, and estuarine waters, where fluctuations in salinity pose a constant
challenge to the adaptive response of the organism and V. parahaemolyticus is moderately
halophilic in nature, making this bacterium more osmotolerant than many other Vibrio
species (Whitaker et al., 2010). Urtaza et al., (2008) mentioned that the highest incidence
of V. parahaemolyticus characteristically occurred during the phases of decreasing salinity,
whereas high levels of the organism mainly occurred when high seawater temperatures
overlapped with periods of reduced salinity. The factor affecting the incidence and
distribution of V. parahaemolyticus in the environment include water temperature, salt and
oxygen concentration, interaction with the plankton, the presence of the sediment, the
organic matter in suspension and tidal action of estuarine waters (Garcia et al., 2004).

2.6  Slt-I, Slt-II, rfbE, and fliC_{H}, Genes of E.coli

Two different set of PCR primers specific for SLT genes can be used to determine
potential toxigenicity of enterohemorrhagic E.coli (EHEC) isolates (FDA’s Bacteriological
E.coli O157:H7 was developed by amplifying a DNA sequence encoding Shiga-like toxin
(SLT) I and II. Rfb and FLIC{H7 primer set were targeted for the gene involved in
biosynthesis of O157 antigen and H7 antigen (Apun et al., 2011).

2.7  toxR Gene of V.parahaemolyticus

toxR gene fragment is specific for V.parahaemolyticus as it is a toxin operon gene. toxR
gene appears to be well conserved among this bacterium (Zulkifli et al., 2009). Kim et al.,
(1999) has stated that identifying V.parahaemolyticus strain through PCR based method
which targets the conserved region of V.parahaemolyticus such as gyrB and toxR gene is
more efficient, reliable and faster compare to biochemical test.
3.0 MATERIALS AND METHODS

3.1 Samples collection

Samples of marsh clams or locally known as *lokan* (Figure 1) were collected from Sg Melaban nearby Faculty of Resource Science and Technology (FRST) External Laboratory and Kg Melaban area on 2 December 2013 and 9 April 2014 respectively. These sites were chosen because it is a marsh clams harvesting area. Sg Melaban nearby FRST External Laboratory is a non-populated area while Kg Melaban is a populated area.

Total of 15 samples of marsh clams were collected from both sites. As the marsh clam presence at the surface of mud, small spade are used to dig into the mud to collect it. Water samples also were collected in sterilized plastic bottle from the sampling sites. The pH and salinity of water was measured by pH meter and refractometer respectively. All samples were placed in a cooler box and transported immediately to the laboratory for sample processing.

![Figure 1 Polymesoda spp. collected from the two sampling sites.](image)
3.2 **Samples processing**

All marsh clam samples were washed and brushed under running tap water before sterilized with 70% ethanol to avoid cross contamination. Then, the samples were opened and obtain the flesh by using sterile scalpel. Each of flesh was weighed before it will be homogenized by cutting the flesh into very small pieces.

3.3 **Isolation of *E.coli* and *V.parahaemolyticus* from samples**

The homogenized flesh from each sample was divided into two portions to enrich in Alkaline Peptone Water (APW) (Merck) and EC broth (Oxoid) for selective enrichment for *V.parahaemolyticus* and *E.coli* respectively. For *V.parahaemolyticus*, the sample was enriched for 18 hours in 37°C and serial dilution was carried out in double distilled water. An amount of diluted sample, 100µl, was plated onto Thiosulphate Citrate Bile Salt (TCBS) agar (Merck). For *E.coli*, the sample was enriched for 7 hours in 37°C. Next, serial dilution was carried out in Phosphate Buffered Saline (PBS) and 100µl of diluted sample was cultured directly on Eosin Methylene Blue (EMB) agar (Oxoid) by using spread plate method.

In order to examine the presence of *E.coli* and *V.parahaemolyticus* in water sample, 100µl of water sample was pipetted out and spread on EMB and TCBS agar respectively. The plates were labelled and incubated at 37°C for 24 hours.
3.4 Plate count and subculturing of *E. coli* and *V. parahaemolyticus*

After 24 hours incubation, the plates were examined and all the colonies were counted and recorded. A green bluish colony on TCBS agar (Figure 2 and Figure 3) shows the presence of *V. parahaemolyticus* in the samples while a metallic green colony on EMB agar (Figure 4) indicates the presence of *E. coli*. The desired colony was picked and streaked on new EMB and TCBS plate agar to obtain pure culture.

3.5 Bacteria identification

Bacteria isolates were tested through gram-staining for morphology identification. Colonies which show the same pink colour and same shape (rod shape for *E. coli*, curve rod shape for *V. parahaemolyticus*) under microscopic view was regarded as pure culture. Then, the presumptive bacteria isolates were further confirmed by using commercial identification kits, API 20E and API 20NE (BioMerieux, France).

3.6 DNA extraction

Bacterial DNA of the *E. coli* and *V. parahaemolyticus* isolate was extracted by using boiling extraction method as described by Zulkifli et al., (2009) with minor modification. Bacterial isolates was cultured in 5 ml Luria-Bertani broth for overnight at 37 °C with agitation 150 rpm. 1 ml of overnight broth suspensions was then centrifuged at 13,000 rpm for 5 minutes to collect the pellets. Next, the pellets were resuspended in 100μl of distilled water before boil for 20 minutes to lyse the cells. The cells were cooled in ice for another 20 minutes. Lastly, the cells were centrifuged again at 13,000 rpm for 3 minutes. The supernatant containing DNA was used as template in PCR.
Figure 2 Colonies on TCBS agar after spread plate method

Figure 3 Presumptive positive *V. parahaemolyticus* after subculture

Figure 4 Presumptive positive *E. coli* after subculture
3.7 Polymerase Chain Reaction experiments and Agarose Gel Electrophoresis (AGE)

Species specific PCR for *V. parahaemolyticus* was targeting the *toxR* gene, which is regulatory gene that is present in all the strains irrespective of their ability to produce TDH or TRH (Kim *et al.*, 1999). The PCR reaction comprise a 25μl reaction mixture containing 15.5μl sterile distilled water, 2.5μl of 10x PCR buffer, 2μl 25 mM MgCl₂, 0.5μl 10Mm dNTP, 1 μl of toxR-R and toxR-F, 0.5μl of 5 U TaqDNA and 2μl of template DNA. PCR reactions were carried out in a Eppendorf MastercycJe® Personal (Germany) with the following reaction conditions: pre-denaturation at 96°C for 5 minutes, followed by 34 cycles of denaturation at 94°C for 1 minute, annealing at 63°C for 1.5 minutes, extension at 72°C for 1.5 minutes and final extension was performed at 72°C for 7 minutes. The PCR products were resolved by electrophoresis in 1% agarose gel and ran in 1x TAE buffer. 5 μL of PCR product was mixed with 1μl of loading dye and then loaded into the wells prepared. After that, 100bp marker was included in the gel as indicator of band size. PCR products were electrophoresed at 100 V for 1 hour. Then, gel was stained with ethidium bromide and visualized using transmitted ultraviolet illumination (Vilber Lourmat). The expected size of amplicons for *toxR* is 368bp.

Detection of *E.coli* O157:H7 was carried out by multiplex PCR method as described by Apun *et al.*, (2011). Four sets of primer were used which are SLT-I, SLT-II targeted for shiga toxin producing gene, rfbE and fliC₇ targeted in gene involved in biosynthesis of O157 antigen and H7 antigen respectively. The PCR reaction comprised a 25μl reaction mixture containing 9.25μl sterile distilled water, 2.5μl of 10x PCR buffer, 2.5μl of 25 mM MgCl₂, 1.25μl of 10Mm dNTP, 0.5 μl of each primers Sltl-F/Sltl-R, Sltll-F/Sltll-R, RfbE-F/RfbE-R, FLIC₇-F/FLIC₇-R, 5μl of 5 U TaqDNA and 5μl of template DNA. *E.coli* reference strain EDL933 was included as a positive control. PCR
amplification was performed according the condition as follows: initial denaturation at 94°C for 5 minutes, followed by 35 cycles of denaturation at 94°C for 0.5 minute, annealing at 59°C for 1 minutes, extension at 72 °C for 1 minutes and final extension was performed at 72°C for 7 minutes. The PCR products for E.coli were resolved by electrophoresis in 1% agarose gel and were electrophoresed at 90 V for 1 h 15 mins. Then, gel was stained with ethidium bromide and visualized using transmitted ultraviolet illumination. The expected size of amplicons for SltI, SltII, rfbE, and fliC₇₇ are 210 bp, 292 bp, 484 bp and 625 bp respectively.
4.0 RESULTS

4.1 Plate count of *E.coli* and *V.parahaemolyticus* from marsh clam samples

Plate count was carried out for the presence of *E.coli* and *V.parahaemolyticus* on EMB and TCBS agar respectively. Table 1 shows the bacterial count of *E.coli* and Table 2 shows the bacterial count of *V.parahaemolyticus* from marsh clams sample from 2 sampling sites. There were 2 samples analysed from Sg Melaban while 10 to 14 samples were analysed from Kg.Melaban. Both sites recorded bacteria count in the range of $10^6$ cfu/ml.

For *E.coli* count, the highest count is from Sample 14 (from Kg Melaban) with the count $1.31 \times 10^8$ cfu/ml, while the lowest count is from Sample 2 (from Sg Melaban) with the count of $1.2 \times 10^6$. From 16 samples which have analysed for the presence of *E.coli*, 15 samples show positive result. The percentage of samples presumptive positive for *E.coli* is 93.75%.

For *V.parahaemolyticus* count, the highest count is from Sample 3 (from Kg. Melaban) with the count $1.65 \times 10^7$. The lowest count is from Sample 10 (from Kg Melaban) with the count $6.5 \times 10^5$. No green colonies were observed from Sg Melaban samples indicating no presence of *V.parahaemolyticus*. Seven out of twelve samples (58.34%) showed the presence of *V.parahaemolyticus*. 
Table 1: Plate Counts for the presence of *E.coli* in Marsh Clams Samples

Source: Sg Melaban (nearby FRST External Laboratory)
Date of sampling: 2 December 2013

<table>
<thead>
<tr>
<th>Sample number</th>
<th>Appearance on EMB agar</th>
<th>Cfu/ml</th>
<th>API 20E test</th>
<th>Possibility/presumptive</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1</td>
<td>Metallic green sheen colonies</td>
<td>$1.7 \times 10^6$</td>
<td>Not done</td>
<td>Positive <em>E.coli</em></td>
</tr>
<tr>
<td>S2</td>
<td>Metallic green sheen colonies</td>
<td>$1.2 \times 10^6$</td>
<td>Not done</td>
<td>Positive <em>E.coli</em></td>
</tr>
</tbody>
</table>
Source: Kg Melaban (populated area)  
Date of sampling: 9 April 2014

<table>
<thead>
<tr>
<th>Sample number</th>
<th>Appearance on EMB agar</th>
<th>CfU/ml</th>
<th>API 20E test</th>
<th>Possibility/ presumptive</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1</td>
<td>Metallic green sheen colonies</td>
<td>TNTC</td>
<td>Not done</td>
<td>Positive <em>E. coli</em></td>
</tr>
<tr>
<td>S2</td>
<td>Metallic green sheen colonies</td>
<td>TNTC</td>
<td><em>E. coli</em> 1 (ID: 99.5%)</td>
<td>Positive <em>E. coli</em></td>
</tr>
<tr>
<td>S3</td>
<td>Metallic green sheen colonies</td>
<td>TNTC</td>
<td><em>E. coli</em> 1 (ID: 99.8%)</td>
<td>Positive <em>E. coli</em></td>
</tr>
<tr>
<td>S4</td>
<td>Metallic green sheen colonies</td>
<td>TNTC</td>
<td>Not done</td>
<td>Positive <em>E. coli</em></td>
</tr>
<tr>
<td>S5</td>
<td>Metallic green sheen colonies</td>
<td>TNTC</td>
<td><em>E. coli</em> 1 (ID: 99.9%)</td>
<td>Positive <em>E. coli</em></td>
</tr>
<tr>
<td>S6</td>
<td>Metallic green sheen colonies</td>
<td>$3 \times 10^6$</td>
<td><em>E. coli</em> 1 (ID: 99.8%)</td>
<td>Positive <em>E. coli</em></td>
</tr>
<tr>
<td>S7</td>
<td>Metallic green sheen colonies</td>
<td>$4 \times 10^6$</td>
<td><em>Serretia odorifera (?)</em></td>
<td>Positive <em>E. coli</em></td>
</tr>
<tr>
<td>S8</td>
<td>Metallic green sheen colonies</td>
<td>$5 \times 10^6$</td>
<td><em>Citrobacter braakii (?)</em></td>
<td>Positive <em>E. coli</em></td>
</tr>
<tr>
<td>S9</td>
<td>Metallic green sheen colonies</td>
<td>$4 \times 10^6$</td>
<td>Not done</td>
<td>Positive <em>E. coli</em></td>
</tr>
<tr>
<td>S10</td>
<td>No metallic green sheen colonies</td>
<td>-</td>
<td>Not done</td>
<td>Negative <em>E. coli</em></td>
</tr>
<tr>
<td>S11</td>
<td>Metallic green sheen colonies</td>
<td>$5.6 \times 10^7$</td>
<td><em>Enterobacter cloacae (?)</em></td>
<td>Positive <em>E. coli</em></td>
</tr>
<tr>
<td>S12</td>
<td>Metallic green sheen colonies</td>
<td>$1.5 \times 10^7$</td>
<td><em>Enterobacter cloacae (?)</em></td>
<td>Positive <em>E. coli</em></td>
</tr>
<tr>
<td>S13</td>
<td>Metallic green sheen colonies</td>
<td>$4.5 \times 10^7$</td>
<td>Possible <em>Enterobacter cloacae</em></td>
<td>Positive <em>E. coli</em></td>
</tr>
<tr>
<td>S14</td>
<td>Metallic green sheen colonies</td>
<td>$1.31 \times 10^8$</td>
<td><em>E. coli</em></td>
<td>Positive <em>E. coli</em></td>
</tr>
</tbody>
</table>

Number of samples analysed: 16
Number of samples presumptive positive for *E. coli*: 15/16

: 93.75%
Table 2: Plate Counts for the presence of \textit{V.parahaemolyticus} in Marsh Clams Samples

Source: Sg Melaban (nearby FRST External Laboratory)
Date of sampling: 2 December 2013

<table>
<thead>
<tr>
<th>Sample number</th>
<th>Appearance on TCBS agar</th>
<th>Cfu/ml</th>
<th>API 20NE test</th>
<th>Possibility/ presumptive</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1</td>
<td>Yellow colonies Plate agar turns yellow</td>
<td>TNTC</td>
<td>Not done</td>
<td>Negative \textit{V. parahaemolyticus}</td>
</tr>
<tr>
<td>S2</td>
<td>Yellow colonies Plate agar turns yellow</td>
<td>TNTC</td>
<td>Not done</td>
<td>Negative \textit{V. parahaemolyticus}</td>
</tr>
</tbody>
</table>