# GROWTH AND SURVIVAL OF *Vibrio parahaemolyticus* RECOVERED FROM CRAB MEAT HOMOGENATE AND INCUBATED AT VARIOUS TEMPERATURES

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***ABSTRACT***
*Vibrio parahaemolyticus isolated from crab was tested to determine the best temperature for growth and survival. Best holding temperature for all inocula of Vibrio parahaemolyticus was observed at 30 $^oC$ . Cell counts increased progressively throughout this incubation temperature and reached a peak of 2.9 x$10^8$ CFU/g after 25 min. with an inoculum level of 2.5 x$10^6$ CFU/g. The bacterium also increased in counts when held at 40$^oC$ at all inoculum levels. When incubated att 50$^oC$, Vibrio parahaemolyticus cells reduced slightly at all inoculum levels and the most notable reduction at this temperature was observed at an inoculum level of 1.5x$10^5$ CFU/g at which only 1.8x$10^3$ CFU/g was isolated after 25 min. The growth of Vibrio parahaemolyticus in crab meat homogenate at 60$^oC$ shows that after 5 min. at an inoculum level of 1.5x$10^5$ CFU/g, only 130 cells were recovered and this level declined with the incubation time. Incubation at 70$^oC$ showed that the cell counts reduced greatly at all inoculum levels. Counts of 71, 168 and 160 cells were obtained for inoculum levels of 1.5 x$10^5$ CFU/g, 2.0 x$10^6$ CFU/g and 2.5 x$10^6$ CFU/g respectively; while at 80 $^oC$, after 5 min of incubation at inoculum level of 1.5x$10^5$ CFU/g, 66 cells were obtained whereas at an inoculum level of 2.0 x$10^6$ CFU/g, 108 cells were recovered after 5 min. incubation. Growth of Vibrio parahaemolyticus at 5$^oC$ showed that viable cell counts fell to 5.8 log CFU/g after 7 days. This decrease in counts continued after 14 day incubation and the lowest viable cell count of 2.9 log CFU/g was recorded after 35 day incubation.*

**Key words**: *Vibrio parahaemolyticus*, crab meat, temperature, bacterial growth.

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## 1. INTRODUCTION

Members of the *Vibrio* genus are a Gram-negative, halophilic bacteria which are indigenous to coastal marine environments (Thompson *et al.*, 2003). Vibrios are among the most abundant culturable bacteria and are found in large numbers in a great many aquatic environments from estuary and coastal waters to the deep sea (Vezzulli et al., 2015). *Vibrio parahaemolyticus* is one of the most common *Vibrio* species associated with illnesses resulting from consumption of raw or partially cooked seafoods worldwide (Kim et al., 2012). *Vibrio parahaemolyticus* is a food-borne pathogen which multiplies rapidly at room temperature and this organism is a major cause of gastroenteritis in areas where the consumption of raw and semi-processed sea food is common (Miles et al; 1997). This organism has been implicated in food poisoning out breaks associated with sea foods and human transmission of *Vibrio parahaemolyticus* is mainly through the consumption of raw, poorly cooked or mishandled seafoods (Mudoh et al., 2014). This organism can cause headaches, diarrhea, fever, gastroenteritis and even life-threatening sepsis (Makino et al., 2003). Human diseases caused by *Vibrio parahaemolyticus* displays as a moderate to severe gastroenteritis although septicemia may occur in individuals with impaired hepatic and renal capacity and in immunocompromised persons (Fernandez-Piquer et al., 2011).

In some developing countries of the world, untreated sewage is dumped into water bodies and this action impacts negatively on the water quality. Sea animals including crabs usually feed by filtering out water and thereby concentrating Vibrios and other pathogenic microorganisms in their bodies. This work aims to test the influence of various incubation temperatures on the growth and survival of

*Vibrio parahaemolyticus* isolated from crab meat homogenate cultured in broth media.

## 2. MATERIALS AND METHODS

*Vibrio parahaemolyticus* was isolated from fresh crab samples. The inoculum was prepared by growing the organism for 24 h at 35°C on Trypticase soy salt agar with 2.5% sodium chloride followed by serial transfer of pure cultures to Trypticase soy salt broth. Crab meat homogenate was prepared for inoculation by blending the meat for 3 min in a Waring blender in a ratio of 1:15 Tripticase soy salt broth at pH 6.8. About 9ml of crab meat homogenate was sterilized by autoclaving at 121°C for 15 min. and added to screw-capped test tubes and placed in shaker incubators for 24 h. at 30°C, 40°C, 50°C, 60°C, 70°C and 80°C. One ml each of broth suspension in test tubes was inoculated with 1.5 $x10^5$ cells/ml, 2.0 $x10^6$ cells/ml and 2.5 $x10^6$ cells/ml of *Vibrio parahaemolyticus* and incubated at each temperature. After each time interval of exposure at each temperature, the tubes were removed, cooled in an ice bath and 0.1ml replicates from each tube was plated on Trypticase soy salt agar and incubated at 35°C for 24 h for cell counts.

For low temperature studies, crab meat homogenates were prepared as described above and added to screw-caped test tubes and inoculated with 1.5 $x10^5$ cells/ml of *Vibrio parahaemolyticus* and the tubes were placed in refrigerated shaker incubators at 5°C. Incubation was done for 35 days while sampling was performed at 7 day intervals. The tubes were thereafter removed from the incubators, placed on the laboratory bench for 2 h and aliquots were withdrawn for total count determination on Trypticase soy salt agar plates.

## 3. RESULTS AND DISCUSSION

The best holding temperature for all inocula of *Vibrio parahaemolyticus* was observed at 30 °C Cell counts increased progressively throughout the incubation temperature and reached a peak of 2.9 $x10^8$ colony forming units/g (CFU/g) after 25 min. cultivation at an inoculum level of 2.5 $x10^6$ CFU/g (Table 1). *Vibrio parahaemolyticus* increased progressively when held at 40°C.

**TABLE 1: Growth of *Vibrio parahaemolyticus* in broth at 30 °C**

| | Inoculum level | | |
|---|---|---|---|
| **Time (min.)** | **1.5 $x10^5$** | **2.0 $x10^6$** | **2.5 $x10^6$** |
| 5 | 1.8 $x10^5$ | 5.0 $x10^6$ | 5.6 $x10^6$ |
| 10 | 1.6 $x10^6$ | 6.2 $x10^6$ | 6.8 $x10^6$ |
| 15 | 2.3 $x10^6$ | 1.2 $x10^7$ | 5.0 $x10^7$ |
| 20 | 3.1 $x10^6$ | 2.0 $x10^7$ | 1.3 $x10^8$ |
| 25 | 2.8 $x10^7$ | 1.8 $x10^7$ | 2.9 $x10^8$ |

At all inoculum levels, cell counts increased with the highest cell counts of 6.3$x10^8$ CFU/g recovered after 25 min. incubation at an inoculum level of 2.5$x10^6$ CFU/g (Table 2).

**TABLE 2: Growth of *Vibrio parahaemolyticus* in broth at 40 °C**

| | Inoculum level | | |
|---|---|---|---|
| Time (min.) | 1.5 $x10^5$ | 2.0 $x10^6$ | 2.5 $x10^6$ |
| 5 | 2.3 $x10^5$ | 2.9 $x10^6$ | 3.5 $x10^7$ |
| 10 | 3.0 $x10^6$ | 3.8 $x10^7$ | 4.0 $x10^7$ |
| 15 | 3.9 $x10^6$ | 4.7 $x10^7$ | 4.7 $x10^8$ |
| 20 | 5.0 $x10^7$ | 4.1 $x10^8$ | 5.6 $x10^8$ |
| 25 | 6.1 $x10^7$ | 5.0 $x10^8$ | 6.3 $x10^8$ |

At 50°C, *Vibrio parahaemolyticus* cells reduced slightly at all inoculum levels in comparison to counts observed at 30 °C and 40 °C. Most notable reduction was observed at an inoculum level of 1.5$x10^5$ CFU/g at which only 1.8$x10^3$ CFU/g was isolated after 25 min. incubation (Table 3).

**TABLE 3: Growth of *Vibrio parahaemolyticus* in broth at 50 °C**

| | Inoculum level | | |
|---|---|---|---|
| Time (min.) | 1.5 $x10^5$ | 2.0 $x10^6$ | 2.5 $x10^6$ |
| 5 | 3.0 $x10^5$ | 4.8 $x10^5$ | 6.5 $x10^6$ |
| 10 | 2.6 $x10^5$ | 3.1$x10^5$ | 3.9 $x10^5$ |
| 15 | 5.0 $x10^4$ | 1.5 $x10^5$ | 2.5 $x10^4$ |
| 20 | 3.2 $x10^4$ | 1.3$x10^4$ | 1.6 $x10^4$ |
| 25 | 1.8 $x10^3$ | 1.2 $x10^4$ | 1.3$x10^4$ |

Growth of *Vibrio parahaemolyticus* in crab meat homogenate held at 60°C is shown in Table 4.

**TABLE 4: Growth of *Vibrio parahaemolyticus* in broth at 60 °C**

| | Inoculum level | | |
|---|---|---|---|
| Time (min.) | 1.5 x$10^5$ | 2.0 x$10^6$ | 2.5 x$10^6$ |
| 5 | 130 | 167 | 185 |
| 10 | 35 | 47 | 61 |
| 15 | 24 | 20 | 20 |
| 20 | 17 | 8 | 7 |
| 25 | 0 | 1 | 1 |

After 5 min. at an inoculum level of 1.5x$10^5$ CFU/g, only 130 cells of *Vibrio parahaemolyticus* were recovered. This level declined progressively and after 25 min. incubation, no *Vibrio parahaemolyticus* colony was isolated. Inoculum levels of 2.0 x $10^6$ and 2.5x$10^6$ CFU/g showed similar trend in *Vibrio parahaemolyticus* cell count reduction. At 70 °C, cell counts reduced greatly at all inoculum levels. Counts of 71, 168 and 160 cells were obtained for inoculum levels of 1.5 x$10^5$ CFU/g, 2.0 x$10^6$ CFU/g and 2.5 x$10^6$ CFU/g respectively. These counts reduced further with incubation time and after 25 min.no *Vibrio* was recovered for all inoculum levels (Table 5).

**TABLE 5: Growth of *Vibrio parahaemolyticus* in broth at 70 °C**

| | Inoculum level | | |
|---|---|---|---|
| Time (min.) | 1.5 x$10^5$ | 2.0 x$10^6$ | 2.5 x$10^6$ |
| 5 | 71 | 168 | 160 |
| 10 | 8 | 15 | 21 |
| 15 | 0 | 1 | 1 |
| 20 | 0 | 0 | 0 |
| 25 | 0 | 0 | 0 |

Incubation of sterile crab meat homogenate with various cell concentrations of *Vibrio parahaemolyticus* held at 80°C is shown in Table 6.

**TABLE 6: Growth of *Vibrio parahaemolyticus* in broth at 80 °C**

| | Inoculum level | | |
|---|---|---|---|
| Time (min.) | 1.5 x$10^5$ | 2.0 x$10^6$ | 2.5 x$10^6$ |
| 5 | 66 | 108 | 135 |
| 10 | 0 | 2 | 2 |
| 15 | 0 | 0 | 1 |
| 20 | 0 | 0 | 0 |
| 25 | 0 | 0 | 0 |

After 5 min of incubation at inoculum level of 1.5x$10^5$ CFU/g, 66 cells were isolated; whereas at an inoculum level of 2.0 x$10^6$ CFU/g, 108 cells were recovered after 5 min. incubation. These levels reduced with time of incubation and after 20 min. no *Vibrio* cell was isolated at all inoculum levels.

Fig 1. shows the survival of *Vibrio parahaemolyticus* at 5°C. *Vibrio* counts of 1.5x$10^5$ CFU/g were used to inoculate sterilized crab meat homogenate and incubated at 5°C. Viable cell counts fell to 5.8 log CFU/g after 7 days. This decrease in viable cell counts continued after 14 day incubation. Lowest cell count of 2.9 log CFU/g was observed after 35 day incubation.

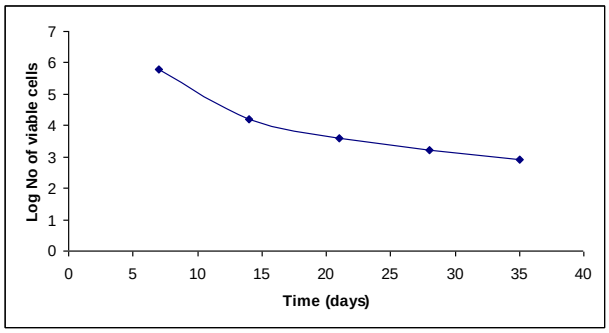


**Figure 1: Survival of *Vibrio parahaemolyticus* in sterilized crab meat homogenate incubated at 5°C**

Temperature is an important factor on food deterioration reactions especially for microbial spoilage since specific growth rate and lag phase are highly dependent on temperature (Giannuzzi et al; 1998). Response to heat increase of the bacterial population is important because certain foods are thermally processed to ensure safety (Breand et al; 1997).*Vibrio* species in general are considered to be relatively sensitive to heat (Hackney and Dicharry 1988) although some Vibrios showed some heat resistance in hot foods (Makukutu and Guthrie, 1986). Vanderzant and Nickelson (1972) reported that a 2x$10^5$ CFU inoculum of *Vibrio parahaemolyticus* survived in shrimp with 3% NaCl after heating at 60°C and 80°C for 15min. Counts of *Vibrio cholerae* 01 inoculated into oysters that were subsequently shucked, canned and heated at 57.2°C for 15 min decreased by 1.2 log units or less, where as the bacterium was not detected when the heat

treatment was extended to 30 min. (Boutin et al., 1982). In contrast, 01 and non-01 strains of *Vibrio cholerae* remained viable in foods held for up to 1 h at 60°C (Makukutu and Guthrie, 1986). *Vibrio cholerae* died off at a rather slow rate when inoculated into shrimp homogenate or crab meat and heat-treated in the temperature range of 48.9-66°C (Liew et al.; 1998). Miles et al, 1997 found the minimum observed temperature for the growth of *Vibrio parahaemolyticus* to be 8.3°C and the maximum was 45.3°C, optimum occurring between 37-39°C. Mudoh et al., (2014) observed the highest total *Vibrio parahaemolyticus* count on day 10 at a storage temperature of 20 °C with about 3.4-7.5 log CFU/g difference from day 0. The authors also reported that total *Vibrio* counts remained at approximately 3.3 log CFU/g or below at 5 and 10°C which indicated that low temperature suppresses *Vibrio* growth. In shellfish, heating to produce an internal temperature of at least 60°C for several minutes appears sufficient to eliminate pathogenic Vibrios (Yang et al., 2009). The effect of storage of *Vibrio parahaemolyticus* in shrimp tissue at –18°C to 10°C and exposed to heat at 60°C and 80°C was investigated by Vandezant and Nickelon (1972). The authors did not recover viable *Vibrio parahaemolyticus* from shrimp homogenate containing 500 cells/ml after heating for 15 min. at 60 °C or 80 °C, or for 5 min. at 100 °C; but when the initial inoculum increased to 2.5 $x10^6$ cells/ml, *Vibrio parahaemolyticus* was recovered from samples heated at 60 or 80 °C. Liston et al., 1971 reported killing times of 30 min. at 60°C while Sakazaki 1973 reported a killing time of 15 min. at 60 °C. Beuchat (1975) reported optimum growth temperature for *Vibrio parahaemolyticus* to be in the range of 35-37 °C while some strains grew well at 44°C. Kaneko and Colwell (1973) observed large numbers of *Vibrio parahaemolyticus* in and on zooplankton particularly when water temperatures rose above 14 °C. The maximum temperatures for the growth of *Vibrio parahaemolyticus* ranged from 42-44°C (Liston et al., 1971), while some strains grew well at 42°C (Twedt et al., 1969) and 43°C (Johnson and Liston, 1973).

Chilling and refrigeration are critical control measures to prevent growth of Vibrios. The survival of *Vibrio parahaemolyticus* in fish homogenate at temperatures below 0°C was studied by Matches et al., (1971). Lowest growth temperature reported for *Vibrio parahaemolyticus* was 5 °C (Beuchat 1973). The minimum growth temperature of *V. parahemolyticus* in broth was reported to be 5°C and 8.3 °C (Miles et al., 1997). Yoon *et al.* (2008) also reported that pathogenic and nonpathogenic *V. parahaemolyticus* grew at 10°C in broth. Burnham *et al.* (2009) observed that five of eight *V. parahaemolyticus* strains and seven of eight *V. vulnificus* strains had increases in viable counts during 10 days of storage at 8°C and 10°C, respectively, with significant differences in growth between the various strains.

## 4. CONCLUSIONS

*Vibrio parahaemolyticus* was found to be very sensitive at temperatures of 60, 70 and 80°C and these sensitivities was time dependent. The bacterium displayed best growth at 30°C and 40°C. At 50°C, cells counts reduced slightly at all inoculum levels and the most notable reduction at this temperature was observed after 25 min. with inoculum level of $1.5x10^5$ CFU/g. When incubated at 5°C, bacterial counts fell to 5.8 log CFU/g after 7 days and the decrease continued after 14 days. The lowest viable cell count of 2.9 log CFU/g was recorded after 35 days. Result of this investigation should assist food scientists to develop guidelines on the storage of crab meat and to formulate regulatory policies on its handling and the information from this study can be used to improve food processing and preservation conditions and reduce exposure to *Vibrio parahaemolyticus*.

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