DETECTION, GROWTH AND SURVIVAL OF *VIBRIO PARAHAEMOLYTICUS* IN SHRIMPS (*PENAEUS MONODON*)

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INTRODUCTION

Vibrio parahaemolyticus is a major food borne pathogen that causes rejection of consignments of shrimps at the export market. There are various methods available for the detection of *V. parahaemolyticus* in food. Quantitative procedures either direct plating or most probable number (MPN) are occasionally used for the enumeration of *V. parahaemolyticus* (Klontz et al. 1993). It has been noted that different methods to detect *V. parahaemolyticus* have different sensitivities (Anon 2001). There are different methods using different enrichments such as alkaline peptone water (APW), alkaline salt peptone water (ASPW), glucose salt teepol broth (GSTB), saline glucose sodium dodecyl sulphate peptone water (GST) etc. for selective isolation of *V. parahaemolyticus*. These methods are designed for the detection of normal cells of *V. parahaeolyticus*. The efficiency of these methods to detect sublethally stressed cells needs to be evaluated. One objective of this study was to find the most recoverable method for quantitative detection of freeze stressed *V. parahaemolyticus* in shrimps. It is important to study the growth and survival rates of *V. parahaemolyticus* in relation to shrimp applicable to industry conditions.

MATERIALS AND METHODS

Comparison of methods

Brackish water shrimps (*Penaeus monodon*) which are commonly known as black tiger shrimps were used in this study. Eight different standard methods for testing of *V. parahaemolyticus* were evaluated in this study. Shrimp homogenates (1:1) were spiked with 2 strains (Vp-1 and Vp-2) of *V. parahaemolyticus* at two inoculation levels 10^3 to 10^4 (low) and 10^5 to 10^6 (high) cfu/ml separately. After 24h frozen storage at -20^{0}C samples were analyzed separately following standard procedures (ISO 1990; Andrews 1992 (FAO); Aus/NZ Standard 1997; Charles & Angelo 2001 (APHA) for quantitative detection of *V. parahaemolyticus*.

Growth study

Shrimp homogenates were inoculated with *V. parahaemolyticus* to obtain approximately 10^3 10^4cfu/g cells separately for 2 strains (Vp-1 and Vp-2) and incubated at 37^{0}C and 30^{0}C. Samples were with-drawn at 1 hr intervals and enumerated on thiosulfate citrate bile salts sucrose (TCBS) agar.

Freeze thaw experiments

Shrimp homogenates were inoculated with stationary phase cells of *V. parahemolyicus* (for 2 strains separately) approximately at 10^8 cells per g. This was transferred in 10g

portions in dilution bottles and frozen to -20°C. Three samples were analysed in each occasion in 0, 1, 3, analysis was MPN in ASPW (ISO 1990).

RESULTS AND DISCUSSION

Three tube MPN method using enrichment ASPW gave significantly higher recovery of 31.6%, 30.5% and 32.4%, 28.2 for 2 strains at high and low inoculation level respectively (Figure 1). Based on these results, ASPW enrichment broth was more efficient than other media for recovery of freeze stressed *V. parahaemolyticus* in shrimps.

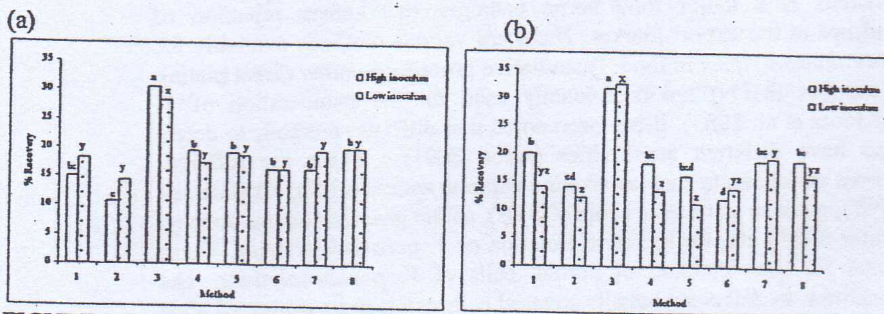

FIGURE 1 Percentage Recovery of Freeze Stressed *V. parahaemolyticus* (a) Vp-1 (b) Vp-2
1) ISO:ASPW-TCBS, 2) ISO:ASPW-SDS, 3) ISO:ASPW- MPN, 4) ISO:GST-TCBS, 5) ISO:GST-SDS, 6) FAO: GSTB-MPN, 7) Aus/NZ: APW-MPN, 8) APHA: APW-MPN
Bars sharing the same letter do not differ significantly from each other at the P>0.05 (a-d for high inoculum and x-z for low inoculum)

Generation times for *V. parahaemolyticus* (Vp-1 and Vp-2) in shrimp homogenate at 37°C were10 min and 8.4 min respectively. Generation times at 30°C were 11.4 min and 12.5 min respectively (Figure 2). *V. parahaemolyticus* is a bacterium with one of the fastest generation time of 8-13 minutes, so that low levels of *V. parahaemolyticus* in raw shrimp could be a potential health hazard if the factors for growth of the organism are favourable.

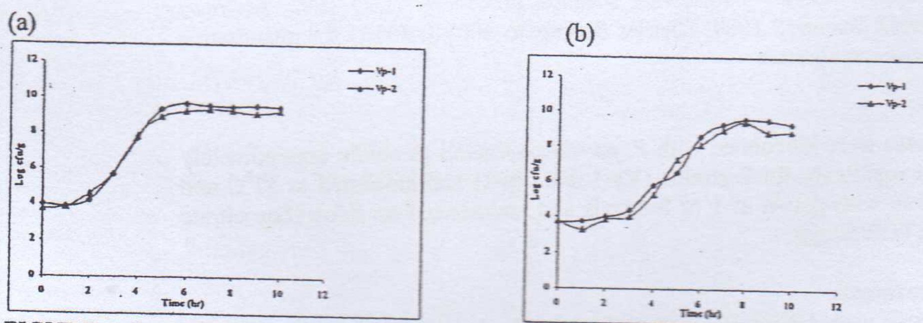

FIGURE 2 Growth of *V. parahaemolyticus* In Shrimp Homogenate At 37°C (a) and 30°C (b)5-6 $\log_{10}$ MPN reduction of *V. parahaemolyticus* in shrimp homogenate has been observed after three weeks of frozen storage (Figure 3). It can be concluded that although

freezing significantly inactivated high numbers of *V. parahaemolyticus* in shrimp, it cannot be relied upon as a method to eliminate *V. parahaemolyticus* in shrimp.

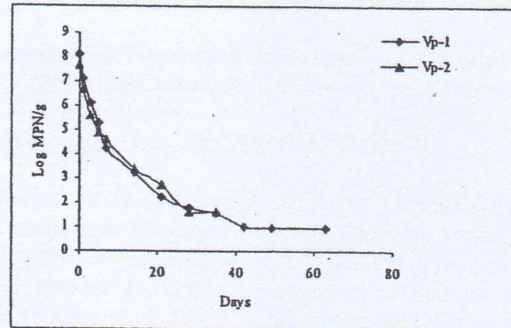

FIGURE 3 Survival of *V. parahaemolyticus* Under Frozen Condition

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