Fishery Technology
2010, Vol. 47(2) pp: 179 - 184

Growth Kinetics and Survival of Urease positive and negative Strains of *Vibrio parahaemolyticus*

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Two each of urease positive and negative *Vibrio parahaemolyticus* strains along with their respective type strains were used for studying the growth kinetics and survival at various temperatures, NaCl concentrations and pH. Maximum growth was obtained at 37°C for both urease positive and negative isolates. The urease postive and negative strains were found to grow only at pH 7 and 9 among the pH's tested. Urease negative isolates were found to grow between 3% and 8% NaCl while urease positives could grow between 3% and 10% NaCl.

Keywords: *Vibrio parahaemolyticus*, **seafoods, survival, urease positive, growth kinetics**

Vibrio parahaemolyticus occurs as natural flora in zooplankton, coastal fish and shellfish (Colwell *et al.*, 1984). It is found to cause food-borne gastroenteritis and septicemia (Anderson *et al.*, 2004). Human infections caused by *V. parahaemolyticus* have increased globally in the recent years (Lee *et al.*, 2001) and in India it has almost doubled in the last 5 years (Chowdhury *et al.*, 2000). It grows within a temperature range typical of mesophiles, with a minimum growth temperature of 9 to 10°C, and an optimum between 35 and 37°C (Joseph *et al.*, 1982). *V. parahaemolyticus* is seldom isolated when the temperature of the seawater drops below 13–15°C (Kaneko & Colwell, 1974). The lowest temperature reported for the growth of the organism in laboratory media was 5°C (Beuchat, 1973).

Almost all the clinical isolates of *V. parahaemolyticus* display a haemolytic pattern called the Kanagawa phenomenon (KP) on wagatsuma agar (Sakazaki *et al.*, 1968). Huq *et al.* (1979) reported a diarrhoeal disease outbreak caused by a strain of urease positive *V. parahaemolyticus*. This biotype was unrecognized previously with regard to pathogenicity and it is found to cause severe gastroenteritis (Kelly & Stroh, 1989), which is generally associated with the KP negative strains of *V. parahaemolyticus* (Okuda *et al.*, 1997).

The microbial food safety and quality assurance in the seafood samples necessitate the study on growth kinetics of this pathogen. The study was aimed to determine the comparative growth kinetics at various temperatures, NaCl concentrations and pH of urease positive and negative *V. parahaemolyticus* isolated from seafood samples collected from markets.

Materials and Methods

Growth kinetics of two urease positive and negative *V. Parahaemolyticus* isolates were studied at various temperature, salinity and pH using tryptic soy broth (TSB), (Difco, USA). Urease negative (MTCC-451) and urease positive (NCMB-1902) type strains along with the isolates were used for carrying out the study. Cultures grown in

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TSB broth (10 ml, 3% NaCl, w/v) at 37°C for 24 h were harvested by centrifugation, and the cells were washed and with 3% NaCl (w/v) to get a inoculum concentration of approximately 10^2 cell/ml in TSB.

A set of 50 ml TSB was inoculated in duplicate with an initial inoculum of 10^2 cfu/ml of young *V. parahaemolyticus* suspended in saline. The flasks were incubated at various temperatures; 0 ± 1°C, 8 ± 1°C, 30 ± 1°C (RT ± 1), 37 ± 1°C, 42 ± 1°C, and 56 ± 1°C and the bacterial growth was monitored.

To the TSB, NaCl (Analytical grade) was added to get different concentrations; 0 (without NaCl), 3, 6, 8 and 10% NaCl (w/v). The pH of the medium was adjusted to 7.3 + 0.1.

TSB broth was adjusted at different pH ranges (3, 5, 7, 9, and 12) using HCl (2N) and NaOH (2N). The medium (50 ml) was dispensed in flasks, and sterilized by autoclaving at 121°C for 15 min.

From each flask, 20 µl cultures were drawn aseptically at regular intervals, and quantitatively analyzed by the drop plate method (Miles & Misra, 1938) on TCBS agar plates to record the counts and purity of the isolates. Each test was conducted in duplicate and bacterial growth was monitored at 24, 48, 72, 192, 384, 720, and 1080 h of incubation and average counts were recorded at specified intervals.

Results and Disscussion

Growth pattern of representative cultures of *V. parahemolyticus* at 0, 8, 30, 37, 42, and 56°C showed maximum growth at 37°C for both urease positive (Fig. 1A) and negative (Fig. 3A) isolates. Sudha *et al.* (1998) reported the optimum temperature as 37°C for growth of many species of vibrios. Urease positive cultures were found to grow better at 42°C than at 30°C (Fig. 1A and Fig. 2A) between 72 and 720 h. Heterogeneity among the *Vibrio* strains is widely reported,

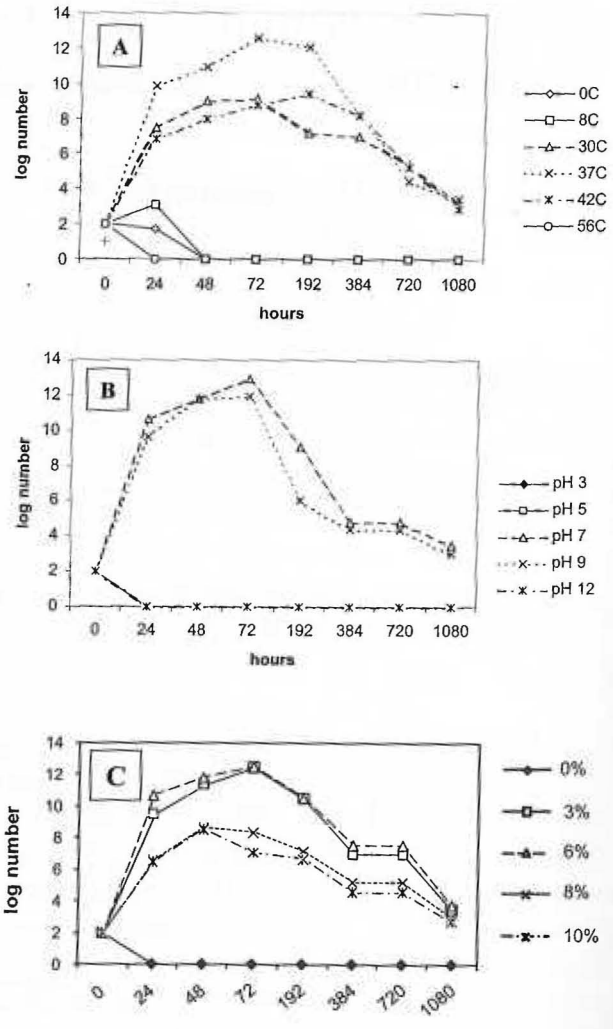

Fig. 1. Growth and survival of urease positive *Vibrio parahaemolyticus* (A: Temperature, B: pH and C: Salinity)

especially in case of metabolic properties (Hoi *et al.*, 1998). For all the test cultures, growth was observed at 30, 37, and 42°C. At 30 and 37°C urease positive *V. Parahaemolyticus* showed characteristic growth curve while at 8°C there was no growth on TCBS media after 24 h while urease negative cells showed no growth after 48 h (Fig. 1A, 2A, 3A and 4A). Survival of *V. parahaemolyticus* at refrigerated temperature (0°C) revealed minimum or no growth (Sudha *et al.*, 1998). In this study, initial inoculum size was 10^2cfu/ml and scanty growth (turbidity) was observed after 24 h but after 48 h at 0°C no colonies were recovered. At this temperature, cells might have attained dormancy, further leading to a state called Viable But

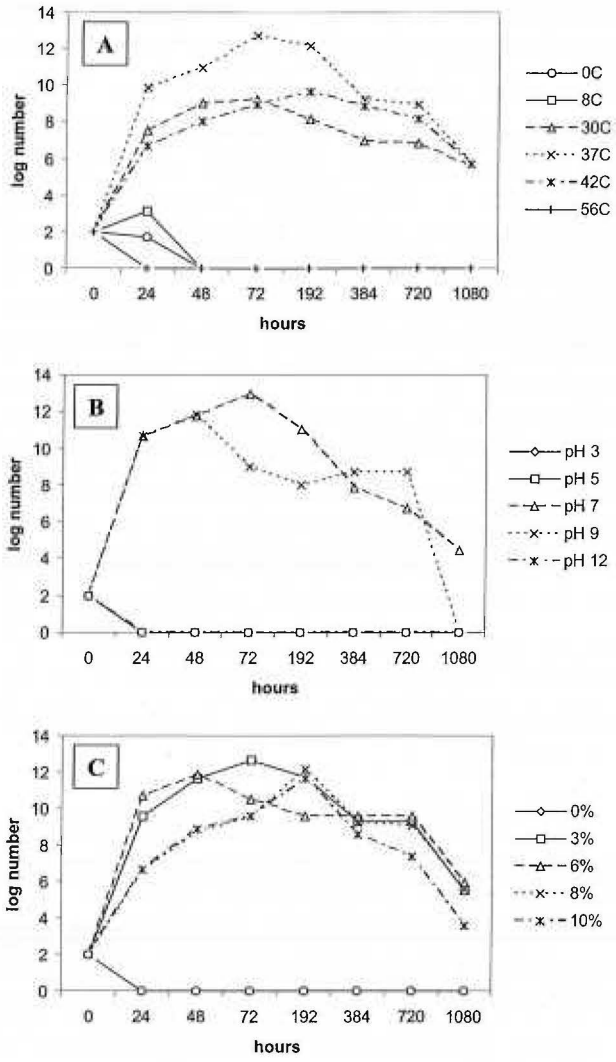

Fig. 2. Growth and survival of urease positive *Vibrio parahaemolyticus* (NCMB - 1902) (A: Temperature, B: pH and C: Salinity).

Non-Culturable (VBNC) as time proceeds. This observation corroborates with the earlier reports (Venugopal *et al.*, 1999). Even though there was no visible growth in cultures at 0 and 8°C, existence of viable cells reported after long-term refrigeration (Venugopal *et al.*, 1999), can be explained due to the formation of VBNC state. Mizunoe *et al.* (2000) reported that in VBNC state *V. parahaemoyticus* could survive for a minimum of 12 days at 4°C, which suggests that the hazard exists even when the products are stored at low temperature.

Growth pattern of selected urease positive and negative isolates of *V. parahaemolyticus* at various pH ranging from 3 to 12 was studied. In all the tested pHs, visible growth was noted only at pH 7 and 9. It is evident from Fig. 1B that urease positive isolate, showed highest count at pH 7 and 9 at 72 h of incubation while drastic reduction was noticed after 72 h. Similar pattern of growth was noticed in the urease positive type strain (NCMB - 1902) but a difference of two-log count in growth was observed between pH 7 and 9 and also growth was found to be slightly higher at pH 9 at the end of 720 h (Fig. 2B). In the case of urease negative isolates highest count was noticed at 48 and 72 h of test period after which it was found to be declining (Fig. 3B, 4B). Information on the pattern of growth at different physico-chemical parameters will help to follow the fate of vibrios in the

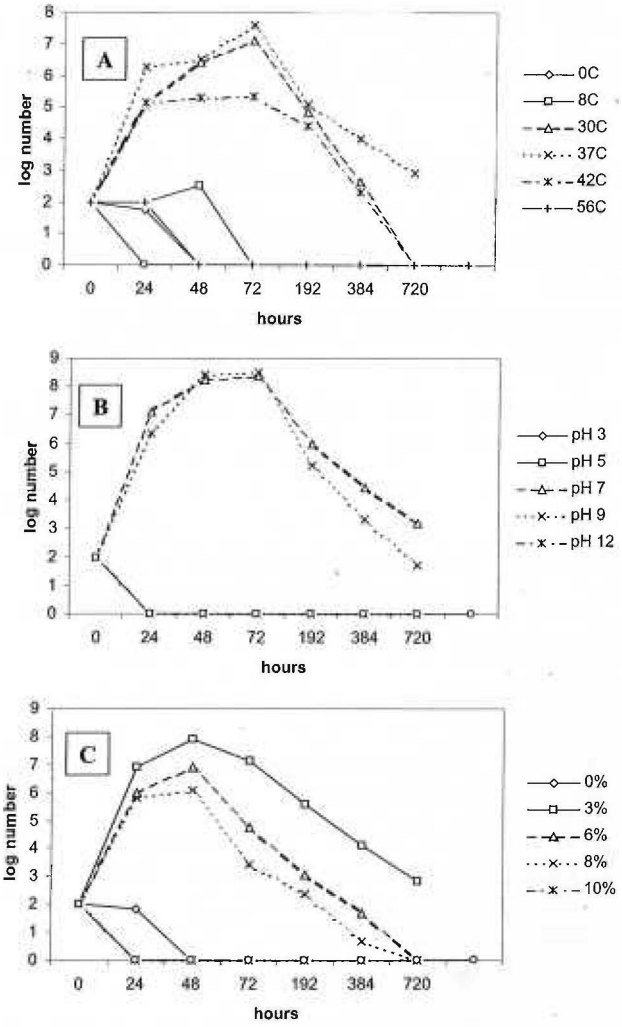

Fig. 3. Growth and survival of urease negative *Vibrio parahaemolyticus* (A: Temperature, B: pH and C: Salinity).

seafoods. While manipulating these invitro patterns of growth to chalk out better preservation technique it should also be considered that in natural conditions the growth and sensitivity will be influenced by many other chemical and biological factors.

Growth patterns of *V. parahaemolyticus* at various salt concentrations ranging from 0 to 10% were studied. In urease negative isolates and type culture (MTCC - 451) the lowest NaCl level at which growth was observed was 3% (w/v) and the highest was found to be 8% (Fig. 3C, 4C). In urease positive type culture (NCMB - 1902) higher counts were noticied at 3% NaCl at the end of 72 h (Fig. 2C). The urease positive isolates, could grow at as high as 10% NaCl (w/v) concentration until 48 h (Fig. 1C). This study is in agreement with Sudha (2001) that *V. parahaemolyticus* could tolerate upto 10% NaCl (w/v) while optimum growth was apparent at 3% level. Sakazaki (1968) recommended, growth in 10% NaCl as one of the methods for distinguishing *V. alginolyticus* (+) from *V. parahaemolyticus* (-). Results of the present study and those reported by Twedt *et al.* (1969), and Vanderzant & Nickelson, (1972) showed that growth of *V. parahaemolyticus* in media with 10% NaCl was variable and this characteristic should not be used as a key in identification. Generally salting above 15% NaCl (w/v) was reported to be lethal for all *Vibrio* species, which is considered as a safe method of preservation of seafoods from the risk of pathogenic *V. parahaemolyticus* (Twedt *et al.*, 1969).

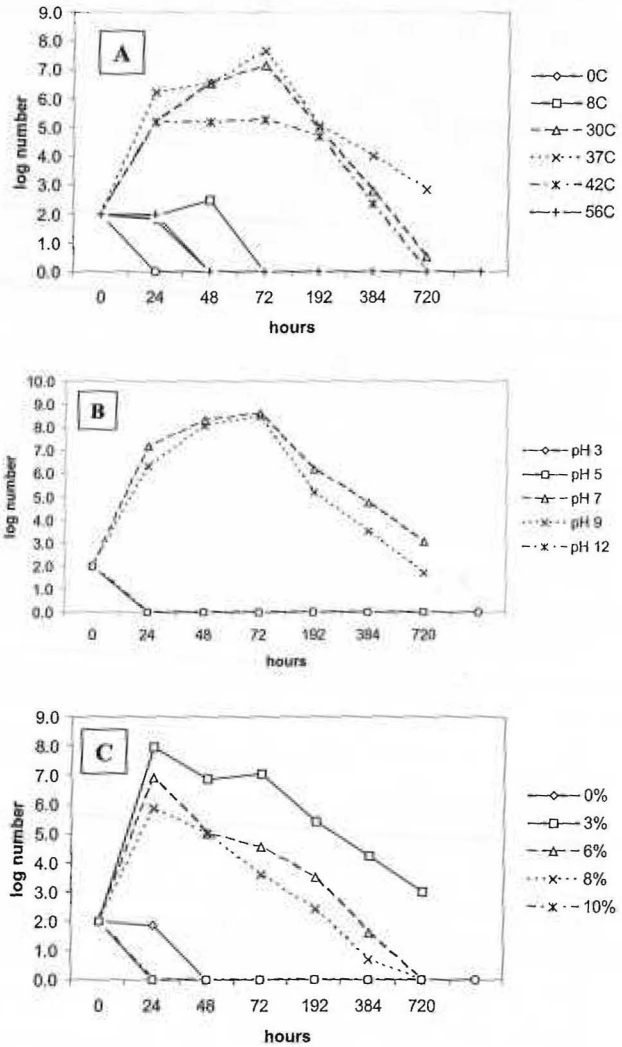

Fig. 4. Growth and survival of urease negative *Vibrio parahaemolyticus* (MTCC - 451) (A: Temperature, B: pH and C: Salinity).

The present study showed that urease positive and negative *V. parahaemolyticus* isolates grow between 30 and 42°C, pH between 7 and 9 and salt concentration between 3 and 8. But urease positive *V. parahaemolyticus* and urease positive type culture (NCMB - 1902) could grow even at 10% NaCl concentration.

The authors are grateful to Dr. K. Devadasan, former Director, Central Institute of Fisheries Technology, Cochin and to Prof. (Dr.) Mohan Joseph Modayil, former Director, Central Marine Fisheries Resarch Institute for providing necessary facilities and encouragement for carrying out the study.

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