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Effect of Temperature on Growth and Biochemical Properties of Selected Species of Pathogenic Vibrio

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Growth pattern of *Vibrio parahaemolyticus, V. vulnificus, V. alginolyticus, V. mimicus* and *V. harveyi,* isolated from fish from the Arabian Sea off Cochin was studied at 4, 15, 28±2 (room temperature), 37 and 42°C in Trypticase Soy Broth with 3% NaCl. Optimal temperature for growth of all species was 37°C. *V. parahaemolyticus* and *V. alginolyticus* exhibited growth at 42°C also. All the species studied grew slowly at 15°C, but failed to grow at 4°C. Twenty important biochemical reactions were also studied at the above temperatures. Observations of the biochemical activity were in accordance with the growth except at 15°C where, although there was growth, most of the biochemical reactions gave negative results.

Key words: Pathogenic vibrio, growth, biochemical reactions

Pathogenic vibrios are hazardous in seafood. Karunasagar *et. al.* (1990) showed the occurrence of *Vibrio parahaemolyticus, V. vulnificus, V. cholerae* and *V. mimicus* in fish caught along the Karnataka coast. The ability of vibrios to survive and remain infective in various environments has been extensively studied. *V. parahaemolyticus* and *V. vulnificus* are the most widely studied species (Kasper and Tamplin, 1993; Boutin *et. al.,* 1985; Covert and Woodburn, 1972). However, only very limited information is available on the growth, metabolic activities and survival of other pathogenic marine vibrios. The effect of temperature on growth and metabolic activities of selected pathogenic marine vibrios was studied and is reported.

Materials and Methods

V. parahaemolyticus, V. alginolyticus, V. vulnificus, V. mimicus and *V. harveyi* isolated from fresh marine fish caught from the Arabian Sea off Cochin and maintained as stock culture at the Central Institute of Fisheries Technology were used in the study. These cultures were maintained in Trypticase Soy agar with 1.5 % sodium chloride at room temperature (RT), 28±2°C. Inocula for growth studies were prepared by growing cultures for 18 h in 3% NaCl-Trypticase Soy Broth (TSB) at room temperature. Broth cultures were centrifuged at 3000 rpm for 30 min, washed with 3% NaCl solution and resuspended in the same diluent to obtain a cell suspension with optical density (OD) of 0.2 (approximately 10^7 - 10^8 cells/ml) at 650 nm.

Trypticase Soy Broth with 3% NaCl was used as the basal medium for the study. Temperatures of incubation were 42°C (serological water bath), 37°C (air incubator), 15°C and 4°C (BOD incubator (Yoma, Indian Instruments Mfg. Co., Calcutta) and

room temperature. Samples were drawn periodically until the growth became almost stationary. Growth was monitored by measuring the optical density in a Spectronic 20+ Spectrophotometer (Milton Roy, Rocheston, New York) at 650 nm.

Effect of temperature on the presence of enzymes arginine dehydrolase, lysine decarboxylase, ornithine decarboxylase, gelatinase, production of acid and gas from arabinose, glucose cellobiose, mannitol and sucrose and reactions on Triple Sugar Iron agar were studied as per standard methods (Elliot *et. al.*, 1995). Inoculum for the biochemical studies was prepared following the same procedure as for the growth study. Cell suspension was prepared in sterile distilled water and inoculated in Tryticase Soy broth containing 0, 3, 6, 8 and 10 % sodium choloride for studying the effect of NaCl on growth. Incubation temperatures were the same as those for the growth study. Cultures showing negative results were incubated at the respective temperature upto 10 days.

Results and Discussion

Effect of temperature of incubation on the growth of *Vibrio* species are presented in Figs 1 to 5. Plotting optical density against the incubation time gave elongated sigmoid curves for *Vibrio harveyi* at incubation temperature 37°C (Fig. 1). At 15°C, the bacterial culture started growing only after 24 h (lag phase) and then maintained a steady increase in the growth rate. Cultures kept at 4 and 42°C showed no growth suggesting the inability of the culture to grow at these two temperatures.

Patterns of growth of *V. alginolyticus* at different temperatures are presented in Fig. 2. The culture showed maximum growth at 37°C followed by RT. It did not grow at 4°C. Unlike *V. harveyi, V. alginolyticus* culture incubated at 42°C showed a pattern of growth parallel to the growth at 37°C, although at a much slower rate. Optimal growth temperature for *V. mimicus* was 37°C (Fig. 3). There was no growth at 4 and 42°C, whereas at 15°C growth was very slow till 72 h and fast thereafter.

Growth of *V. parahaemolyticus* (Fig. 4) at room temperature followed the same pattern as in the other species. The maximum growth was at 37°C. Growth at 15 and 42°C was very slow and showed same pattern upto 3rd day, after which an abrupt increase was observed at 15°C. There was no growth at 4°C corroborating the observation that V. parahaemolyticus is sensitive to 4°C (Covert and Woodburn, 1972; Johnson and Liston, 1973; Kelly, 1982).

For *V. vulnificus* (Fig. 5), growth at 37°C was slow for the first two days, then showed a rapid increase and reached the peak on 6th day. This observation is in agreement with the findings of Kelly (1982) in *in vitro* studies. The growth at RT was fairly fast while at 15°C it was very slow.

Survival of *V. vulnificus* has been reported to be optimum at temperatures between 13 and 22°C in 10 ppt sterile sea water and temperature outside this range (25 to 42°C) reduced the time of survival (Oliver, 1981). Reduced growth in 30 ppt saline at 15°C and fairly good growth at 37°C and RT indicated in the present study do not agree with the above observation. The enhanced growth and metabolic activity of the isolate at RT and 37°C could be attributed to its tropical origin. Earlier studies

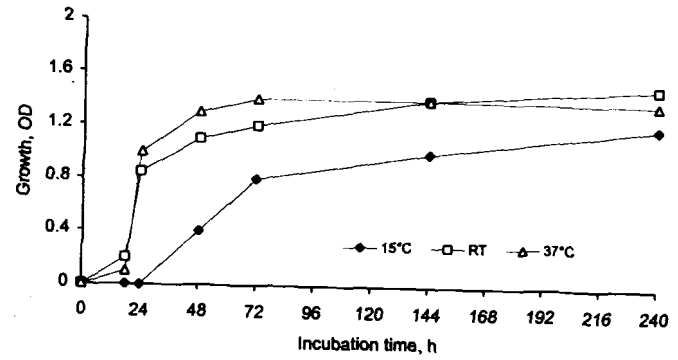

Fig. 1

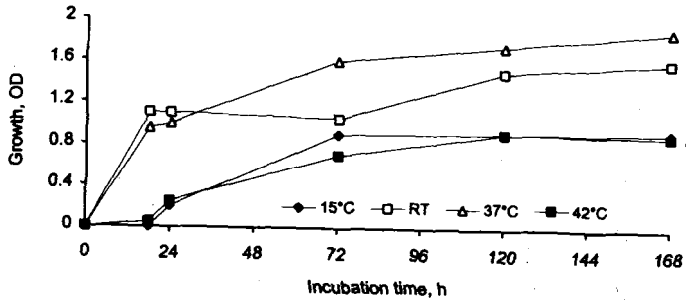

Fig. 2

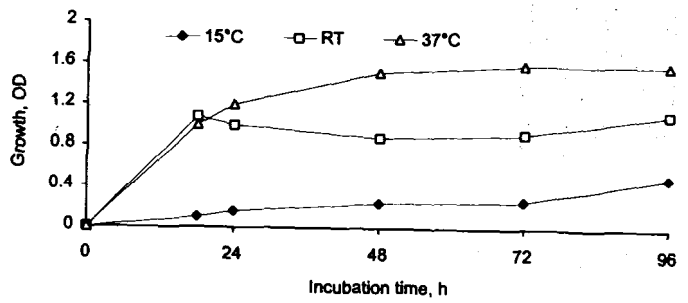

Fig 3

Effect of temperature of incubation on the growth of V. harveyi (Fig. 1), *V. alginolyticus* (Fig. 2) and *V. mimicus* (Fig. 3)

(Oliver, 1981; Kaspar and Tamplin, 1993) also reported that *V. vulnificus* showed substantial decrease in growth with complete elimination of viable cells within 24 h at 4°C probably due to cold shock injury. This conclusion agreed with the present observation of complete elimination of viable cells within 6 days of incubation at 4°C as evidenced by direct plating of the cell suspension (unpublished data).

In the early stages of growth i.e., upto 18 h a slightly higher optical density was observed at room temperature than at 37°C for all test cultures. However,

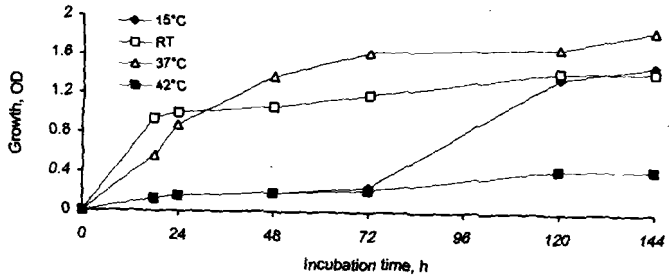

Fig 4

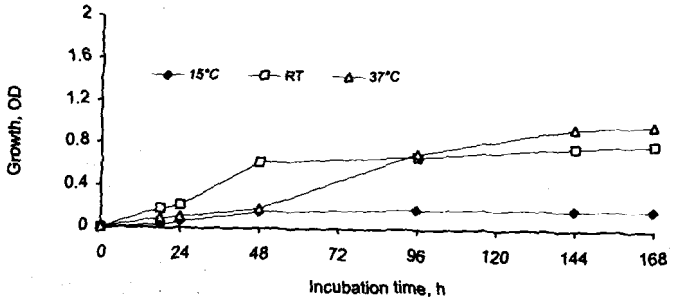

Fig 5

Effect of temperature of incubation on *V. parahaemolyticus* (Fig. 4) and *V. vulnificus* (Fig. 5)

after 24 h the growth rate was slightly higher at 37°C than at room temperature. All test species showed 37°C as optimal growth temperature, thereby confirming their pathogenic nature. None of the *Vibrio* spp. could survive at refrigerated temperatures. *V. alginolyticus* and *V. parahaemolyticus* were able to tolerate higher temperature.

The results of biochemical studies are given in Table 1. Observations of biochemical reactions at different temperatures are in accordance with the results obtained in the growth study. All test cultures were biochemically active at room temperature and 37°C and the results were comparable. The discrepancies noted between biochemical reactions at room temperature and 37°C were few. *V. mimicus* was mannitol positive at room temperature and mannitol negative at 37°C. Similar observation was noted for glucose fermentation of *V. harveyi*. These results point to the necessity of selecting the optimum incubation temperature.

V. parahaemolyticus is reported tolerant to 6 and 8 % NaCl at 37°C (Alsina and Blanch, 1994; Elliot *et. al.*, 1995). But at 15°C, it was found sensitive to both 6 and 8 % NaCl. Same effect can be seen for *V. alginolyticus* at 10% and *V. vulnificus* at 6 % NaCl. The present observation of high sensitivity at higher salt concentration at low temperature can be attributed to the enhanced cold stress injury. High salt concentrations are found to enhance the cold stress injury occurring to the cells (Oliver, 1981; Covert and Woodburn, 1972; Johnson and Liston, 1973).

Table 1. Effect of temperature on biochemical reactions of *Vibrio* spp.

Biochemical reactions	*V. parahaemolyticus*					*V. alginolyticus*					*V. vulnificus*					*V. mimicus*					*V. harveyi*				
Temperature, °C	4	15	RT	37	42	4	15	RT	37	42	4	15	RT	37	42	4	15	RT	37	42	4	15	RT	37	42
Arginine dehydrolase	*	*	-	-	-	*	*	-	-	-	*	*	-	-	*	*	*	+	+	*	*	*	-	-	*
Lysine decarboxylase	*	*	+	+	+	*	*	+	+	+	*	*	+	+	*	*	*	+	+	*	*	*	+	+	*
Ornithine decarboxylase	*	*	+	+	+	*	*	-	-	-	*	*	+	+	*	*	*	+	+	*	*	*	+	+	*
Gelatinase	*	-	+	+	+	*	-	+	+	+	*	-	+	+	*	*	-	+	+	*	*	-	+	+	*
Voges proskauer	*	-	-	-	-	*	-	+	+	+	*	-	-	-	*	*	-	-	-	*	*	-	-	-	*
Indole	*	-	+	+	+	*	-	+	+	+	*	-	+	+	*	*	-	+	+	*	*	-	+	+	*
Glucose..gas	*	-	-	-	-	*	-	-	-	-	*	-	-	-	*	*	-	-	-	*	*	-	-	-	*
Growth in 0% NaCl	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
3% NaCl	*	+	+	+	+	*	+	+	+	+	*	+	+	+	*	*	+	+	+	*	*	*	+	+	*
6% NaCl	*	*	+	+	+	*	+	+	+	+	*	*	+	+	*	*	+	+	+	*	*	*	+	+	*
8% NaCl	*	*	+	+	*	*	+	+	+	+	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
10% NaCl	*	*	*	*	*	*	*	+	+	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Acid from																									
Arabinose	*	-	+	+	-	*	-	-	-	-	*	-	-	-	*	*	-	-	-	*	*	-	-	-	*
Cellobiose	*	-	-	-	-	-	-	-	-	-	*	-	+	+	*	*	-	-	-	*	*	-	-	-	*
Glucose	*	-	+	+	-	*	-	+	+	-	*	-	+	+	*	*	-	+	+	*	*	-	+	-	*
Mannitol	*	-	+	+	+	*	-	+	+	-	*	-	-	-	*	*	-	+	-	*	*	-	-	-	*
Sucrose	*	-	-	-	-	*	-	+	+	+	*	-	-	-	*	*	-	-	-	*	*	-	-	-	*
Triple sugar iron agar	*	*	KA	KA	KA	*	*	AA	AA	AA	*	*	KA	KA	*	*	*	KA	KA	*	*	*	KA	KA	*
Simmons citrate	*	*	+	+	+	*	*	+	+	*	*	*	+	+	*	*	*	-	-	*	*	*	-	-	*
NO_3 production	*	-	+	+	+	*	-	+	+	+	*	-	+	+	*	*	-	+	+	*	*	-	+	+	*

+ Positive reaction; - Negative reaction; * No growth; KA Alkaline slant acid butt; AA Acid slant acid butt; RT Room temperature

All the tested species showed growth at 15°C but were unable to exhibit some important biochemical activities at this temperature, viz. amino acid decarboxylation, sugar fermentation, gelatinase production, indole production and citrate utilization. *V. vulnificus*, *V. mimicus* and *V. harveyi* were found metabolically inactive at 4 and 42°C.

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