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Influence of Temperature on The Chemotaxis of *Vibrio cholerae* 0139 Towards *Anabaena* sp.

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Abstract: The present investigation was designed to assess the role of temperature, an important environmental parameter on the chemotaxis ***Vibrio cholerae*** 0139 towards homogenates of ***Anabaena*** sp., amino acids (L-serine and L-alanine) and a carbohydrate (D-glucose). A series of temperature (4, 25, 37 and 44°C) was assessed in a capillary tube technique to observe its effect on the chemotaxis of ***V. cholerae*** 0139 at six different incubation periods of 15, 30, 45, 60, 75 and 90 min. The highest effect was observed at 25°C irrespective of the agents tested followed by 37 and 44°C. However, no significant chemotactic movement was recorded at 4°C. Among the various types of attractants used, maximum response was observed with L-serine as compared to L-alanine and D-glucose and ***Anabaena*** sp. homogenates.

Key words: Chemotaxis, ***Vibrio cholerae***, Blue-green algae

Introduction

Bangladesh is an endemic zone for cholera, where cholera epidemics occur twice a year with a regular seasonality (Glass ***et al.***, 1982). Cholera, a dreadful diarrhoeal disease caused by both the serotypes of ***Vibrio cholerae*** 01 and 0139, characterized by the passage of voluminous stool of rice water that rapidly leads to dehydration. Hypovolemic shock, acidosis and death can ensue both in adult and in children within 12-24 h in severe cases (Kaper ***et al.***, 1979). Various reports have been published on the isolation of ***V. cholerae*** 0139 from various aquatic and clinical sources in different parts of the world including Bangladesh (Islam ***et al.***, 1994b; Lee ***et al.***, 1996; Pokhrel and Kubo, 1996; Sengupta ***et al.***, 1994; Tay ***et al.***, 1994). During the inter-epidemic period, ***V. cholerae*** becomes non-cultivable on routine culture media (Islam ***et al.***, 1994a; Khan ***et al.***, 1984). It has been reported that in the small intestine of human body, both chemotaxis and adhesion act as an important prerequisite for attachment and colonization of ***V. cholerae*** (Jones, 1952; Freter, 1981). But it is a great mystery to the scientists to define the cause of association of such pathogen with a large number of aquatic flora and fauna. Islam ***et al.*** (1994a)

suggested that chemotaxis plays an important role in such association. Such phenomenon imparts momentous role for motile organisms to search for their carbon and energy sources in relationship to develop micro-habitats in the ecological system (Pearl, 1978; Pearl and Gallucci, 1985). As ***V. cholerae*** 0139 has been referred to be the causative agent of eighth pandemic of cholera (Swerdlow and Ries, 1993) and in Bangladesh there were 107,000 cholera cases to be reported by the end of March (1993), among which 1473 deaths occurred (Albert, 1994) therefore, it was utmost importance to find out its reservoir in the environment. ***V. cholerae*** 0139 was isolated from various aquatic components in Bangladesh including snail, duckweed, water, fish (Islam, 1994b) and this pathogen also has been reported to be survived as both culturable and non-culturable form in association with cyanobacteria (***Anabaena*** sp., ***Nostoc*** sp. and ***Hapalosiphon*** sp.) in laboratory microsome (Islam ***et al.***, 1996). Chemotactic movement of ***V. cholerae*** 0139 towards homogenates of various aquatic flora and fauna, among them ***Anabaena*** sp. shows the strongest response (Mizanur ***et al.***, 2001; Mizanur ***et al.***, 2002). It has been reported that both motility and chemotaxis of bacteria are influenced by a number of physico-chemical parameters of the respective environment (Adler, 1969). However, little is known about temperature, one of the most important environmental factors, which might influence the chemotaxis of ***V. cholerae*** 0139 towards the homogenates of ***Anabaena*** sp. as well as to some chemicals. Since it is difficult to assess the effect of environmental factors on microorganisms under in situ conditions, the present study envisages undertaking research in laboratory controlled conditions to assess the role of various components in relation to the chemotactic attachment of ***V. cholerae*** 0139.

Materials and Methods

Microorganisms growth condition and chemicals

An environmental isolate of ***V. cholerae*** 0139 (DWP-341) (Islam ***et al.***, 1996) isolated from duckweed, was obtained from the Environmental Laboratory, ICDDR, B. Purity and identity of the strain was reconfirmed by cultural, biochemical and serological tests. The strains of ***V. cholerae*** serotype 0139 was maintained in T1N1 soft agar (Trypticase [BBL] 1%, NaCl 1% and agar 0.8%) in vials added with paraffin oil at room temperature and grown at 37°C in trypticase soy broth (TSB) (BBL). The vibrio selective medium used was thiosulfate citrate bile sucrose (TCBS) agar (Difco Laboratories, USA). The soft agar used for assaying the motility of vibrio strain was TSB added with 0.3% agar. The ***Anabaena*** sp. used in this study was grown and maintained in pure culture on BG-11 medium (Valdis and Laurence, 1986) at room temperature. The chemotaxis and washing medium used in this study was phosphate-buffered saline (PBS)(pH 7.4, NaCl, 0.8%). All compounds tested in the chemotaxis assay were purchased from Sigma Chemical Co. (USA). Glass-distilled deionized water was used in all experiments.

Preparation of homogenates of *Anabaena* sp.

A defined amount (0.05 g) of ***Anabaena*** sp. cultured in BG-11 medium was picked up with a sterile spatula from the culture flask, washed three times with previously autoclaved PBS and finally mixed with 1 ml of PBS to make 5% suspension. It was then homogenized using a glass-made hand homogenizer and finally with an ultrasonicator. This homogenized solution was then diluted to make desired solution for chemotaxis assay.

Chemotaxis assay

A modified method of the previously described quantitative capillary assay (Adler, 1973) was used to measure the chemotaxis of ***V. choleare*** 0139. The overnight grown cells of ***V. cholerae*** 0139 was diluted 10 fold in TSB and reincubated for up to 4 h to maximize the number of motile cells before proceeding to the assay. The bacterial cells were harvested at 8000 × g for 5 min and resuspended in an equal volume of PBS. This washing step was repeated three times and the final suspension was made in chemotaxis medium (PBS) to give an estimated cell density of 10^{10} cfu/ml. Serial dilutions of the bacterial suspension in PBS were used to obtain counts of viable and culturable cells (colony forming unit). A bacterial suspension of estimated 10^{7}/ml viable cells was dispensed in 200 µl aliquots into 1 cc syringe ([B-D] R Brand, Becton Dickinsion medical products Ptc. Ltd. Singapore). 1 µl capillary tube (Drummond Scientific Co.) heat sealed at one end and containing the substrate (in PBS) to be tested in half the length of the tube, was inserted horizontally into the syringe to approximately 1.0 cm below the surface of the bacterial solution. After incubation for 15, 30, 45, 60, 75 and 90 min at different temperatures (4, 25, 37 and 44°C) the capillaries were removed and the exterior rinsed with a thin stream of PBS and their contents were expelled into a specified amount of chemotaxis medium. A ten fold dilutions were made in the same medium and plated on TCBS agar plate and counts of viable cells were performed. In each experiment, substrates were simultaneously tested in triplicate and control capillaries containing PBS were included. The chemotactic activity of a particular substrate was expressed as percent accumulation (PA) i.e., the ratio of bacterial cells accumulated in substrate-containing capillaries to those contained in 200 µl suspension in the syringe multiplied by 100.

Results

Chemotactic response of *V. choleare* 0139 towards a number of individual chemical components

Commercial preparations of compounds i.e. L-serine, L-alanine and D-glucose those are also reported to be present in large amount in the extra-cellular materials of BGA (Fogg, 1952; Fogg and Pattnaik, 1966; Jones ***et al.***, 1952; Hellebust ***et al.***, 1974) were individually tested in the chemotaxis assay to evaluate their effect on the chemotaxis of ***V. cholerae*** 0139 at different

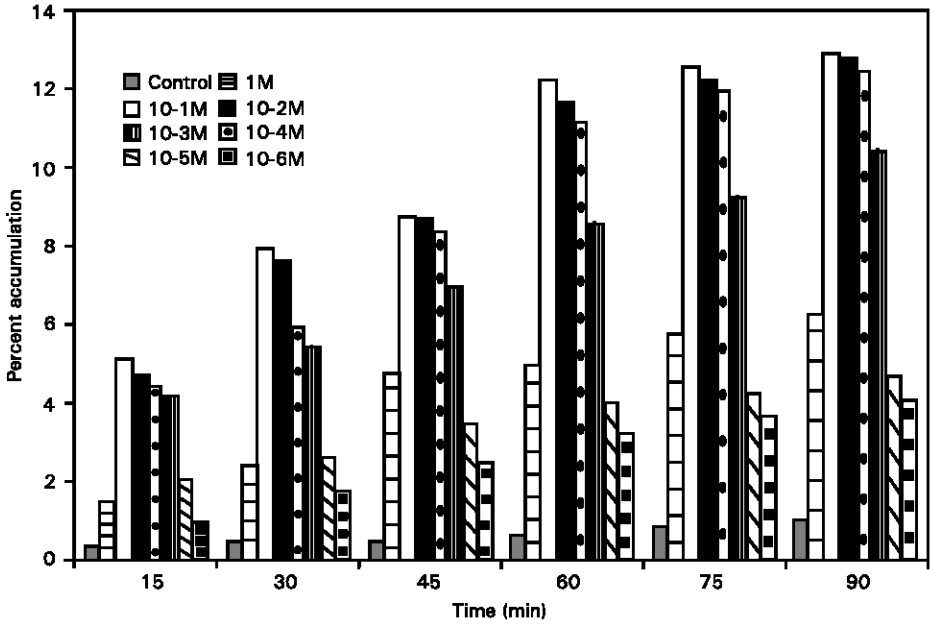

Fig. 1: Percent accumulation of *V. cholerae* 0139 in different concentration of L-serine at 25°C
Note: Legends (10-1M to 10-6M) written inside figure should be read as 10^{-1}M to 10^{-6}M, respectively

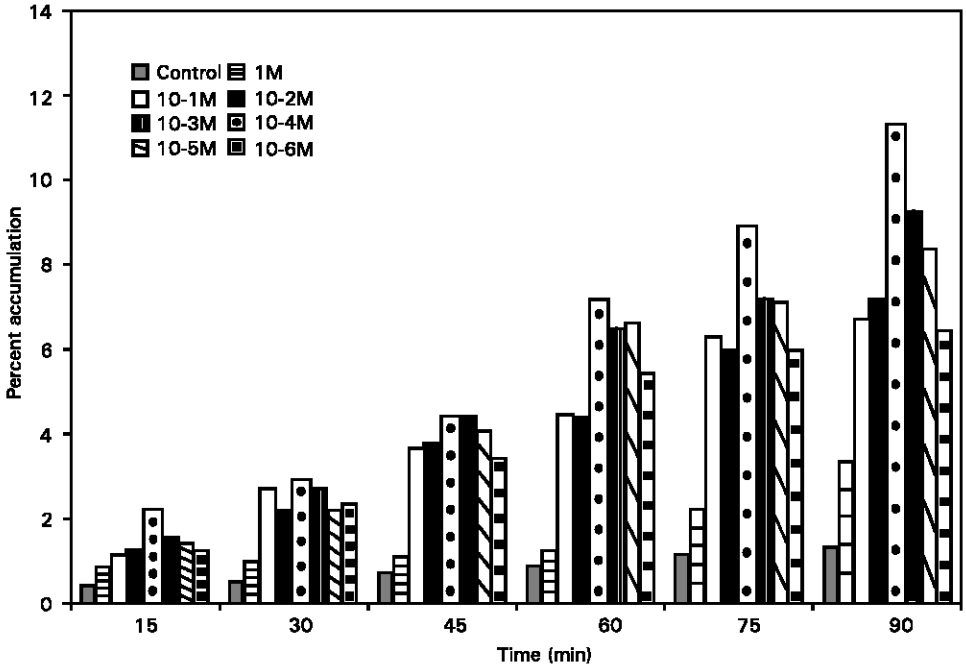

Fig. 2: Chemotaxis of *V. cholerae* 0139 towards different concentrations of L-alanine at 25°C

temperatures. The chemotactic response to L-serine and L-alanine at 25°C is illustrated in Fig. 1 and 2, respectively since at this temperature the highest chemotactic response of ***V. cholerae*** 0139 was recorded. Among the six different concentration of two amino acids used in the assay, the strain 0139 showed maximum response towards 10^{-1} M and 10^{-3} M for L-serine and L-alanine, respectively and the lowest response was observed at 1 M solution for both the amino acids (Fig. 1 and 2). As shown in Fig. 1 bacterial accumulation at 10^{-1} M was increased gradually up to 60 min and after that it was almost static. On the other hand, the percent accumulation of the strain 0139 in different concentration of L-alanine was increased consistently throughout the six different incubation periods. The capillaries containing no amino acid as attractant (control, Fig.1 and 2) showed a relatively small percent of bacterial accumulation at different time interval. Fig. 3 shows the influence of temperature on the percent accumulation of ***V. cholerae*** 0139 in capillaries containing 10^{-3} M glucose. The rate of accumulation of the strain to the capillaries containing 10^{-3} M glucose solution was found to increase with time and the maximum was observed at 90 min. Among the temperature used for chemotaxis assay the highest percentages of bacterial cell was found to be observed at 25°C. Various concentrations ranging from 10^{-8} M to 1 M solutions of the aforementioned chemicals were subjected to chemotaxis capillary assay in order to determine their threshold and peak concentration for chemotaxis of ***V. cholerae*** 0139

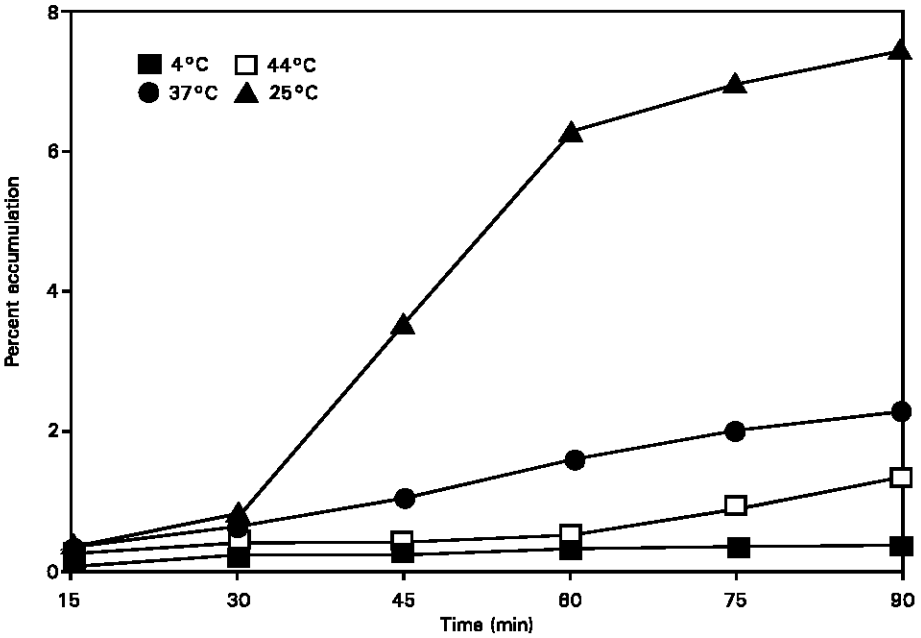

Fig. 3: Percent accumulation of ***V. cholerae*** 0139 towards 10^{-3}M glucose solution at various temperature

at various temperatures. The 'threshold concentration' the lowest concentration of attractant that gives an accumulation in the capillary greater than that obtained in the absence of attractant, was 10^{-8} M for both L-serine and L-alanine and 10^{-7} M for glucose (Fig. 4). The 'peak concentration' where the maximum response to attractant occurs, was 10^{-1} for L-serine and 10^{-3} for both for both L-alanine and glucose (Fig. 4).

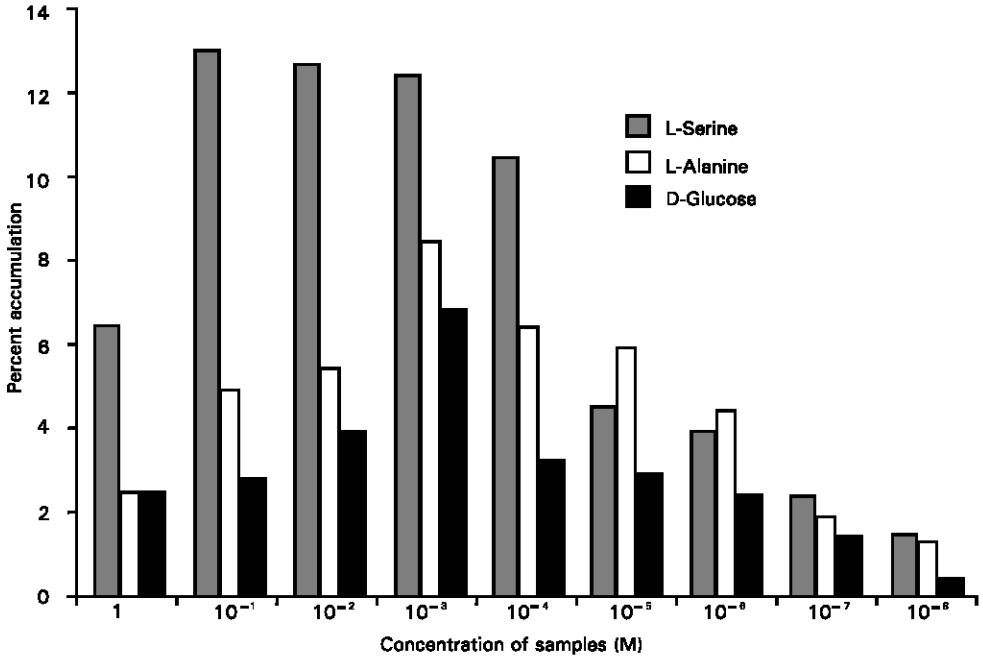

Fig. 4: Chemotactic response of *V. cholerae* 0139 towards different concentrations L-serine, L-alanine and L-glucose at 25°C

Chemotactic response towards *Anabaena* sp. at different temperatures

Four different concentrations of *Anabaena* sp. homogenate (0.5, 1.0, 2.0 and 4.0%) were used as chemoattarctants to demonstrate the effect temperature on the chemotaxis of *V. cholerae* O139. As shown in Table 1 variable percentage of accumulation of the strain in the capillaries containing homogenates of *Anabaena* sp. was observed at different temperature. The highest percentages of bacteria were recorded in the capillaries containing 4% homogenates of *Anabaena* sp. incubated for 90 min at 25°C followed by 37°C. At 44°C the accumulation is much lower than that was recorded at 25°C and no significant response was observed at 4°C.

Table 1: Chemotaxis of ***V. cholerae*** 0139 towards homogenates of ***Anabaena*** sp. at different temperature

		Percent of cells accumulated at various temperature			
Time (min)	Samples	4°C	25°C	37°C	44°C
15	Case-1	0.10	0.23	0.20	0.10
	Case-2	0.11	0.69	0.32	0.14
	Case-3	0.13	0.85	0.59	0.19
	Case-4	0.16	1.05	0.85	0.53
	Case-5	0.21	1.12	0.93	0.98
30	Case-1	0.10	0.32	0.23	0.13
	Case-2	0.13	0.72	0.42	0.17
	Case-3	0.13	1.07	0.63	0.46
	Case-4	0.17	1.67	0.99	0.60
	Case-5	0.23	2.82	1.04	1.19
45	Case-1	0.11	0.44	0.35	0.14
	Case-2	0.14	0.73	0.52	0.35
	Case-3	0.15	1.21	1.11	0.54
	Case-4	0.18	2.70	1.43	0.64
	Case-5	0.24	3.60	1.60	1.25
60	Case-1	0.12	0.48	0.35	0.14
	Case-2	0.15	0.87	0.64	0.54
	Case-3	0.16	2.30	1.29	0.97
	Case-4	0.20	2.81	1.61	1.07
	Case-5	0.25	5.24	1.69	1.31
75	Case-1	0.14	0.58	0.44	0.17
	Case-2	0.16	1.48	0.72	0.98
	Case-3	0.17	2.80	1.42	1.09
	Case-4	0.23	4.12	1.76	1.16
	Case-5	0.28	5.37	1.88	1.41
90	Case-1	0.16	0.80	0.56	0.26
	Case-2	0.19	1.80	1.01	1.09
	Case-3	0.22	3.00	1.45	1.19
	Case-4	0.25	4.52	1.97	1.97
	Case-5	0.37	5.45	2.70	1.51

Note: Case-1, PBS (pH) 7.4) as control, Case-2 to Case-5, homogenates of Anabaena sp. Of 0.5, 1.0, 2.0 and 4%, respectively. Each value was the mean of three replicates

Discussion

In this study, the effect of temperature on the chemotactic responses of ***V. cholerae*** O139 to the extracts of ***Anabaena*** sp. was tested in a chemotaxis capillary assay. Alanine, serine and glucose are important constituents of the extracellular products of ***Anabaena*** sp. (Fogg, 1952 ; Fogg and Pattnaik, 1966 ; Jones ***et al.***, 1952; Hellebust ***et al.***, 1974). ***Vibrio cholerae*** 0139 showed chemotactic movement towards all these substances in the present study. However, the percent accumulation of the strain 0139 varied in different substances at different temperature used (Fig. 1-3). The chemotactic response of the strain 0139 to L-serine and L-alanine has been shown in Fig 1 and 2 for 25°C, since this temperature was found to be the optimum for chemotactic movement. Earlier it has been reported that the highest chemotactic response of ***V. cholerae*** 0139 was observed at 10^{-3} M glucose solution (Mizanur ***et al.***, 2001). Therefore, in this report, 10^{-3} M glucose solution was used to evaluate the effect of temperature on the chemotaxis of the strain 0139. Four different concentrations of homogenates of ***Anabaena*** sp. (0.5, 1.0, 2.0 and 4.0%) were used to observe the effect of temperature on chemotaxis of ***V. cholerae*** 0139. Results of the study indicated that irrespective of the attractant used, maximum chemotaxis occurred at 25°C followed by 37, 44°C and no chemotaxis was recorded at 4°C. It has been reported that bacterial chemotaxis is not detectable at or below 15°C and raising the temperature from 20 to 30°C resulted in an increase chemotaxis (Adler, 1967). It could be assumed that, at this temperature the synthesis of chemotactic machinery is favoured. It was reported that the synthesis of lateral flagella of ***Vibrio*** spp. was maximal at temperature between 15 and 37°C (Belas and Colwell, 1982) which ultimately supports our findings. Among the attractant used, the highest response was observed in L-serine followed by L-alanine, glucose and 4% homogenates of ***Anabaena*** sp. (Fig. 1-3 and Table 1). Bacterial chemotaxis was demonstrated to be higher in pure amino acids tested in this study compared to glucose and ***Anabaena*** sp. homogenates. Such observation is probably due to that amino acids stimulate flagellar movement of the strain. Previous study indicated that glucose prevent the synthesis of flagella (Adler, 1973). In addition, the rate of diffusion of glucose from the capillary tube might be higher than that of other chemicals used in this experiment. This is also supported by the reports presented earlier (Adler, 1967), which suggested that glucose have poor chelating power at neutral pH. The reason behind comparatively lower response towards ***Anabaena*** sp. homogenates (Table 1) than that was observed with the chemicals used is not clear. It might be due to that, along with attractants there should have some inhibitory components present in the homogenates to lower the chemotactic activity. In this study it was not possible to investigate the concentration of homogenates beyond 4.0% suspension, as the concentrated homogenates above 4.0% could not be dispensed inside the capillaries. For statistical analysis, t-test was performed to analyze the results at 5% level of significance. In conclusion, temperature has strong effect on the chemotactic movement of ***V. cholerae*** 0139 towards various chemicals including extracellular products of ***Anabaena*** sp. and thus helps this pathogen to get attach to ***Anabaena*** sp. and use

as sanctuary of this cyanobacterium to survive against adverse environmental condition. Thus, finally favor these pathogens to spread cholera disease in the epidemic period.

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