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Chemotaxis between *Vibrio cholerae* O1 and a blue-green alga, *Anabaena* sp.

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SUMMARY

The chemotactic response of *Vibrio cholerae* O1 towards the mucilaginous sheath of *Anabaena* sp. was investigated by capillary tube method using a virulent strain of *V. cholerae* O1, El Tor, Ogawa (3083-T) and its isogenic mutant (*HAP*-1-T) that lacks the *hap* gene, which codes for mucinase (HA/protease). Homogenates of *Anabaena* sp. and purified mucin were used in this study as chemoattractants. Results showed 5·7% bacterial accumulation of wild-type *V. cholerae* O1 towards 4% homogenates of *Anabaena* sp. whereas, its mutant (*hap*$^-$) showed 2·9% accumulation after 90 min. The higher percentage of attraction of wild-type *V. cholerae* O1 than the mutant (*hap*$^-$) towards mucin and the homogenates of *Anabaena* sp. might be due to the activity of mucinase. These results indicate the role of mucinase in the chemotactic motility of *V. cholerae* O1 towards *Anabaena* sp.

INTRODUCTION

A blue-green alga, *Anabaena* sp. can provide a microenvironment for long-term survival of *V. cholerae* O1 in both the microcosm and the aquatic environment of Bangladesh [1]. In a recent study, it was demonstrated that mucinase, a soluble haemagglutinin/protease (HA/protease) present in both classical and El Tor biotypes, played an important role in the association of *V. cholerae* O1 with *Anabaena* sp. [2].

The mucilaginous sheath of blue-green algae has been considered as a potential microenvironment for bacteria. Paerl and Keller [3] observed 1000- to 5000-fold more motile, rod-shaped, Gram-negative bacteria attached to the heterocyst of both *Anabaena oscillarioides* and *Aphanizomenon flos-aquae* than in water columns of eutrophic lakes. Paerl [4] hypothesized that chemotaxis might be functional in making the association between cyanobacteria and bacteria.

Paerl and Gallucci [5] demonstrated the interaction between Gram-negative bacteria and the filaments of *A. oscillarioides*. They showed that when bacteria approached the filaments, motility in terms of flagellar movement increased markedly over planktonic conditions. When they encountered the heterocyst–vegetative cell junction, flagellar rotation increased for approximately 5–10 min and upon attachment flagellar movement appeared functionally stopped. At this stage, both the host and the epiphytes started to grow [5].

Heterocyst–vegetative cell junctions often excrete copious amount of mucilage, which contains carbohydrates, peptides [6] and free amino acids [7]. All these compounds of *Anabaena* culture filtrates were found to be chemotactically attracted by the bacteria

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when they remained free in the microenvironment. Therefore, in the present study, we attempted to compare the chemotactic response of both wild-type *V. cholerae* O1 (*hap*$^+$, i.e. mucinase positive) and its isogenic mutant *V. cholerae* O1 (*hap*$^-$, i.e. mucinase negative) to the mucilaginous sheath of *Anabaena* sp.

MATERIALS AND METHODS

Two strains of *V. cholerae* O1 were used in this study. A wild, hypertoxigenic strain of *V. cholerae* O1, El Tor, Ogawa (3083-T) containing the *hap* gene (HA/protease), was isolated from a cholera patient in Saigon, Vietnam, in 1964 [8]. The other strain (*HAP*-1-T) was an isogenic mutant of *V. cholerae* O1, El Tor Ogawa (3083-T), which was HA/protease negative, i.e. the *hap* gene deleted [9].

Partially purified type III mucin (Sigma Chemical Co., St. Louis, MO, USA), extracted from porcine stomach was used in this study. The wash medium used in this study was phosphate buffered saline (PBS, pH 7·4).

V. cholerae O1 preserved in T_1N_1 soft agar was used for the preparation of bacterial suspension and the chemotaxis experiments were carried out following the procedure described earlier [10, 11].

Anabaena used in this study was maintained as pure culture in standard algal media. Four different concentrations of *Anabaena* homogenates were prepared, i.e. 0·5, 1·0, 2·0 and 4·0% following the procedure described earlier [11] and were stored in different Eppendorf tubes for the chemotaxis assay.

Mucin (0·05 g) was carefully weighed and mixed with 1 ml pre-sterilized PBS in an Eppendorf tube by vortex. Thus, 5·0% stock solution of mucin was prepared. From the stock solution, 1, 2 and 4% mucin solutions were prepared with pre-sterilized PBS for the chemotaxis assay.

A series of pre-sterilized capillary tubes (10-μl Pyrex disposable micro-sampling pipette) were used in the experiment. The capillary tubes were plunged into the Eppendorf tubes containing different concentrations of *Anabaena* homogenates. The capillary tube was inserted into the syringe containing the bacterial suspension in such a way that the open tips of the capillary tubes were submerged in bacterial suspension. The syringes with the capillary tubes were then kept for 15, 30, 45, 60, 75 and 90 min at room temperature (25 °C). As a previous study [11] had shown that chemotactic response of *V. cholerae* and *Anabaena* homogenate was highest at 25 °C, all the experiments in the present study were carried out at room temperature (25 °C). The room temperature was controlled by using an air conditioner. These procedures were followed as described by Adler [10] and Rahman et al. [11]. Ten-fold dilutions of the chemotaxis medium containing *V. cholerae* O1 were prepared and the samples were plated on TTGA medium following the drop-plate technique [12]. After incubation for 18–24 h at 37 °C, colonies of *V. cholerae* O1 were counted. Each experiment was repeated three times and the mean count was recorded. Capillary tubes with control (PBS) were also used in this experiment. The number of bacteria entered into the capillary tube was calculated as per cent accumulation using the formula described earlier [10, 11].

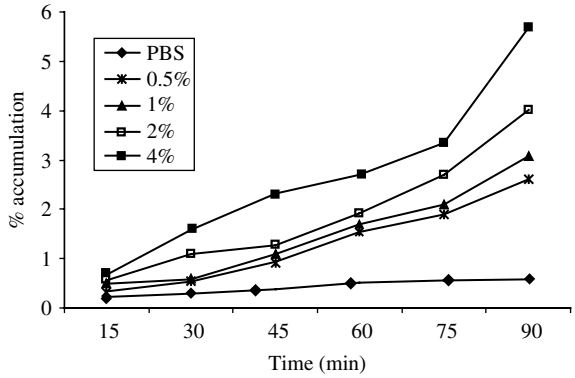

Fig. 1. Chemotactic response of wild-type *V. cholerae* O1, El Tor Ogawa (3083-T) towards different concentrations of *Anabaena* homogenates.

RESULTS

The accumulation of wild-type *V. cholerae* O1 (*hap*$^+$) was observed for different concentrations of homogenates of *Anabaena* used (Fig. 1). The percentage of accumulation of *V. cholerae* O1 gradually increased with the increased percentage of *Anabaena* homogenates. The highest (5·7%) percentage of bacterial accumulation was observed at 4% homogenates of *Anabaena* sp. at 90 min.

The accumulation of mutant strain was also observed at different concentrations (0·5, 1·0, 2·0 and 4·0%) of homogenates of *Anabaena* (Fig. 2). The percentage of accumulation was much lower than that observed in wild-type strain (Fig. 1). The highest accumulation (2·9%) was observed for 4% homogenates at 90 min, which is almost 50% lower than the wild-type strain.

The accumulation of wild-type *V. cholerae* O1 (*hap*$^+$) was observed at different concentrations of

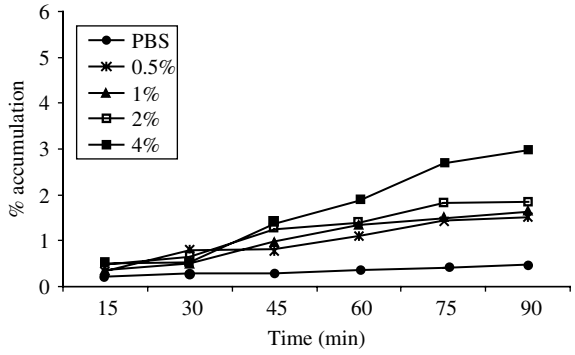

Fig. 2. Chemotactic response of mutant *V. cholerae* O1, El Tor Ogawa (HAP-1-T) towards different concentrations of *Anabaena* homogenates.

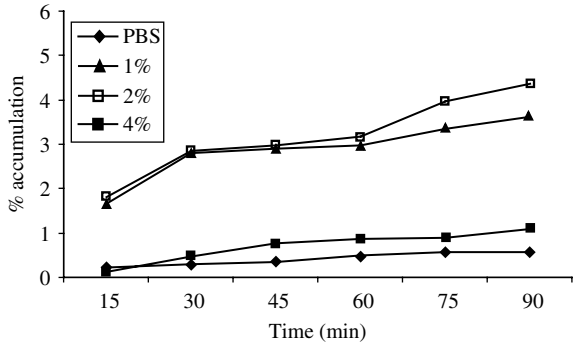

Fig. 3. Chemotactic response of wild-type *V. cholerae* O1, El Tor Ogawa (3083-T) towards different concentrations of mucin.

mucin used (Fig. 3). The highest (4·4%) bacterial accumulation was observed at 2% concentration of mucin after 90 min, whereas in control (PBS), virtually no accumulation was observed, even after 90 min.

In Figure 4, the percentage accumulation of mutant type *V. cholerae* O1 (*hap*$^-$) towards different concentrations of mucin has been depicted. No significant bacterial accumulation was evident towards any concentration of mucin. The highest (1·4%) bacterial accumulation was observed at the lowest (1%) concentration of mucin compared to the higher concentrations of 2 and 4%, which were found at 1 and 0·8% respectively.

DISCUSSION

In the present study, results showed that the rates of bacterial accumulation in capillary tubes towards the homogenates of *Anabaena* sp. were gradually increased with the function of time. The wild-type strain of *V. cholerae* O1 showed the highest

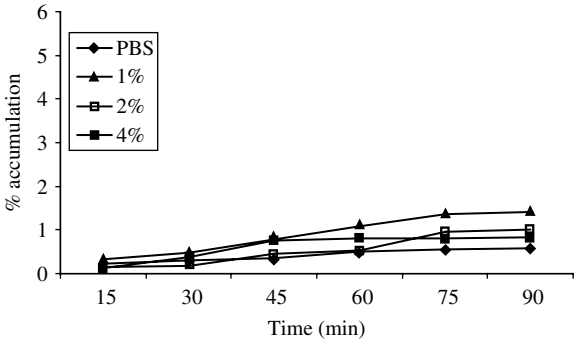

Fig. 4. Chemotactic response of mutant *V. cholerae* O1, El Tor Ogawa (HAP-1-T) towards different concentrations of mucin.

percentage (5·7%) of bacterial accumulation at 4·0% homogenates after 90 min. On the other hand, the mutant strain of *V. cholerae* O1 (*hap*$^-$) showed comparatively poor (2·9%) chemotactic response towards 4% homogenates of *Anabaena* sp. after 90 min. However, no significant bacterial accumulation was observed in the case of control suggesting that wild-type *V. cholerae* O1 with its ability to produce mucinase showed a stronger chemotactic response than that of the mutant type.

In a recent study [2], it has been demonstrated that the mutant strain of *V. cholerae* O1 (*hap*$^-$) could not persist longer in association with *Anabaena* sp. These findings led to the idea that chemotactic motility of *V. cholerae* O1 towards the mucilaginous sheath of *Anabaena* sp. could be influenced or stimulated by the chemoattractant mucin that covered the mucilaginous sheath.

Partially purified mucin extracted from porcine stomach was also used in different concentrations (1, 2 and 4%) for chemotaxis assay. The bacterial accumulation of wild-type strain of *V. cholerae* O1 increased with exposure to 1 and 2% mucin but was dramatically reduced when exposed to 4% mucin. The change in the direction of flagellar rotation from counterclockwise to clockwise may influence chemotaxis and motility. Armed with a single polar flagellum, *V. cholerae* can randomly reorient itself and swim in a new direction as described by Butler and Camilli [13] and, therefore, the 4% concentration of mucin may change the *V. cholerae* flagellar rotation which might cause less accumulation of *V. cholerae*. Another explanation may be due to rate-limiting of mucinase when exposed to a higher concentration of mucin.

The present study showed that chemotactic motility of *V. cholerae* O1 towards the mucilaginous sheath of

Anabaena sp. might be the function of mucinase (HA/protease). This study provides some insight into the association of *V. cholerae* O1 with cyanobacteria.

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DECLARATION OF INTEREST

None.

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