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Quantitative Assay for Algal Chemotaxis

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A quantitative capillary assay is described for measuring chemoreception in the neritic and littoral unicellular alga *Dunaliella tertiolecta*. Lucite chemotaxis plates were used in the assay with 3-μl capillaries. A Coulter Counter was employed to determine algal cell numbers. *D. tertiolecta* is attracted to ammonium ion with a maximum positive response at 10^{-3} M. Inclusion of calcium and L-methionine in the chemotaxis medium stimulates algal chemoreception, although neither chemical is essential for motility. Attraction of the chlorophyte to ammonium is dependent on time of incubation, cell density, and pH. The optimum pH for attraction was found to be 6.25.

Pfeffer (8) in 1888 used a capillary assay to observe chemotactic responses of bacteria, and Adler (1) adapted and modified the assay to allow for quantitative evaluation of bacterial chemoreception. Lucite chemotaxis plates recently have been designed by Palleroni (7) to facilitate the assay. Armitage et al. (2) developed a rapid quantitative assay for bacterial chemotaxis in which a Coulter Counter was used to measure the percentage of motile bacteria passing through a polycarbonate membrane in a chemotactic response.

We have developed an assay to quantify algal chemotaxis with Palleroni's Lucite chambers. A Coulter Counter was employed to determine the number of algal cells entering microcapillaries filled with solutions of attractant. The motile marine alga *Dunaliella tertiolecta* was used in the assay. Some of the parameters which affect chemotaxis of the alga are described in this report.

MATERIALS AND METHODS

Algal growth. An axenic culture of *D. tertiolecta* was provided by R. R. L. Guillard, Woods Hole Oceanographic Institution. The alga was grown in seawater medium enriched with B vitamins, trace elements, glycerophosphate, and urea (modified from [5]). The seawater was obtained from the Northeastern University Marine Research Station, Nahant, Mass. It was initially treated with 1.5 g of activated carbon (Aqua Nuchar A, West Virginia Pulp and Paper Co., Covington, Va.) per liter and stirred for 30 min. The activated carbon was removed by filtration, and the seawater was further treated by filtering through a membrane of 0.45-μm pore size (Millipore Corp., Bedford, Mass.). The treated seawater is designated as NSW.

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Cultures of *D. tertiolecta* were grown in stationary 250-ml Erlenmeyer flasks containing 50 ml of seawater medium (pH 8.3). The flasks were incubated at 30°C over 5 klx of continuous fluorescent illumination.

Preparation of algae for chemotaxis. The algal suspension was incubated until a density of at least 10^6 cells per ml was attained. The cells were centrifuged at 270 × *g* and 20°C for 5 min, the supernatant was decanted, and the algal pellet was carefully brought to the original volume by resuspension in a buffered artificial seawater medium (ASW).

The wash medium was an artificial seawater solution containing 24.0 g of NaCl, 7.0 g of $MgSO_4 \cdot 7H_2O$, 5.3 g of $MgCl_2 \cdot 6H_2O$, 1.1 g of $CaCl_2 \cdot 2H_2O$, and 0.7 g of KCl per liter of distilled water. The artificial seawater was buffered at pH 8.5 with 10^{-2} M sodium carbonate-sodium bicarbonate, and L-methionine (Eastman Organic Chemicals, Rochester, N.Y.) was added at a final concentration of 10^{-5} M. Unless otherwise stated, the wash medium also served as the chemotaxis medium. By definition, the chemotaxis medium is used to suspend the algal cells and also to dissolve the chemicals added to the capillaries.

Chemotaxis assays. Lucite chemotaxis plates designed by Palleroni (7) and 3-μl capillaries (Microcaps, Drummond Scientific Co., Broomall, Pa.) were used in all our assays. The plates were washed in warm detergent, rinsed in distilled water, and stored in glass petri dishes. The microcapillaries were cleaned by thorough washing in reagent grade acetone (Fisher Scientific Co., Pittsburg, Pa.) followed by extensive rinsing in distilled water.

The washed algal cells were incubated for 2 h at 23 ± 1°C and were then pipetted into the excavated chambers of each chemotaxis plate. The 3-μl capillaries were submerged in the algal suspension after filling with the chemotaxis medium containing appropriate concentrations of NH_4Cl. Control capillaries with no added NH_4Cl were included in each assay. The Lucite chemotaxis plates were kept in the covered, glass petri dishes during the assay to minimize evaporation. The plates were incubated at 23 ± 1°C and for 45 min unless otherwise noted.

After incubation, the capillaries were removed from the cell suspension with fine-tipped tweezers, and the

outside surfaces were carefully, but thoroughly, dried with a paper wipe. The contents of each capillary were dispensed into 10 ml of a balanced electrolyte solution (Isoton, Coulter Electronics, Inc., Hialeah, Fla.). After the capillary was rinsed in Isoton six times, the volume in each counting vial was brought up to 20 ml. Algal numbers in each vial were determined with a Coulter Counter, model Zf (Coulter Electronics, Inc.). Five counts were made per vial, and the results represent an average of the five values. Before each determination, the vial was capped and mixed by inverting several times.

RESULTS

Preliminary experiments showed that the chlorophyte *D. tertiolecta* is attracted to NH_4Cl. A typical concentration-response curve is shown in Fig. 1. The peak attraction of the alga was observed when the capillary contained 10^{-3} M NH_4Cl. The lowest concentration needed to elicit an observable response (the threshold concentration) was approximately 10^{-6} M. The algal suspension contained 2.5×10^6 cells per ml. Approximately 2.7×10^3 algae accumulated in capillaries containing no added NH_4Cl. Experiments with KCl and NaCl showed that NH_4^+, rather than Cl^-, is responsible for eliciting the positive chemotactic response. Furthermore, ammonium sulfate [$(NH_4)_2SO_4$] attracted *D. tertiolecta,* whereas $MgSO_4$ elicited no response.

Figure 2 shows the effect that incubation time has on the attraction of *D. tertiolecta* to 10^{-3} M NH_4Cl. A rapid increase of cells entering the capillary was observed during the first 20 min of incubation, followed by a slower, but steady, positive rate of accumulation during the next 60 min. Levels of cells in capillaries containing no added NH_4Cl remained constant after 20 min. Thus, 45 min was chosen as a reasonable assay incubation period in subsequent experiments.

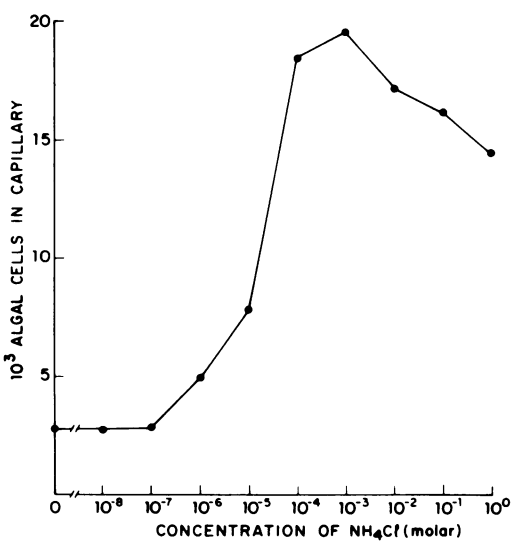

FIG. 1. *Attraction of D. tertiolecta to NH_4Cl.*

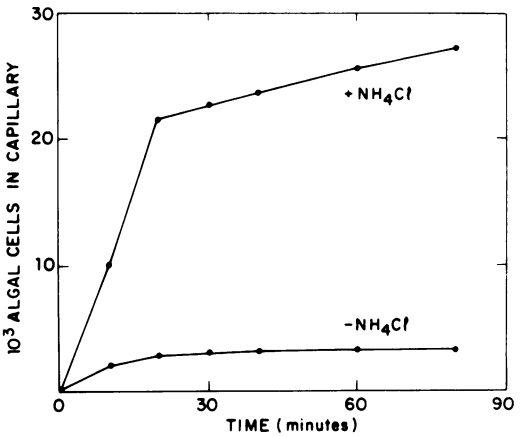

FIG. 2. *Effect of incubation time on the attraction of D. tertiolecta to 10^{-3} M NH_4Cl.*

The effect of cell concentration on algal chemoreception was determined by diluting a washed algal suspension which initially contained 6.0×10^6 cells per ml. The diluted samples were incubated with capillaries containing 10^{-3} M NH_4Cl. Figure 3 shows that the number of attracted algae is related to cell density. The total number of cells entering capillaries containing no NH_4Cl slowly rises as the population of algae in the chemotaxis chamber becomes more dense. *D. tertiolecta* suspensions containing 2×10^6 to 3×10^6 cells per ml would certainly be sufficient in most assays, because higher population densities do not give an increased ratio of attracted cells in the test versus control capillaries.

The effect of varying the chemotaxis medium components and the reversible loss of chemoreceptive capabilities induced by the washing procedure are shown in Fig. 4. The algae were washed as described previously. However, some of the components were omitted from the chemotaxis medium. Assays were performed at 0, 1.5, 2.5, and 3.5 h after washing. In each case the medium in the capillary was the same as that used to wash the cells, except that the capillary contained 10^{-3} M NH_4Cl. As can be seen from Fig. 4, omission of calcium or L-methionine from the medium resulted in a reduced chemotactic response. It appears that calcium is essential for eliciting a significant response with *D. tertiolecta* even though microscopic examination indicated that omission of $CaCl_2$ from the chemotaxis medium did not noticeably curtail algal motility. Addition of 10^{-4} M disodium ethylenediaminetetraacetic acid to the chemotaxis medium did

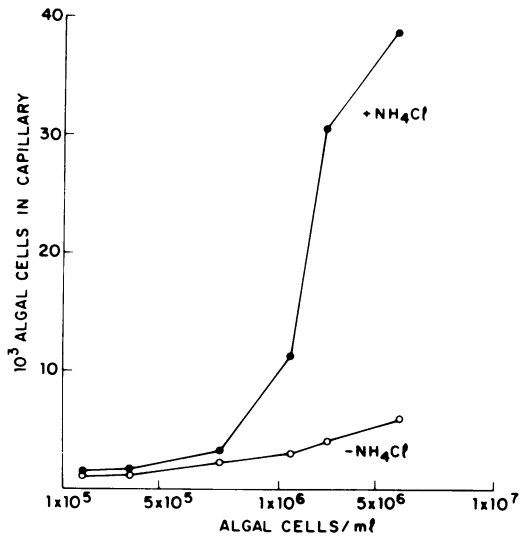

FIG. 3. *Effect of cell concentration on attraction of D. tertiolecta to 10^{-3} M NH_4Cl.*

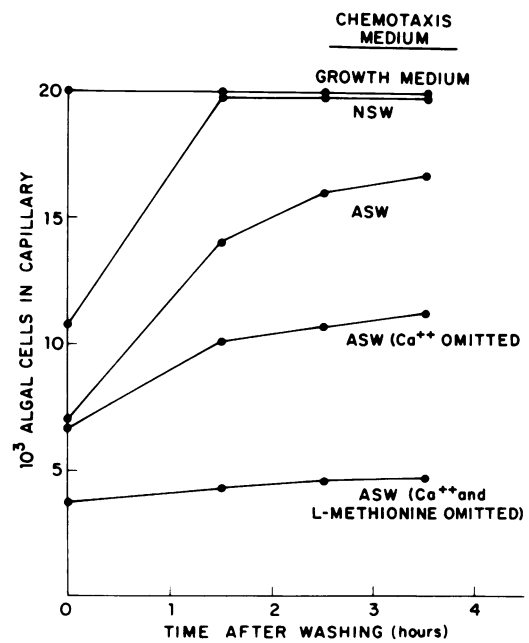

FIG. 4. *Temporary loss of attraction to 10^{-3} M NH_4Cl induced by washing procedure and effects of chemotaxis medium components on chemotaxis of D. tertiolecta. Preparation of NSW and ASW is described in the text.*

not enhance the chemotactic response. Furthermore, inclusion of a mixture of 96 mg of KBr, 24 mg of $SrCl_2$, 26 mg of H_3BO_3, and 3 mg of NaF per liter of chemotaxis medium did not affect attraction of *D. tertiolecta* to NH_4^+. L-Methionine at 10^{-5} M also did not attract the alga.

The procedure of washing the algal cells apparently was sufficient to temporarily reduce the attraction of *D. tertiolecta* to NH_4^+. A period of 3.5 h after the time of washing was not long enough to allow for complete recovery of algal chemotaxis in any of the ASW wash systems. However, full chemotactic potential (the response of unwashed cells—labeled "growth medium" on the figure) was restored within 1.5 h after washing the algae with NSW.

The optimum pH for chemotaxis to 10^{-3} M NH_4Cl was determined for *D. tertiolecta*. A population of 2.5×10^6 algae per ml was suspended in the chemotaxis medium buffered at pH 8.5 with 10^{-2} M sodium carbonate-sodium bicarbonate. The capillaries were filled with chemotaxis medium adjusted to various pH values with either 10^{-2} M citric acid-sodium citrate, mono- and dibasic sodium phosphate, boric acid-sodium borate, or sodium carbonate buffer systems. In this study, the assay plates were incubated for 30 min. Figure 5 shows that the optimum pH for the attraction of *D. tertiolecta* was 6.25, with 3.2×10^4 algal cells entering the capillary. Only 10^3 to 4×10^3 algae accumulated in control capillaries containing the various buffered ASW solutions with no added NH_4Cl. Thus, attraction of *D. tertiolecta* was observed even at pH values as low as 3.0 and as high as 9.5. The reason for the secondary peak of attraction at pH 3.5 is not clear.

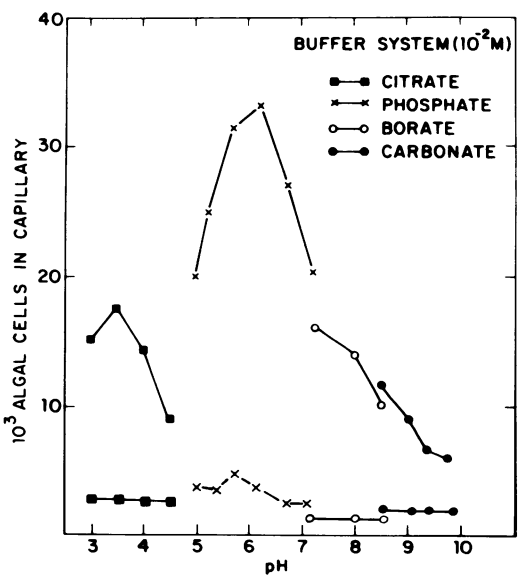

FIG. 5. *Effect of pH on attraction of D. tertiolecta to 10^{-3} M NH_4Cl. Capillaries in four upper curves contained buffer systems with 10^{-3} M NH_4Cl added; the capillaries in the bottom four curves contained buffer alone.*

Attempts were made to employ a chemotaxis medium composed of ASW containing 10^{-5} M L-methionine and adjusted to pH 6.25 with 10^{-2} M sodium phosphate buffer. However, when the cells were washed in this medium, aggregation occurred. The algae also rapidly settled onto surfaces in the chemotaxis chamber.

DISCUSSION

We have developed a rapid assay to quantify attraction of the unicellular alga *D. tertiolecta* to NH_4^+. Parameters which affect optimum conditions for attraction of the alga to this chemical were also assessed. Although calcium and L-methionine were not essential for algal motility, their addition to the chemotaxis medium stimulated positive chemotaxis. A concentration of 10^{-3} M NH_4Cl resulted in the maximum positive response, and an assay incubation time of 45 min with at least 2×10^6 algae per ml in the cell suspension was found to be sufficient for studying attraction to NH_4^+.

A temporary reduction in the attraction to NH_4^+ caused by washing the cells must be considered in assessing the possible attraction of *D. tertiolecta* to other chemicals even though the reason for the decreased response caused by the washing procedure is obscure. It appears that there are similarities between the mechanisms of chemotactic behavior of the eucaryotic *D. tertiolecta* and procaryotic bacteria. Both calcium ion (6) and L-methionine (9) can regulate chemotactic behavior in bacteria, and these chemicals also influenced the attraction of *D. tertiolecta* to NH_4^+.

Although much effort has been devoted to the development of assays for bacterial chemotaxis, we know of no systematic studies that have assessed the parameters which affect chemotactic responses in algae. It has been suggested that chemoreception in bacteria may serve as a simple, but useful, model for sensory responses in more complex organisms (3, 4). Thus, it becomes of interest to further assess mechanisms of chemotactic behavior in eucaryotic algae and to compare algal chemoreception with bacterial chemoreception.

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