DIEL VERTICAL MOVEMENTS OF BACTERIA IN INTERTIDAL STREAMS OF SIPPEWISSETT MARSH

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Environmental conditions within the sediment of intertidal streams of salt marshes change drastically in 24 hrs. Light intensity and quality; surface temperature; dissolved oxygen and sulfide concentrations in pore-waters; and depth of watercover all play a role in determining the survival of bacteria living in this environment.

The purpose of this report is to observe how bacteria in these areas respond to environmental changes in the course of a day and to attempt to determine what causes these responses. To simplify the system, the work described here was confined to monitoring the effects of light intensity and sulfide under laboratory and field conditions.

MATERIALS AND METHODS

The area studied was a stream approximately 20 cm wide (at low tide) which was covered by about 1 cm of water at low tide. The stream was composed entirely of loose sand with no interwoven mat material. For some studies, this area was recreated in the laboratory by filling aluminum pans (28 cm x 18cm x 4cm) with sulfide-rich sand from this area. Over the surface of this was deposited approximately 0.5 cm of the surface sand from this area. Seawater was allowed to flow across this continuously. One pan was set up outside to get maximal exposure to the sun. Other pans were set up in the laboratory under illumuniation from either a 150 watt or a 75 watt flood lamp from a distance of approximately one meter. These lamps were on for 14 hrs and off for 10 hrs per day.

Shading experiments were conducted in the marsh using filters made of mason jar lids with attached wire screens of varying thickness, a layer of dark plastic, or metal lids. After leaving these areas covered for 2 hrs, the surfaces of the shaded areas were sampled and examined microscopically.

Other experiments in the marsh involved covering areas with mason jar lids (38.5 cm^2) and applying 2.5 ml of 10^{-4} M DCMU to the surface. After 2 hrs under direct sunlight, the surface was collected and examined microscopically.

Cores of 0.5 mm diameter were collected from the laboratory pans and extruded onto a microscope slide for sectioning. Slices of 1 lmm were removed using a razor blade. These were disperses onto another slide and examined microscopically. Slices were refrigerated in vials if not examined immediately.

Slices of six replicate cores used for spectrophotometric studies were suspended in 0.5 ml of TSM buffer and attached cells removed by sonication in an ultrasonic bath while on ice for 5 min. The supernatant was decanted and

An experiment to determine the light intensity favored by the Oscillatoria was set up as follows. A U-shaped glass tube (5 mm i.d.) was filled with a suspension of the Oscillatoria filaments in 0.2% agar. The tube was shaded from a 150 watt lamp by varying thicknesses of plastic screen.

An experiment to determine chemotaxis toward sulfide involved forming a slant with 8 mM sulfide in 2% agar in a plastic petri dish. Over this was placed a suspension of Oscillatoria in 0.2% agar. The plate was evenly illuminated with a fluorescent lamp.

RESULTS AND DISCUSSION

In the marsh, the surface population changes dramatically during the course of a day. The sand surface appeared slightly pink to yellow in the afternoon of a sunny day. The surface at this time was populated by naviculoid diatoms and microcolonies of purple sulfur bacteria on the grains. These purple sulfur bacteria have been tentatively identified as Thiocapsa. At dusk, the surface develops dark patches. These are formed as the result of an increase in the number of a filamentous, green phototrophic bacterium similar in morphology to Chloroflexus and clusters of the green sulfur bacterium Chloroherpeton. In the morning white patches composed of masses of Beggiatoa filaments are evident on the surfce as well as the green patches mentioned above.

To assess the effects of reduced light on migrations, light filters were placed on the sand. When approximately 70% of the incident light (final intensity of 240 watts m^{-2}) was blocked, dark patches of an Oscillatoria were seen on the surface. Clear filaments of 4.8 µm diameter were also observed in slightly greater abundance under these conditions. This is also the diameter of the predominant Beggiatoa species found in these sediments. Under total darkness (0.3 watts m^{-2}), there was an increase in the Thiocapsa concentration in one area, giving rise to a pink patch.

To determine whether oxygenic photosynthesis might inhibit the migration of Beggiatoa to the surface during the day, DCMU was applied to a limited area of the sand. After two hrs, no increase in the number of Beggiatoa was evident, probably due to the negative phototactic response that has been observed in Beggiatoa (Nelson and Castenholz, 1982).

Observations of the sand pans set up in the lab showed similar patterns of migration. Cores of 5 mm diameter were removed at the end of the "day" and sectioned at 1 mm intervals. The Oscillatoria was found in the upper 1 mm of the core. It is apparently most abundant at about 0.5 mm because it is not visible on the surface. Beggiatoa numbers increased with depth to 3 mm. At 4 mm, the Beggiatoa were much less abundant and many clear filaments of a similar diameter were present, suggesting that the filaments may be Beggiatoa that have utilized internally stored sulfur granules. Other investigators have observed similar accumulations of Beggiatoa, but found clear filaments to be scarce (Jorgenson, 1977).

A similar core was taken from the same area after the "night". The Oscillatoria were found to be distributed more evenly through the upper 6 mm, but more so in the 3-5 mm level. Beggiatoa was very abundant on the surface and the filaments were full of sulfur granules.

Similar cores were taken from a pan left outdoors. Seven cores were taken from different areas at different times of the day. One core was sectioned and examined microscopically. The other six were sectioned and the analogous sections combined and examined spectrophotometrically as described previously. Changes in the spectrum were not obvious through the course of a day, but it did appear that a bacteriochlorophyl c (bchl c)-containing organism did migrate toward the surface at dusk. The technique was quite useful in demonstrating the abundance of chl a and bchl a in the upper 2 mm. At 3 mm, a large nchl c peak was evident as shown in Figure 1. This appeared to be due to an abundance of Chloroherpeton at this level. At 4 mm the sand was blackened, presumably due to the presence of ferrous sulfide. Below this level little pigment was detectable by the spectrophotometric assay.

Attempts to correlate chl a to bchl a ratios with migrations proved unsuccessful in initial trials. The high background absorbance due to suspended particles could be compensated for by obtaining spectra of duplicate samples in which the pigment was bleached using hydrogen peroxide and exposure to light. This method proved useful for observations of pigment distribution within single cores and further trials may yet show it to be useful for time-course studies.

Oscillatoria migration was monitored in two experiments using masses of filments gathered from field material. Table I shows the results of a migration experiment using the U-shaped tube described previously. It was apparent that after four days, the Oscillatoria had migrated to the low light intensity regions. Under continuous illumination of a sulfide-gradient plate, the Oscillatoria migrated toward the high sulfide end of the plate. The filaments continued to migrate for one week until they were all clumped at the high sulfide end. These results indicate that this Oscillatoria prefers low light and some form of reduce sulfur. This has been observed in other cyanobacteria (Parker, 1982). Enrichments of this organism did better when given low light, sulfite, and a surface to adhere to.

Enrichments for the Beggiatoa, photosynthetic bacteria and Oscillatoria were started. None have yielded pure cultures yet. The use of thioacetimide as a source of sulfide has proven useful in the enrichment of the photosynthetic sulfur bacteria (Irgens, 1983). Until isolates are obtained, it is difficult to determine what conditions may cause these organisms to migrate.

These sandy streams allow easier observations of bacterial movements than do the surrounding algal mats. The lower populations density makes changes more obvious. Their intertidal nature makes field studies more difficult, but by setting up laboratory simulations, the field conditions can be reproduced. This procedure undoubtedly produces some artifacts, however. Concentrating on the organisms described here will allow future investigators to unravel the essential parameters involved in these diel vertical migrations.

TABLE I. *Oscillatoria* migration in U-tube experiment

Layers of Screen	Light Intensity (watts m^{-2})	Accumulation of *Oscillatoria*
0	177	+
1	138	++
3	45	++
5	30	+
7	9.1	-
8	0.9	-

FIGURE 1

In vivo spectra of sand extract
(7:45 pm)