APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Jan. 1984, p. 135–143
0099-2240/84/010135-09$02.00/0

Vol. 47, No. 1

# Hydrophobicity as an Adhesion Mechanism of Benthic Cyanobacteria

ALI FATTOM AND MOSHE SHILO*

*Division of Microbial and Molecular Ecology, Life Sciences Institute, Hebrew University, Jerusalem, Israel*

Received 20 June 1983/Accepted 7 October 1983

The capacity of benthic cyanobacteria to adhere to solid substrates was examined in terms of their cell surface properties. By using a biphasic water-hydrocarbon test system, it was demonstrated that benthic cyanobacteria from divergent habitats were all hydrophobic, whereas all the planktonic cyanobacteria tested were hydrophilic. Divalent cations were found more efficient than monovalent cations in effecting the expression of hydrophobicity. Mechanical shearing of the cell surface, as well as chemical removal of the cell wall, demonstrated that the hydrophobicity was confined to the outer surface layers. The hydrophobic sites were distributed along the whole length of the cyanobacterial filament. Hydrophilic hormogonia of benthic cyanobacteria became hydrophobic within 48 h when grown in the light; chloramphenicol, 3(3,4-dichlorophenyl)1,1 dimethylurea, or incubation in the dark prevented this transition. Hydrophobicity of *Phormidium* filaments was masked in late stationary phase; this effect was removed by gentle washing.

Aquatic cyanobacteria can be divided into two types with respect to position in the euphotic zone. Whereas the planktonic types float freely in the water column, benthic cyanobacteria adhere to submerged solid surfaces: the bottom sediments at the water-soil interface, rocks and stones along the littoral, and algae and aquatic plants (20). The ways in which these two groups differ from each other and the general characteristics of the benthic group have hardly been investigated and deserve closer study.

A prerequisite for understanding benthic behavior is the elucidation of the mechanisms involved in adhesion of the organisms to solid surfaces. Since adhesion is a surface property, this study concentrates on the cell envelope characteristics of benthic cyanobacteria.

Cell surface hydrophobicity is considered an important factor in the adhesion and proliferation of microorganisms on solid surfaces (31). In natural ecosystems, in which adhesion to inert surfaces such as plastics, metal sheets, water-air interfaces (11), and even teeth (37) are common, adhesion is nonspecific, in contrast to biological surfaces, where specific receptors and lectins play a major role (36, 45). Hydrophobicity seems to be the most important factor in the nonspecific adhesion of bacteria to interfaces. Moreover, several recent studies with pathogenic bacteria, certain of which have specific surface receptors, indicate that their surface hydrophobicity may influence the cell-bacteria interaction (14, 15).

In this study we examined the hydrophobic characteristics of cell envelopes of benthic cyanobacteria in comparison with those of planktonic cyanobacteria; we describe their dispersal mechanisms.

## MATERIALS AND METHODS

**Cyanobacteria and culture conditions.** The cyanobacterial strains and their habits and culture conditions are summarized in Table 1. Growth medium BG11, described by Stanier et al. (49), and modified BG11 containing in addition $Na_2SiO_3 \cdot 9H_2O$ (58 mg/liter) were used. The media salts were either dissolved in distilled water or added to the double-concentrated Turks Island salt solution described in the Merck Index (p. 1088, 8th ed., 1968). The pH of the medium was adjusted to pH 7.5 with HCl (1 N) before autoclaving. The strains were grown on a gyrorotary shaker in 250-ml flasks containing 100 ml of culture medium, continuously illuminated with cool white fluorescent lamps (incident light intensity, 20 to 30 microeinsteins/$m^2$ per s). Solid medium for isolation and colony formation of the different strains was obtained by adding 15 g of agar (Difco Laboratories, Detroit, Mich.) to each liter of appropriate liquid medium.

**Hydrophobicity test in the biphasic system.** A modification of the method described by Rosenberg et al. (43) for measuring cell surface hydrophobicity in bacteria was employed in our tests with cyanobacterial strains. This method is based on the partitioning of cells having hydrophobic surface characteristics in the hydrocarbon phase of a biphasic hydrocarbon-aqueous system after brief mixing. Since the buoyancy of some hydrophilic gas-vacuolated cyanobacteria might produce misleading results in such a system, the gas vacuoles were collapsed before testing. This was done by applying sudden hydrostatic pressure to each culture before samples were taken. In our test, various volumes of *n*-hexadecane were added to flat-bottomed Klett tubes containing 5 ml of cell suspension in appropriate growth medium. Unless otherwise mentioned, the cultures were tested in the early stationary phase, i.e., after 7 to 10 days of growth. The mixture was vigorously shaken manually for 1 min, allowed to stand for 5 min, and then gently agitated for 10 s (Vortex-Genie; model 550; GBS Instruments). This second shaking was found necessary to avoid partitioning in the hydrocarbon phase of certain cyanobacteria determined to be hydrophilic by other methods, which included contact angle measurements and adherence to phenyl-Sepharose and to polystyrene. The tubes were again allowed to stand for 5 min to allow complete separation of the phases. The optical density of the aqueous phase was determined in a Klett-Summerson colorimeter (filter 54), and the ratio of readings before and after the addition of the hydrocarbon served as the measure of hydrophobicity.

**Hydrophobicity measurement by contact angle of air bubbles with cell layers.** Cyanobacterial cells after 5 days of growth were filtered on a Millipore filter (0.8 μm) and placed

* Corresponding author.

TABLE 1. Cyanobacterial strains used in this study

| Cyanobacteria | Characteristics and source | Culture conditions | Hydrophobic[a] |
|---|---|---|---|
| *Anabaena* sp. | Filamentous, planktonic sample from brackish fish pond, Nir David, Israel | 26°C, BG11 | − |
| *Anabaena azollae*[b] var. *pinnate, caroliniana, filiculoides* | Filamentous, symbiotic in leaf cavities of water fern *Azolla*; nonaxenic isolates (34) | 26°C, BG11 | + |
| *Anabaena variabilis*[c] | Filamentous, planktonic; axenic strain M3, Tokyo University Culture Collection | 26°C, BG11 | − |
| *Anabaenopsis circularis* | Filamentous, hormogonia-producing, benthic; strain 6720, Paris Culture Collection (PCC) | 26°C, modified BG11 | +[d] |
| *Anacystic nidulans* | Unicellular, planktonic; strain 6311, PCC, axenic | 26°C, BG11 | − |
| *Aphanotheca halophytica* | Unicellular, benthic; axenic strain 7418, PCC, isolated from hypersaline Solar Lake Sinai by Y. Cohen | 26°C, BG11 in TIS[e] | +[d] |
| *Calothrix desertica* | Filamentous, hormogonia-producing, benthic; axenic strain 7102, PCC | 26°C, modified BG11 | +[d] |
| *Freymyella diptosiphon*[f] | Filamentous, hormogonia-producing, benthic; axenic isolate from fresh water | 26°C, BG11 | +[d] |
| *Microcoleus* sp. | Filamentous, nonaxenic isolate from Solar Lake by Y. Cohen, Hebrew University | 35°C, BG11 in TIS | + |
| *Oscillatoria* sp. | Filamentous, planktonic gas vacuolated from brackish fishponds, Neve-Ur, Israel | BG11 | − |
| *Oscillatoria* sp. | Filamentous, benthic; sample from marine fish ponds, Elat, Israel | 26°C, seawater | + |
| *Oscillatoria limnetica* | Filamentous, benthic; axenic isolate from hypersaline Solar Lake, Sinai, Israel, by Y. Cohen | 35°C, BG11 in TIS[g] | + |
| *Phormidium* sp. | Filamentous, benthic; Wadi Natrun salt marsh; nonaxenic isolate (22) | 35°C, BG11 in TIS | + |
| *Phormidium* sp. | Filamentous, benthic; from sulfide-rich hot spring near Dead Sea, Israel | 26°C, sulfide-rich spring water | + |
| *Phormidium* sp. strain J-1 | Filamentous, benthic; axenic isolate from freshwater drainage canal, Huleh, Israel, by A. Fattom | 26°C, modified BG11 | + |
| *Plectonema boryanum* | Filamentous, planktonic; strain 594, Indiana University Culture Collection | 26°C, BG11 | − |
| *P. boryanum* | Filamentous, hormogonia-producing, benthic; axenic strain 6306, PCC | 26°C, BG11 | +[d] |
| *Spirulina* sp. | Filamentous, benthic; axenic isolate from hypersaline Bardawill Salt Marsh, Sinai, Israel, by M. Shilo | 35°C, BG11 in TIS | + |
| *Spirulina platensis* (wild type) | Filamentous, planktonic; axenic isolate from freshwater Lake Chad | 26°C, BG11 | − |
| *Spirulina plantensis* mutants 5FT[r] and ACA[r] | Axenic clumping mutants obtained from wild-type by A. Senangelantoni, Paveia University, Italy (41) | 26°C, BG11 | + |
| *Spirulina tenuis* | Filamentous, benthic; axenic isolate from brackish fishpond, Israel (2) | 26°C, BG11 in TIS | + |

[a] Position in the biphasic test system: +, hydrocarbon phase; −, aqueous phase.
[b] Kindly provided by E. Tel Or, Hebrew University.
[c] Kindly provided by A. Kaplan, Hebrew University.
[d] Mature filaments gave + reaction, and hormogonia gave −; compare with Table 3 and Fig. 9.
[e] TIS, Turk's Island salt solution.
[f] Kindly provided by I. Ohad, Hebrew University.
[g] This isolate can be cultured under aerobic conditions or anaerobically (38).

horizontally upside down at the top of a transparent polystyrene water chamber (30 by 10 by 10 cm). A 10-μl air bubble was injected into the chamber with the aid of an Agla micrometer syringe (Burroughs Wellcome, London, England) so that the bubbles floated from the point of release to rest against the cyanobacterial layer. The bubbles were photographed, and the contact angles were measured.

**Preparation of hormogonia.** The four hormogonia-producing strains were cultured as described in Table 1. Cells harvested from the cultures after 7 days of growth by centrifugation (10,000 × *g* for 10 min) were homogenized in a cone-driven stirrer (W. H. Sargent and Co., Chicago, Ill.) in fresh modified BG11. Clumps and long filaments were allowed to settle for 5 min, and then the water column was collected and centrifuged again. The cell pellet obtained was resuspended in modified BG11, put on a continuous Ficoll gradient of 10 to 30%, and centrifuged (400 × *g* for 5 min) in a swinging bucket rotor. The hormogonia formed a distinct band in the region of 10 to 15% Ficoll. Cells from this band were drawn out, washed, and suspended in modified BG11.

**Preparation of OSS.** Osmotically sensitive spheres (OSS) were obtained by using a modification of the method described by Binder et al. (6). The modification included incubation of the mixture for 2 h at 30°C in the dark without stirring (23). Completion of OSS formation was checked microscopically in a phase microscope at 400× magnification.

**Hydrophobicity measurement by adherence to phenyl-Sepharose beads.** One milliliter of phenyl-Sepharose beads (size, 45 to 165 μm; approximate concentration, 40 μmol of gel beads per ml) containing 0.1% demethoxan as preservative (Pharmacia Fine Chemicals) was added to 5 ml of cyanobacterial suspension. Mixtures were thoroughly mixed, and the optical density of the aqueous phase was measured after 5 min (time needed for the phenyl-Sepharose beads to settle).

## RESULTS

**Hydrophobicity of benthic cyanobacteria.** A series of cultured cyanobacterial strains, as well as samples of natural cyanobacterial populations from different ecosystems (in-

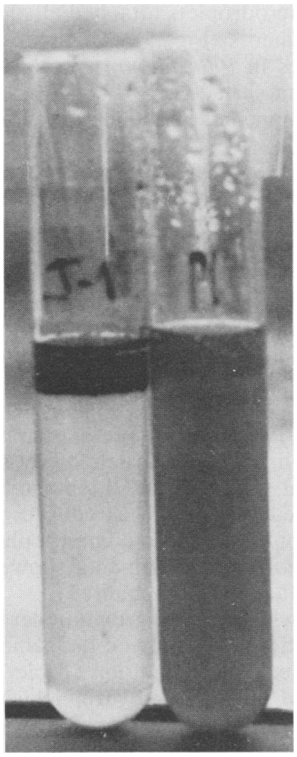

FIG. 1. Partitioning of cyanobacteria in the biphasic system. Test tubes containing *Phormidium* sp. strain J-1 (left) and *P. boryanum* 594 (right) were mixed with *n*-hexadecane. *Phormidium* sp. strain J-1 rose with the hydrocarbon phase, forming a dark green upper layer. *P. boryanum* remained dispersed in the lower water phase.

cluding fresh, brackish, marine, and hypersaline waters), were tested for cell surface hydrophobicity by using partitioning of the cells in a biphasic (aqueous-hydrocarbon) system. In all cases tested, the planktonic cyanobacteria remained in the aqueous phase, whereas all the benthic types concentrated in the hydrocarbon phase (Table 1).

Two typical examples were the filamentous *Phormidium* sp. strain J-1, a benthic form, and the planktonic *Plectonema boryanum* 594. After mixing with *n*-hexadecane, the *Phormidium* filaments transferred to the hydrocarbon upper phase, indicating their hydrophobicity, whereas *P. boryanum* filaments remained in the aqueous phase (Fig. 1). The addition of trace amounts of detergents such as Polo soap (Dorith Chemicals Ltd., Jerusalem, Israel) reversed the phase separation of the benthic phormidium in the water-hexadecane biphasic system.

The cell surface hydrophobicity of the benthic cyanobacteria, shown in the biphasic tests, was confirmed by using several additional independent methods. By using a method proposed by Fletcher and Marshall (17) to measure cell surface hydrophobicity (Fig. 2), the contact angles of gas bubbles in contact with layers of *P. boryanum* 594 were 120 ± 2°, whereas the contact angles with *Phormidium* sp. strain J-1 were 90 ± 2°. These results correlate well with the respective hydrophilicity and hydrophobicity of the strains. In yet another test for hydrophobicity, suspensions of *P. boryanum* 594 and *Phormidium* sp. strain J-1 were thoroughly mixed with phenyl-Sepharose beads. Results showed that none of the *P. boryanum* adhered to the phenyl-Sepharose beads, whereas most (about 90%) of the original *Phormidium* sp. strain J-1 in suspension had been removed.

The adhesion of colonies on solid agar media in petri dishes to polystyrene disks has been described as a replica method for detection of hydrophobic microorganisms (42). By using this method, we found that the benthic *Phormidium* sp. strain J-1 adhered to the polystyrene surface, whereas the planktonic *P. boryanum* 594 did not.

**Localization of hydrophobic sites of *Phormidium* sp. strain J-1.** The behavior of OSS of *Phormidium* sp. strain J-1 was compared to that of untreated *Phormidium* filaments in the biphasic test system. Figure 3 shows that the hydrophobicity displayed by intact filaments was largely abolished by enzymatic treatment with lysozyme, which removed cell wall components.

Table 2 shows that mechanical treatment, in this case shearing off of cell surface layers in an Omnimixer, partially removed the hydrophobic characteristics of the cell surfaces in intact *Phormidium* sp. strain J-1, as well as in filaments of *P. boryanum* 6306 and *Calothrix desertica* 7102. Omnimixer treatment became increasingly efficient in removing hydrophobicity with progressing age of the *Phormidium* culture.

A less radical treatment, consisting of washing and homogenization in fresh medium, did not produce the same effect as mechanical shearing on the cell surface. Table 2 shows that although *Phormidium* sp. strain J-1 remained hydrophobic at all stages of growth, late in the stationary phase (14 to 28 days) a considerable amount of this property was masked. The washing of these late-stationary-phase cells allowed for almost complete expression of the hydrophobicity of the cell surface. Incubation of hydrophobic *Phormidium* sp. strain J-1 cultures in the dark or alternatively in the light in the presence of 10 μg of chloramphenicol per ml for 48 h decreased their hydrophobicity 80 to 22%; reincubation of the cultures in the light or removal of chloramphenicol, respectively, resulted in full initial hydrophobicity expression.

Marshall and Cruickshank (32) pointed out that the orientation of cells to interfaces of biphasic systems is determined

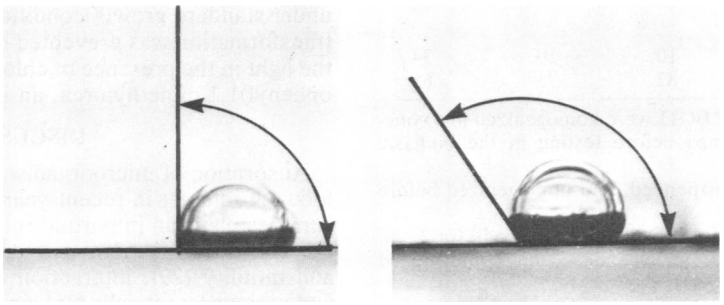

FIG. 2. Curvature of gas bubbles touching inverted *Phormidium* sp. strain J-1 (left) and *P. boryanum* 594 (right) surfaces.

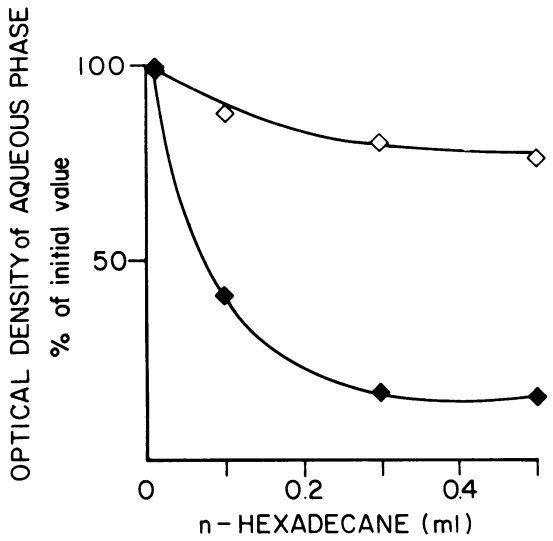


FIG. 3. Partitioning of intact *Phormidium* sp. strain J-1 (◆) and OSS (◇) in the biphasic test system with different amounts of *n*-hexadecane. The biphasic test and the preparation of OSS are described in the text.

by the location of the hydrophobicity sites on the cell surface. *Phormidium* filaments were observed to adhere throughout their lengths to the interfaces with oil droplets (Fig. 4). Additional proof was found in the observation that

TABLE 2. Partitioning of untreated, washed, or sheared cyanobacterial filaments and hormogonia in the biphasic test system

| Cyanobacteria and age (days) | Optical density of aqueous phase (% of initial value) | | |
|---|---|---|---|
| | Untreated[a] | Washed[b] | Sheared in Omnimixer[c] |
| *Phormidium* sp. strain J-1 | | | |
| 3 | 0 | 0 | 30 |
| 7 | 0 | 0 | 30 |
| 14 | 30 | 10 | 50 |
| 21 | 30 | 10 | 50 |
| 28 | 50 | 10 | 65 |
| *Plectonema boryanum* 594 | | | |
| 3 | 100 | 100 | 100 |
| 14 | 100 | 100 | 100 |
| *Plectonema boryanum* 6306 | | | |
| Mature filaments[d] | 15 | 15 | 35 |
| Hormogonia | 100 | 93 | 92 |
| *Calothrix desertica* 7201 | | | |
| Mature filaments[d] | 10 | 10 | 34 |
| Hormogonia | 82 | 75 | 65 |

[a] Culture samples in modified BG11 were homogenized in a cone-driven stirrer to disperse clumps before testing in the biphasic system.

[b] Cells were centrifuged, resuspended, and homogenized before testing.

[c] Washed cells were agitated in an Omnimixer (Sorvall) for 3 min at 12,000 rpm and centrifuged, resuspended, and homogenized before testing.

[d] Hormogonia-producing cyanobacteria cultures were tested after 25 days of growth.

*n*-hexadecane microdroplets attached all along the length of *Phormidium* filaments (Fig. 5). All this indicated that the hydrophobic sites in *Phormidium* sp. are distributed along the entire filament surface.

**Effect of environmental conditions on cell surface hydrophobicity.** Expression of cell surface hydrophobicity required the presence of at least 0.1 mM of a divalent cation, such as $Mg^{2+}$, or much higher concentrations (at least 10 mM) of a monovalent cation, such as $Na^{+}$. Figure 5 shows this effect in the freshwater *Phormidium* sp. strain J-1. Similar tests, but using a 0.5 M sucrose solution for stabilization, were made with *Oscillatoria limnetica* from the hypersaline Solar Lake. The results were similar to those shown in Fig. 6.

When tested at different pH values (range, pH 4 to 11), results with *Phormidium* sp. strain J-1 showed no difference from results obtained with the standard pH, 7.5. *Phormidium* sp. strain J-1 cultured at different temperatures (20 and 35°C) showed cell surface hydrophobicity similar to that of cultures grown at standard (26°C) temperature. Even when grown in modified BG11 with a reduced concentration of nitrate (2% that of the standard content), phosphate (1%), or ferrous (1%), *Phormidium* filaments showed no significant difference in surface hydrophobicity.

*O. limnetica*, which can be grown under either anaerobic or aerobic conditions (38) showed the same hydrophobicity when grown under either condition (Table 1).

**Genetic basis of cell surface hydrophobicity.** A number of mutants obtained from the hydrophilic wild-type *Spirulina platensis* (41) exhibit clumping when grown in liquid media. These same mutants expressed a greater degree of hydrophobicity than the wild type (Fig. 7). Samples of *S. platensis* from open mass culture ponds in which the cyanobacteria tend to clump also showed highly hydrophobic surface characteristics (Fig. 7).

**Hydrophobicity of symbiotic *Anabaena azollae*.** Three varieties of *A. azollae* isolated from the species of the water fern *Azolla* (34) showed marked hydrophobic characteristics (Fig. 8). *A. azollae* occupies a specific cavity in the leaf of the fern and forms close associations with the host cells. In contrast, the planktonic *Anabaena variabilis* exhibited typical hydrophilic characteristics in the biphasic test system.

**Cell surface properties of hormogonia of benthic cyanobacteria.** A number of hormogonia-producing benthic cyanobacteria were tested for their cell surface characteristics. Results indicated that whereas the mature filaments were highly hydrophobic, the hormogonia were hydrophilic (Table 1 and Fig. 9). In an independent test, the hormogonia were found to differ from the mature filaments in that they did not adhere to phenyl-Sepharose beads. Washing or Omnimixer shearing brought a small change in the hydrophilic nature of the hormogonia (Table 2).

The hydrophilic hormogonia of *P. boryanum* 6306 and *C. desertica* became hydrophobic within 48 h when incubated under standard growth conditions in the light (Table 3). This transformation was prevented by incubation in the dark or in the light in the presence of chloramphenicol or 3(3,4-dichlorophenyl)1,1 dimethylurea, an inhibitor of photosystem II.

## DISCUSSION

Adsorption of microorganisms to interfaces has become a focus of interest in recent years (5, 7, 13). Adhesion by cell surfaces plays an important role in many biological processes: contact inhibition, cell differentiation, morphogenesis and motility (29), interaction between pathogenic bacteria and various target cells (25), and the phagocytosis of bacteria (50). Each of these involves highly specialized mechanisms

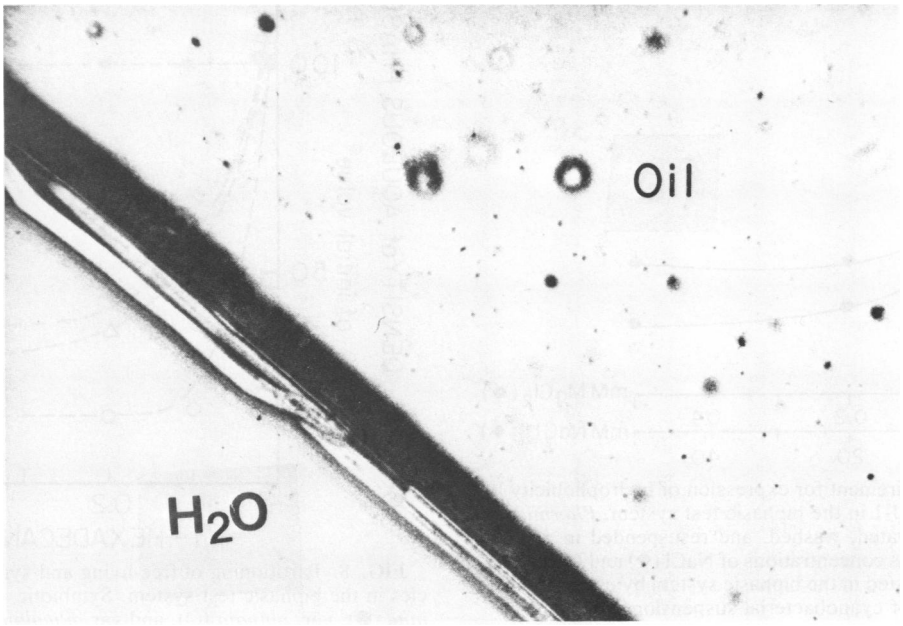


FIG. 4. Photomicrograph of *Phormidium* sp. strain J-1 filament along oil-water interface. A drop of culture suspension was placed alongside an immersion oil droplet. The interface was examined in a Zeiss phase microscope (400×).

of recognition mediated by lectins (sugar and protein carbohydrates) and specific receptors on the cell surfaces. Many microorganisms, such as pathogens and rhizobia on plant root cells (12), adhere to surfaces in this specific way. However, this cannot apply to all these organisms, which attach nonspecifically to many different types of interfaces as well as inert surfaces. Among these are the benthic cyanobacteria.

In addition, there are many microorganisms which depend in nature on the degradation of nonsoluble substrates such as cellulose, chitin, elemental sulfur, and petroleum and which adhere to these substances.

The sorption of bacteria to hydrocarbons and their partitioning in a hydrocarbon-aqueous biphasic system has been suggested as a good method for measuring cell surface hydrophobicity (43). By using this method, we found that all

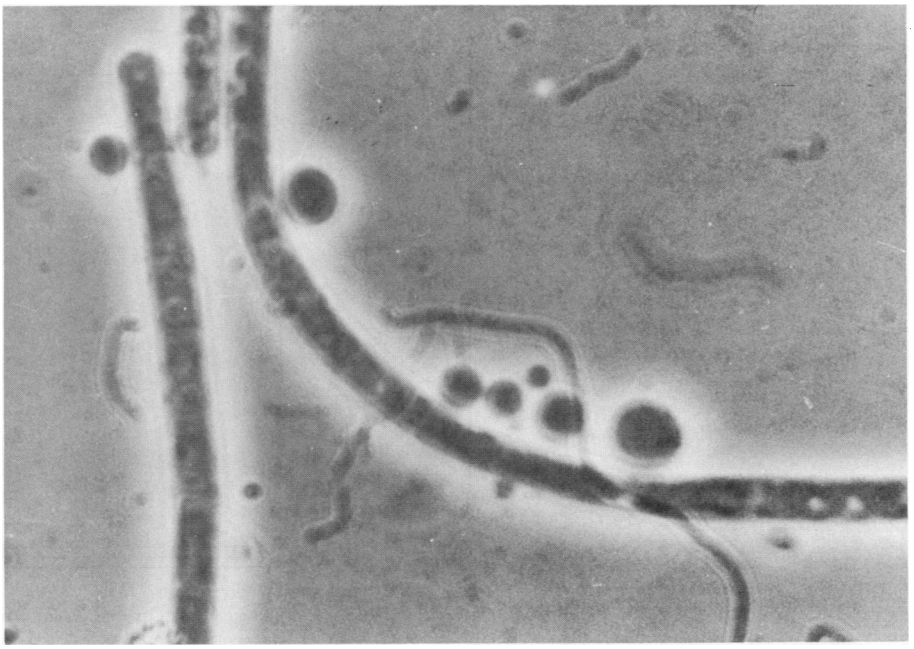

FIG. 5. Photomicrograph of *Phormidium* sp. strain J-1 with *n*-hexadecane microdroplets along filament length (Zeiss phase microscope; 400×).

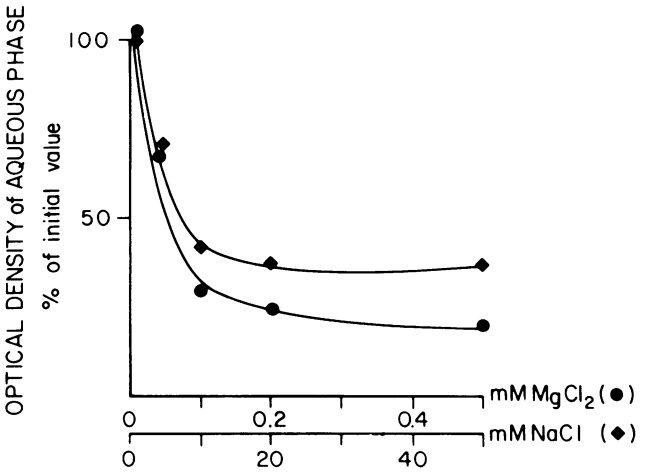


FIG. 6. Cation requirement for expression of hydrophobicity by *Phormidium* sp. strain J-1 in the biphasic test system. *Phormidium* cultures were concentrated, washed, and resuspended in distilled water containing various concentrations of NaCl (◆) and $MgCl_2$ (●). Hydrophobicity was tested in the biphasic system by using 0.5 ml of *n*-hexadecane to 5 ml of cyanobacterial suspension.

the benthic cyanobacteria we tested, whether from natural populations or in axenic cultures, filamentous as well as unicellular, and from various habitats including fresh, brackish, and hypersaline waters, moved into the nonpolar hydrocarbon phase. On the other hand, all the planktonic cyanobacteria tested were hydrophilic and remained in the aqueous phase.

Further confirmation of the hydrophobicity of benthic cyanobacteria was their adhesion to phenyl-Sepharose beads, a typical agent binding hydrophobic cells and molecules, and the contact angle measurements with air bubbles (17). These findings suggest that hydrophobicity plays an important role in the benthic habitat. However, it cannot be concluded that hydrophobicity is the sole mechanism for adhesion of benthic cyanobacteria. Evidence for the role of polymer bridging in the adhesion of bacteria to solid surfaces has been presented (32, 33). Moreover, the hydrophobic nature of *A. azolla* living in close symbiotic contact with the

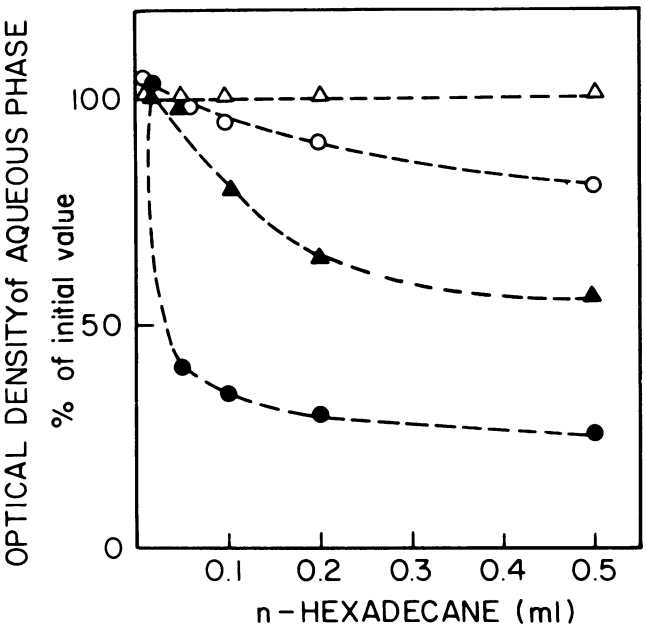


FIG. 7. Partitioning of the planktonic wild-type *S. platensis* and its mutants and mass culture clumping *S. platensis* in the biphasic test system. *S. platensis* wild type (△), mutant $ACA^r$ (○), mutant $5FT^r$ (▲), and clumping mass culture *S. platensis* (●) were tested for cell surface hydrophobicity.

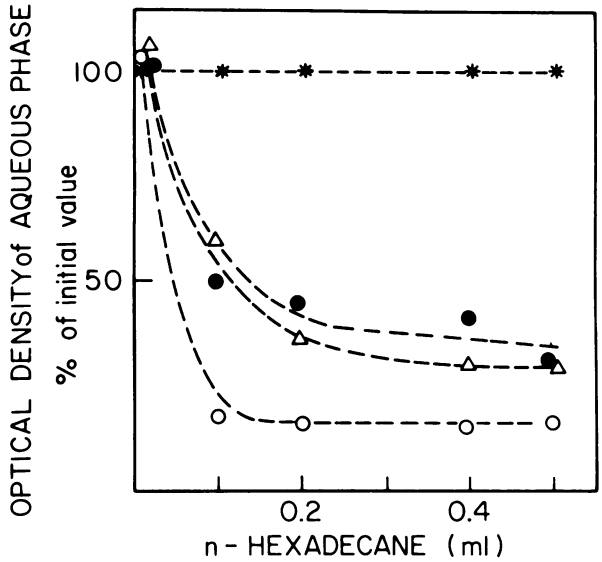


FIG. 8. Partitioning of free-living and symbiotic *Anabaena* species in the biphasic test system. Symbiotic *A. azollae* var. *caroliniana* (●), var. *pinnata* (○), and var. *filiculoides* (△) were tested for cell surface hydrophobicity in the biphasic system. Results are compared to free-living planktonic *A. variabilis* M3 (*).

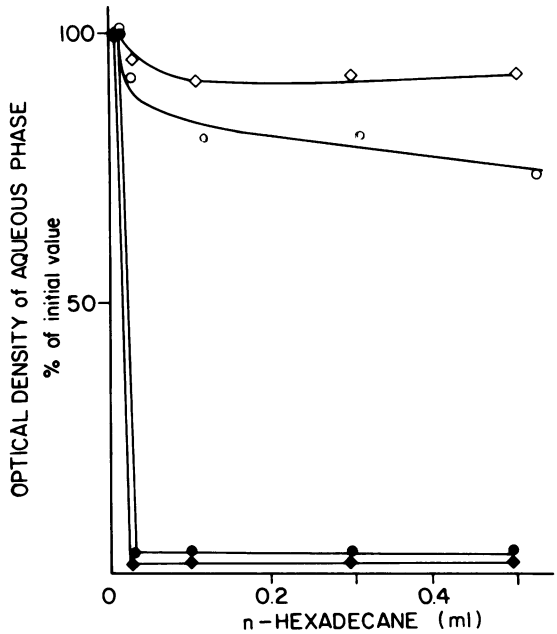


FIG. 9. Partitioning of mature filaments (black symbols) and hormogonia (open symbols) of *C. desertica* (○) and *P. boryanum* 6306 (◇) in the biphasic test system.

TABLE 3. Partitioning of cyanobacteria hormogonia in the biphasic test system before and after incubation under different conditions[a]

| Hormogonia | Optical density of aqueous phase (% of initial value) | | | | |
|---|---|---|---|---|---|
| | No incubation | Incubated for 48 h in modified BG11 at 26°C plus | | | |
| | | Light | Dark | Light + chloramphenicol[b] | Light + DCMU[c] |
| *Plectonema boryanum* 6306 | 88–90 | 20 | 80 | 90 | 90 |
| *Calothrix desertica* 7102 | 90 | 22 | 82 | 90 | 88 |

[a] Hormogonia obtained as described in the text were tested immediately or incubated as indicated for 48 h and then tested.
[b] 10 μg of chloramphenicol per ml of incubation medium.
[c] 5 μM DCMU [3(3,4-dichlorophenyl)1,1 dimethylurea].

cell surfaces of the *Azolla* fern emphasizes that hydrophobicity may play a role in cyanobacterial symbiosis with higher plants and possibly with phycobionts in lichens and with certain diatoms such as *Rhopalodia gibba* (18), *Richellia* sp. (28), *Phozosolema* sp. and *Hemiaules* sp. (30).

Planktonic forms observed to clump together as in bundle formation in *Oscillatoria (Trichodesmium) erythraea* (I. Bryceson and P. Fay, Abstr. IV Int. Symp. on Photosynthetic Procaryotes, Oxford, D2, 1979) or to form large colonial aggregates (*Microcystis* sp.) may represent yet another aspect of the capacity of cyanobacteria to cling to a solid surface, in these cases the cell surface of their cogeners. It would be interesting to test for the correlation between hydrophobicity and the seasonal appearance of bundles, which has been suggested to create a local microaerophilic environment allowing for dinitrogen fixation in the non-heterocystous-forming *O. erythraea* (Bryceson and Fay, Abstr. D2, 1979). The correlation of physiological state and its fluctuations to cell surface hydrophobicity in planktonic cyanobacteria such as colony-forming microcystis also deserves testing.

We observed that the expression of hydrophobicity of benthic cyanobacteria required the presence of cations. Divalent cations such as magnesium and calcium have been shown to influence attachment of microorganisms to surfaces and to be essential for infection of plant roots by phytopathogenic and root nodule bacteria (35). This effect was accounted for by a decrease in the thickness of the electron double layer (33). As a result, repulsion forces between the cell surface and the substratum were reduced (46). In the case of benthic cyanobacteria, the cation might act in neutralizing and masking the negative charges on the cell surface (26), thus decreasing electrostatic repulsion (33). However, this does not exclude other mechanisms indirectly influencing cell physiology or membrane permeability (16). The firm adherence of *Phormidium* sp. strain J-1 filaments to the noncharged surfaces of polystyrene further indicates the role of hydrophobicity in adhesion (42).

It was also shown that the adsorption of hexadecane droplets along *Phormidium* filaments, as well as the positioning of filaments throughout their length along the oil-water interface, indicates that hydrophobic sites are distributed along the entire surface of the filaments. This differs from the situation in many organisms, such as marine *Flexibacter*, *Hyphomicrobium*, and *Caulobacter* sp., which orient themselves perpendicularly to the oil-water interface, indicating the polar location of the hydrophobic site (31, 32, 40).

Removal of the cell wall or part of it by treatment with lysozyme to form OSS or mechanical shearing of the cell envelope in the Omnimixer resulted in the loss of hydrophobicity in *Phormidium* sp. strain J-1. This confirms that hydrophobicity is a surface phenomenon and that the cell envelope alone is responsible for the hydrophobicity expressed in the benthic cyanobacteria.

Cyanobacterial hydrophobicity appears to have a genetic basis. Certain mutants of *S. platensis*, which is hydrophilic in the wild type, have been shown to possess hydrophobic cell envelopes. In another example of genetic control, Simon (48) isolated a mutant from the hydrophobic *Aphanotheca halophytica* which had lost its outer cell envelope. This mutant, without the outer cell envelope layers and lacking the phototactic gliding motility characteristics of the wild-type cyanobacteria, was observed by Simon to have lost its hydrophobic behavior in the biphasic system.

Hydrophobicity in certain cyanobacteria changes phenotypically as a function of culture age (Table 2) or in hormogonia-forming cyanobacteria during the transformation of hormogonia to mature filaments. This may also be the case with baeocyte-forming cyanobacteria, in which the mature cell adheres to the surface, whereas the baeocytes swarm into the water (52).

By their very adherence to a solid surface and their relative immobility, benthic cyanobacteria are exposed to extreme environmental stresses and to environmental fluctuations (24), which the planktonic forms evade through varying their buoyancy and positioning themselves at the optimal conditions. Benthic cyanobacteria, in response to these fluctuating environmental conditions, exhibit extremely varied and flexible modes of metabolism (47). Considering their long evolutionary history (39) and their abundance in a variety of habitats, as well as their important contribution to present-day primary production (8, 19, 27) and nitrogen fixation (9), a more penetrating examination of the physiology of the benthic cyanobacteria seems in order.

Existence at the soil-water interface provides the adhering organism with relatively high concentrations of nutrients. This is an important advantage for surviving in the oligotrophic regime prevailing in oceans and many lakes. At the same time, benthic organisms are exposed to stresses inherent in the sediment-water interface, so that successful adaptation is a prerequisite to their ability to survive in this ecological niche.

Foremost among these is adaptation to low light conditions. This is especially marked in turbid waters rich in suspended particles and plankton. Furthermore, the benthic layer is in a dynamic state, since sediments of particles and cell debris continuously cover the benthic organisms. Although this incessant rain of particles enriches the flow of nutrients in the benthic layer, it further markedly reduces the light reaching benthic organisms. Benthic cyanobacteria have developed positive phototactic gliding motility which allows them to escape burial under sediments and to obtain access to areas with sufficient illumination. This motility is restricted to contact with solid surfaces and may thus involve the hydrophobic faculty of the cell envelope. Another mechanism for ameliorating the illumination condition is the active clarification of waters rich in suspended particles through production of a flocculating agent by the cyanobacteria themselves (3).

Still another problem for benthic organisms is distribution of progeny and colonization of new territory. The dimorphic

life cycle, which includes free-floating forms such as hormogonia or swarming stages, facilitates dispersal and detachment from the benthic interface. Such transformations were shown in this study to require protein synthesis and light energy. Since hormogonia can be obtained synchronously and are produced within a short time period, they represent an ideal system for investigating the sequence involved in the surface changes and in their regulation. It would be interesting to verify to what extent baeocyte formation involves a transformation from hydrophobic to hydrophilic cell surface.

Another mechanism for dispersal of mature filaments by detachment seems to be the masking of cell surface hydrophobicity. This was demonstrated in aged cultures of *Phormidium* sp., which is not biphasic and which formed a substance that prevented expression of the underlying hydrophobicity of the cell envelope. When this substance was washed off, the cyanobacteria displayed almost all their original hydrophobicity. Recently, an additional dispersal mechanism of attached cyanobacteria has been described for *Nostoc muscurom* (1). This involves the induced formation of gas vacuoles in hormogonia but not in mature filaments. This is the only reported case in which gas vacuoles are induced and rapidly lost and are not constitutive as in all other described cases.

Planktonic cyanobacteria can change their position in the water column in response to less than optimal conditions by changes in their buoyancy through intracellular gas vacuoles (51) or their storage granules (R. L. Oliver, H. C. Utkilen, and A. E. Walsby, Abstr. 4th Int. Symp. on Photosynthetic Procaryotes, Bombannes, France, 1982). Benthic cyanobacteria, on the other hand, confined to the interface, must adapt to rapidly fluctuating conditions in their environment (in pH values, Eh, oxygen, $H_2S$, and light) through possessing a versatile metabolism. This requires multiple metabolic patterns which can be activated or stopped quickly. Such shifts have been demonstrated in several benthic cyanobacteria (22). An example of this versatility is *O. limnetica*, which has such a shift in the oxygenic pathway for the photoassimilation of $CO_2$ to a hydrogen sulfide-driven photoassimilation. Its versatility is further demonstrated in its ability to use anoxygenic photosynthetic electron production in the absence of $CO_2$, in hydrogenase-mediated hydrogen production in the absence of $CO_2$, and in nitrogen fixation in the absence of ammonia (4; S. Belkin, Plant Physiol., in press). Furthermore, there are a number of alternative ways to generate energy for, at the least, maintenance in the dark: respiration, fermentation, and even anaerobic respiration (reduction of elemental sulfur to hydrogen sulfide [39]). The marked increase in the level of superoxide dismutase in cells shifted from anaerobic to aerobic growth conditions (21; M. Shilo, A. Oren, and D. Friedberg, Abstr. 3rd Int. Symp. on Photosynthetic Procaryotes, Oxford, 1979) further demonstrates this versatility.

Adhesion to a solid surface has recently become important in studies aimed at industrial processes. Methods were sought for immobilizing cyanobacteria in columns for industrial processes. However, it seems likely, in light of their hydrophobicity and tendency to attach to surfaces, that benthic types would have better potential in such systems. A major problem that has hindered the economic exploitation of mass cultivation of cyanobacteria has been the expense of harvesting the planktonic organisms which were used in most previous studies. The possibility of culturing hydrophobic benthic cyanobacteria and concentrating them in nonpolar solvents may overcome this difficulty.

## ACKNOWLEDGMENTS

This work was supported by the Wolfson Foundation.

We thank J. Bar-Or for his help in the experiment on the effect of age of *Phormidium* sp. on its hydrophobicity and Binah Schor for her advice on the manuscript.

## LITERATURE CITED

1. **Armstrong, R. E., P. K. Hayes, and A. E. Walsby.** 1983. Gas vacuole formation in hormogonia of *Nostoc muscorum*. J. Gen. Microbiol. **128**:263–270.
2. **Aschner, M., H. Leventer, and J. Chorin-Kirsch.** 1967. Off flavour in carp form fishponds in the coastal plain and the Galil. Bamidgeh **19**:23–25.
3. **Avnimelech, Y., B. W. Troeger, and L. W. Reed.** 1982. Mutual flocculation of algae and clay evidence and implications. Science **216**:63–65.
4. **Belkin, S., and E. Padan.** 1978. Sulfide dependent hydrogen evolution and $CO_2$ photoassimilation by the cyanobacterium *Oscillatoria limnetica* p. 381–394. *In* H. G. Schlegel, K. Schneider (ed.), Hydrogenases—their catalytic activity, structure and function. E. Goltze K. C., Gottingen.
5. **Berkeley, R. C. W., J. M. Lynch, J. Melling, P. R. Rutter, and B. Vincent (ed.).** 1980. Microbial adhesion to surfaces. Society of Chemical Industry, London.
6. **Binder, A., E. Tel-Or, and M. Avron.** 1976. Photosynthetic activities of membrane preparations of blue-green algae phormidium luridium. Eur. J. Biochem. **67**:187–196.
7. **Bitton, G., and K. C. Marshall (ed.).** 1980. Adsorption of microorganisms to surfaces. John Wiley & Sons, Inc., New York.
8. **Brock, T. D., and M. L. Brock.** 1967. The measurement of chlorophyll, primary productivity, photo phosphorylation, and micromolecules in benthic algal mats. Limnol. Oceanogr. **12**:600–605.
9. **Capone, D. G., and E. J. Carpenter.** 1982. Nitrogen fixation in the marine environment. Science **217**:1140–1142.
10. **Cohen, Y., W. E. Krumbein, and M. Shilo.** 1977. Solar Lake Sinai. 2. Distribution of photosynthetic microorganisms and primary production. Limnol. Oceanogr. **22**:609–620.
11. **Dahlback, B., M. Hermannson, S. Kjelleberg, and B. Norkrans.** 1981. The hydrophobicity of bacteria—an important factor in their initial adhesion at air-water interface. Arch. Microbiol. **128**:267–670.
12. **Dazzo, F. E.** 1980. Adsorption of microorganisms to roots and other plant surfaces. *In* G. Bitton and K. C. Marshall (ed.), Adsorption of microorganisms to surface. Wiley-Interscience, New York.
13. **Ellwood, D. C., J. Melling, and P. Rutter.** 1979. Adhesion of microorganisms to surfaces. Academic Press, Inc., New York.
14. **Faris, A., M. Lindahl, and T. Wadstrom.** 1982. High surface hydrophobicity of hemagglutinating *Vibrio cholerae* and other vibrios. Curr. Microbiol. **7**:357–362.
15. **Faris, A., T. Wadstrom, and J. H. Freer.** 1981. Hydrophobic adsorptive and hemagglutinatic properties of *E. coli* possessing colonization factor antigen (CFA/I or CFA/II/type 1 pili, or other pili). Curr. Microbiol. **5**:67–72.
16. **Fletcher, M.** 1980. Adherence of marine microorganisms to smooth surfaces, p. 345–374. *In* E. H. Beachy (ed.), Bacterial adherence. Chapman and Hall, London.
17. **Fletcher, M. and K. C. Marshall.** 1982. Bubble contact angle method for evaluating substratum interfacial characteristics and its relevance to bacterial attachment. Appl. Environ. Microbiol. **44**:189–192.
18. **Floener, L., and H. Bothe.** 1980. Nitrogen fixation in *Rhopalodia gibba*, a diatom containing blue-greenish inclusions symbiotically, p. 541–552. *In* W. Schwemmler and H. E. A. Schenk (ed.), Endocytobiology, endosymbiosis and cell biology: a synthesis of recent research, vol I. Volterde Gruyter, Berlin.
19. **Fogg, G. E., and A. J. Horne.** 1970. The physiology of antarctic fresh water algae, p. 632–638. *In* M. W. Holdgate (ed.), Antarctic ecology, vol. 2. Academic Press, Inc., New York.
20. **Fogg, G. E., W. D. P. Stewart, P. Fay, and A. E. Walsby.** 1973.

The blue-green algae. Academic Press, Inc., New York.
21. **Friedberg, D., M. Fine, and A. Oren.** 1979. Effect of oxygen on the cyanobacteria *Oscillatoria limnetica*. Arch. Microbiol. **123**:311–313.
22. **Garlick, S., A. Oren, and E. Padan.** 1977. Occurrence of facultative anoxygenic photosynthesis among filamentous and unicellular cyanobacteria. J. Bacteriol. **128**:623–629.
23. **Ginzburg, D., and E. Padan.** 1972. Light effect on the internal osmotic pressure of the blue-green algae *Plectonema boryanum* and *Phormidium luridium*. Arch. Microbiol. **87**:181–183.
24. **Gnaiger, E., I. Glinth, and W. Wieser.** 1978. pH fluctuation in an intertidal beach in Bermuda. Limnol. Oceanogr. **23**:851–857.
25. **Halt, S. C.** 1982. Bacterial adhesion in pathogenesis: an introductory statement, p. 261–265. *In* D. Schlessinger (ed.), Microbiology—1981. American Society for Microbiology, Washington, D. C.
26. **Harden, V. P., and J. O. Harris.** 1953. The isoelectric point of bacterial cells. J. Bacteriol. **65**:198–202.
27. **Jorgensen, B. B., N. P. Revsbach, T. H. Blackburn, and Y. Cohen.** 1979. Diurnal cycle of oxygen and sulfide micro gradients and microbial photosynthesis in a cyanobacterial mat sediment. Appl. Environ. Microbiol. **38**:46–58.
28. **Kimor, B., F. M. H. Reid, and J. B. Jordan.** 1978. Phycologia **17**:162–166.
29. **Letourneau, P. C., P. N. Ray, and M. R. Bernfield.** 1980. The regulation of cell behavior by cell adhesion. *In* K. F. Goldberger (ed.), Biological regulation and development, vol. 2. Plenum Publishing Corp., New York.
30. **Mague, T. H.** 1977. *In* R. W. F. Hardy and A. H. Gibson (ed.), A treatise on dinitrogen fixation, p. 85–140. John Wiley & Sons, Inc., New York.
31. **Marshall, K. C.** 1976. Interfaces in microbial ecology. Harvard University Press, Cambridge, Mass.
32. **Marshall, K. C., and R. H. Cruickshank.** 1973. Cell surface hydrophobicity and the orientation of certain bacteria at interfaces. Arch. Microbiol. **91**:29–40.
33. **Marshall, K. C., R. Stout, and R. Mitchell.** 1971. Mechanism of the initial events in the sorption of marine bacteria to surface. J. Gen. Microbiol. **68**:337–348.
34. **Newton, J. W., and A. I. Herman.** 1979. Isolation of cyanobacteria from the aquatic fern Azolla. Arch. Microbiol. **120**:161–165.
35. **Nissen, P.** 1971. Choline sulfate permease: transfer of information from bacteria to higher plants. II. Induction processes, p. 201–212. *In* L. Ledoux (ed.), Information molecules in biological systems. North Holland, Amsterdam.
36. **Ofek, I., D. Mirelman, and N. Sharon.** 1977. Adherence of *E. coli* to human mucosal cells mediated by mannose receptors. Nature (London) **265**:623–625.
37. **Olsson, J., and G. Westergren.** 1982. Hydrophobic properties of oral streptococci. FEMS Microbiol. Lett. **15**:319–323.
38. **Oren, A., and E. Padan.** 1978. Induction of anaerobic photoautotrophic growth in cyanobacterium *Oscillatoria limnetica*. J. Bacteriol. **133**:558–563.
39. **Oren, A., and M. Shilo.** 1979. Anaerobic heterotrophic dark metabolism in the cyanobacterium *Oscillatoria limnetica*; sulfur respiration and lactate fermentation. Arch. Microbiol. **122**:77–84.
40. **Poindexter, J.** 1981. The caulobacter: ubiquitous unusual bacteria. Microbiol. Rev. **45**:123–179.
41. **Riccardi, G., A. M. Sanangelantoni, D. Carbonera, A. Savi, and O. Ciferri.** 1981. Characterization of mutants of *Spirulina platensis* resistant to amino acid analogues. FEMS Microbiol. Lett. **12**:333–336.
42. **Rosenberg, M.** 1981. Bacterial adherence to polysterene: a replica method of screening for bacterial hydrophobicity. Appl. Environ. Microbiol. **42**:375–377.
43. **Rosenberg, M., D. Gutnick, and E. Rosenberg.** 1980. Adherence of bacteria to hydrocarbons: a simple method for measuring cell-surface hydrophobicity. FEMS Microbiol. Lett. **9**:29–33.
44. **Schopf, J. W., and E. S. Barghoorn.** 1967. Algae-like fossils from the early Precambrian of South Africa. Science **156**:508–512.
45. **Sharon, N., and H. Lis.** 1972. Lectins: Cell-agglutinating and sugar-specific proteins. Science **177**:949–959.
46. **Shaw, D. J.** 1970. Introduction to colloid and surface chemistry, 2nd ed. Butterworths, London.
47. **Shilo, M.** 1982. Photosynthetic microbial communities in aquatic ecosystems. Phil. Trans. B. Soc. Lond. B **297**:565–574.
48. **Simon, R.** 1981. Gliding motility in *Aphanotheca halophytice*: analysis of wall proteins in mot mutants. J. Bacteriol. **148**:315–321.
49. **Stanier, R. Y., R. Kunisawa, M. Mandel, and G. Cohen-Bazire.** 1971. Purification and properties of unicellular blue-green algae (order Chroccoccales). Bacteriol. Rev. **35**:171–205.
50. **Van Oss, C. J.** 1978. Phagocytosis as a surface phenomenon. Annu. Rev. Microbiol. **32**:19–39.
51. **Walsby, A.** 1972. Structure and function of gas vacuoles. Bacteriol. Rev. **36**:1–32.
52. **Waterbury, J., and R. Y. Stanier.** 1978. Patterns of growth and development in pleurocapsalean cyanobacteria. Microbiol. Rev. **142**:2–44.