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Lysis of Blue-Green Algae by Myxobacter

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Enrichment from local fishponds led to the isolation of a bacterium capable of lysing many species of unicellular and filamentous blue-green algae, as well as certain bacteria. The isolate is an aflagellate, motile rod which moves in a gliding, flexuous manner; the organism is capable of digesting starch and agar, but not cellulose and gelatin. Its deoxyribonucleic acid base pair composition (per cent guanine plus cytosine ~70) shows a close resemblance to that of the fruiting myxobacteria. Algae in lawns on agar plates were lysed rapidly by the myxobacter, but only limited and slow lysis occurred in liquid media, and no lysis took place when liquid cultures were shaken. No diffusible lytic factors would be demonstrated. Continuous observation of the lytic process under a phase-contrast microscope suggested that a close contact between the polar tip of the myxobacter and the alga is necessary for lysis. The lytic action is limited to the vegetative cells of the algae, whereas heterocysts are not affected. The gas vacuoles of the algal host are the only remnant visible after completion of digestion by the myxobacter.

Different investigators have described lysis of microorganisms by bacteria. Certain bacteria in the soil are known to lyse *Azobacteriaceae* (22) and fungi (19). Special emphasis has been placed on studies of myxobacteria which lyse other bacteria (2, 5, 8).

The isolation of myxobacteria which decompose blue-green algae (25, 30) has demonstrated that the range of action of these bacteria is even wider. The lysis of blue-green algae by myxobacteria may possibly be a factor in population dynamics of algae in nature and may contribute to the often-observed sudden disappearance of blue-green blooms in the natural milieu.

The enrichment, isolation, and characterization of a myxobacter which lyses blue-green algae and the mode of lysis of the algae are described in this paper.

MATERIALS AND METHODS

Microorganisms, media, and culture conditions. The blue-green algae employed in this study were *Anacystis nidulans* 6301, *Coccochloris penyocystis* 6307, *Synechococcus cedorum*, and *Nostoc* sp. (from the Department of Bacteriology, University of California, Berkeley); *Plectonema boryanum*, *Anabaena cylindrica* 629, *A. cylindrica* 381, and *Oscillatoria prolifera* (from Culture Collection of Algae at the University of Indiana, Bloomington); *O. amphibiae* (isolated from fishponds in Israel); *Spirulina platensis* (isolated from Lake Bodou in Kanem, Tchad, kindly supplied by J. Leonard, University of Brussels); and *S. tenuis* (a halophilic strain isolated from the Bardawil Lagoon, northern Sinai).

Other algae included *Chlorella pyrenoidosa* (Department of Botany, Hebrew University, Jerusalem, Israel) and *Prymnesium parvum* (isolated from fishponds in Israel and preserved in the collection of this department).

Bacteria included *Staphylococcus aureus*, *Aerobacter aerogenes*, *Escherichia coli* K-12, *E. coli* O111, *Salmonella typhimurium* G30C21, *Bacillus subtilis*, *Pseudomonas fluorescens*, and *B. cereus* (all from our collection).

The blue-green algae and *Chlorella* were grown on the medium of Hughes, Gorham, and Zehnder (7) as modified by Mennes-Allen and Stanier (17); the halophilic *Spirulina tenuis* was grown on the same medium prepared with filtered seawater instead of distilled water. *S. platensis* was grown on a modified medium of Lefèvre (J. Leonard, *personal communication*) containing (g/liter): $NaHCO_3$, 16.8; K_2HPO_4, 0.5; $NaNO_3$, 2.5; K_2SO_4, 1.0; NaCl, 1.0; $MgSO_4 \cdot 7H_2O$, 0.2; $CaCl_2$, 0.4; $FeSO_4 \cdot 7H_2O$, 0.01; ethylenediaminetetraacetate, 0.8; and 1 ml of solutions A_5 and B_6, respectively. [A_5 contained (g/liter): H_3BO_3, 2.86; $MnCl_2 \cdot 4H_2O$, 1.81; $ZnSO_4 \cdot 7H_2O$, 0.222; $CuSO_4 \cdot 5H_2O$, 0.079; MoO_2, 0.015. B_6 contained (g/liter): NH_4VO_3, 229.6 $\times 10^{-4}$; $K_2Cr(SO_4)_4 \cdot 24H_2O$, 960 $\times 10^{-4}$; $NiSO_4 \cdot 7H_2O$, 472.5 $\times 10^{-4}$; $Na_2WO_4 \cdot 2H_2O$, 179.4 $\times 10^{-4}$; $Ti(SO_4)_3$, 400 $\times 10^{-4}$; $Co(NO_3)_2 \cdot 6H_2O$, 439.8 $\times 10^{-4}$.]

Prymnesium parvum was grown on a modified Droop S50 medium (31). All algae were incubated at 24 to 26 C in 250-ml Erlenmeyer flasks containing 100 ml of medium under continuous illumination of white fluorescent lamps giving an incident light intensity of 600 to 800 ft-c without shaking (except for *Spirulina platensis*, which was grown on shaker).

The myxobacter isolated (designated strain FP-1;

deposited with the American Type Culture Collection, and with the National Collection of Industrial Bacteria Torry Research Station, Aberdeen, Scotland) was grown in one-membered culture at 26 C without shaking on a modified Chu No. 10 medium (21) with the addition of 0.2% Casitone (Difco). Formation of fruiting bodies was tested on the Ca^{2+}-water-agar described by McCurdy (9). Other bacteria were grown on Nutrient Broth (Difco) at 37 C (except for *P. fluorescens*, which was incubated at 30 C).

Tests for lysis of blue-green algae. Various techniques were used for testing lytic activity.

Plaque formation was obtained by the soft-agar overlayer technique used in the estimation of cyanophages (23).

Lysis of algae in liquid media was shown by use of algal cultures in the logarithmic growth phase. The myxobacter was harvested after 5 days of growth, resuspended in the algal medium, and added to the algal culture to give a final concentration of 2×10^9 myxobacters/ml. After different incubation times, samples were taken for microscopic examination.

Continuous microscopic observation of the lytic sequence was made with a phase-contrast microscope. Lysis was followed microscopically by use of thin-layer agar preparations made by spreading 0.2 ml of an alga-myxobacter mixture on 10 ml of solid agar medium (1% Difco agar) in a petri dish. Immediately after being spread with a Drigalski rod, a square of the thin-layer agar was cut, placed on a slide, and covered. Microscopic examination started immediately and continued for several hours, until lysis was complete.

Extraction of deoxyribonucleic acid (DNA) from myxobacter FP-1 and determination of base pair ratio. DNA was isolated essentially as described by Marmur (14): 3 to 4 g of wet-packed myxobacterial cells served for the DNA extraction; complete lysis of the cells was obtained by the addition of sodium lauryl sulfate, and lysozyme treatment was not necessary. The DNA was dissolved in a saline-citrate buffer (0.15 M NaCl and 0.015 M sodium citrate) adjusted to *p*H 7, to a concentration of 0.2 mg of DNA/ml; the solution was stored at −20 C in the presence of several drops of chloroform. Since the DNA of our isolate has high guanine plus cytosine (G3) contents, the thermal denaturation was carried out in one-tenth concentration of the saline-sodium citrate buffer. The temperature values under these conditions are 15.4 C lower than in the undiluted buffer. The base pair ratio was calculated from the equation $GC = (T_m - 53.9)\ 2.44$, as described by Mandel and Marmur (13). The base pair ratio was also determined from the buoyant density of DNA in a CsCl density gradient in an analytical ultracentrifuge (Spinco model E) as described by Meselson et al. (18). The conversion into mole % GC was calculated according to Schildkraut et al. (24).

Pigment extraction. Myxobacter FP-1 was grown on the medium described above for 10 days at 26 to 28 C and was harvested by centrifugation. The wet-packed cells were then extracted in the lower phase of a chloroform-methanol-water (8:4:3, v/v) mixture (6). The solution was dried and dissolved in *n*-hexane, and its absorption spectrum was recorded in a Perkin-Elmer spectrophotometer (model 137 UV).

RESULTS AND DISCUSSION

Isolation, enrichment, and characterization of myxobacter FP-1. Bacteria were isolated from water samples collected during blooms of blue-green algae from fishponds in the Jezrael Valley of Israel (at Kibbutz Geva). The samples were filtered and concentrated by the technique of Padan et al. (21) for isolation of cyanophages, and were enriched for bacteria that lyse blue-green algae by incubation with *Plectonema boryanum* as described for cyanophage enrichment (21). Samples from the enrichment culture were plated on lawns of *Plectonema*.

Typical plaques of myxobacters appeared on the lawns after 5 to 7 days (Fig. 1), whereas cyanophage plaques appeared after only 2 to 3 days (21). The bacterial plaques also differed from the cyanophage plaques by being sunken into the agar (Fig. 2).

Bacteria isolated from single typical plaques on

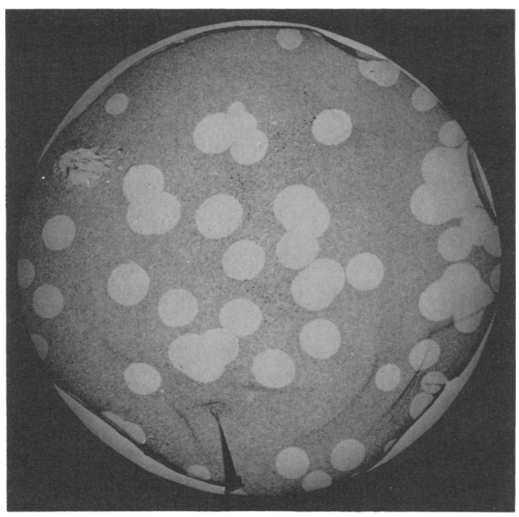

FIG. 1. *Plaques of myxobacter FP-1 on Nostoc lawns.*

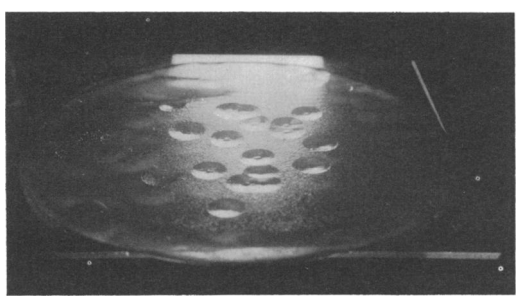

FIG. 2. *Myxobacterial plaques (strain FP-1) on a Plectonema boryanum lawn. Note shallow depressions formed on the agar surface.*

those lawns were grown further in cultures of *Plectonema*. The bacteria were separated from the algae in liquid culture by transfer into the myxobacterial medium (supplemented modified Chu No. 10) and growth in the dark.

One of the bacteria isolated (strain FP-1) was investigated further. It was found to be a gram-negative organism with rounded ends, having two or more dense areas located usually at the two poles, as could be clearly seen by phase-contrast and electron microscopy (Fig. 3 and 4). The isolate was of variable length (3 to 9 μm by 0.6 to 1.0 μm) and usually increased in length with age. It was aflagellate and had a very slow gliding flexuous motion which could be observed directly under a phase-contrast microscope after cell suspensions had been spread on thin solid medium.

Electron micrographs of strain FP-1 (Fig. 4) show mesosome-like structures, either at its polar ends or in the center. In some cases, cell division occurred in the mesosomal area (Fig. 4b).

H. Reichenbach (Department of Microbiology, University of Minnesota Medical School) examined strain FP-1 and found that trails of slime were left behind the gliding organism on thin agar layers. In his experiments, the cells did not cluster together and did not form fruiting bodies. When tested by us on Ca^{2+}-water-agar, no fruiting bodies typical of the fruiting myxobacteria were obtained.

Colonies and liquid cultures of strain FP-1 were salmon-colored. The absorption spectrum of the pigment showed peaks at 455, 485, and 518 nm in Folch's solvent mixture, and at 455, 482, and 516 nm in *n*-hexane (Fig. 5). When grown on solid agar medium or on lawns of blue-green algae, the colonies formed depressions or shallow craters about 5 mm in diameter (Fig. 2) on the agar, with a surrounding gelase field of about 10 mm (demonstrated by KI stain). Strain FP-1 digested starch but not cellulose in liquid or solid medium, and it did not liquefy gelatin.

A typical characteristic of many myxobacteria, recently described by Dworkin (3), is their high sensitivity to actinomycin D. Whereas 1 μg of actinomycin D/ml completely inhibited the growth of *Myxococcus xanthus*, nongliding gram-negative bacteria were inhibited only by 100 μg/ml. Therefore, the sensitivity of strain FP-1 to actinomycin D (Calbiochem) was tested. Different concentrations of the antibiotic were added to 5 ml of culture in the logarithmic growth phase, and the effect was observed for 2 to 3 days. It was found that 2 μg/ml completely inhibited growth, further supporting identification of the bacterium as a myxobacter.

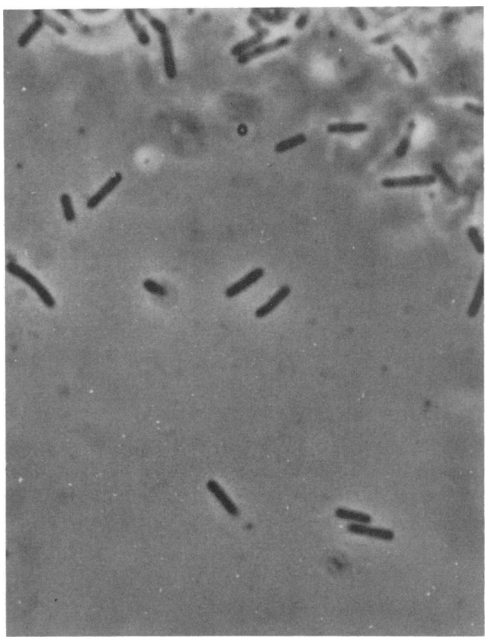

FIG. 3. *Myxobacters (strain FP-1) from soft-agar cultures, 5 days old. Zeiss phase-contrast microscope.* × *4,000.*

According to Mitchell et al. (20), most strains of *Cytophaga* are resistant to polymyxin B, and can be separated from the fruiting myxobacteria by their reaction to this antibiotic. The susceptibility of myxobacter FP-1 to polymyxin B (sulfate; (Pfizer Laboratories, New York, N.Y.) and polymyxin E (Colimicina Laboratori Smit, Torino, Italy) was tested at concentrations of 2.5, 5, 10, and 25 μg/ml in Casitone liquid medium and at a concentration of 25 μg/ml in Casitone solid agar medium. Strain FP-1 was sensitive to the antibiotics at all concentrations tested. Tests for susceptibility to various other antibiotics on paper discs showed that myxobacter FP-1 is sensitive to kanamycin (5 μg) and neomycin (5 μg), but not to streptomycin (2 μg), penicillin G (2 units), erythromycin (2 μg), chloramphenicol (5 μg), and tetracycline (5 μg).

The DNA base pair ratios (%GC) of myxobacteria fall into two discrete groups with respective ranges of 30 to 40% and 70 to 75%. The group having lower values consists of nonfruiting species such as *Cytophaga* and *Flexibacter*, whereas the %GC of the higher fruiting myxobacteria is about 70% (2, 5, 10, 12, 13, 16). Buoyant density of the DNA of myxobacter FP-1 in a CsCl gradient showed a single peak at 1.729 g/cc, indicating a %GC of 70. The schlieren refractive index pattern showed that the DNA sample was free from

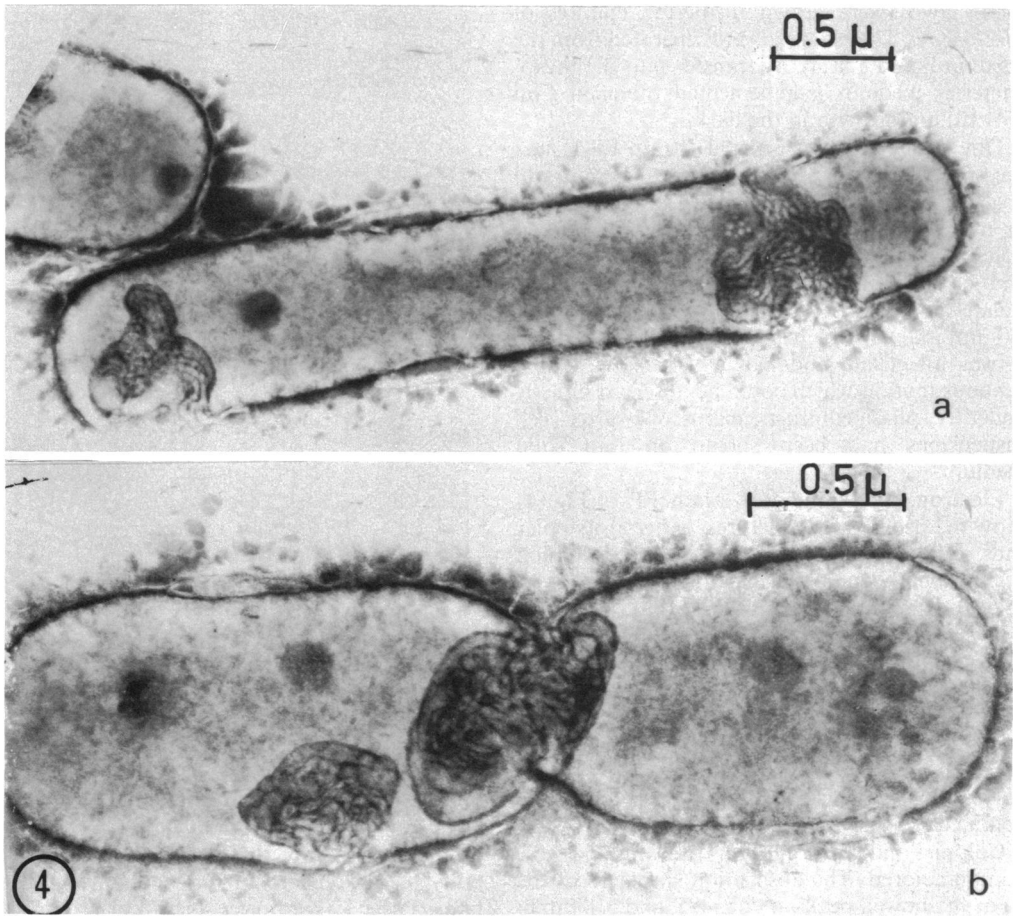

FIG. 4. *Electron micrographs of myxobacter strain FP-1. Note mesosomelike structures close to the poles of the cell (a), or during division (b). Micrographs were made with an AEI EM68 electron microscope operating at 60 kv; 1% phosphotungstic acid was used for negative staining for 20 sec.*

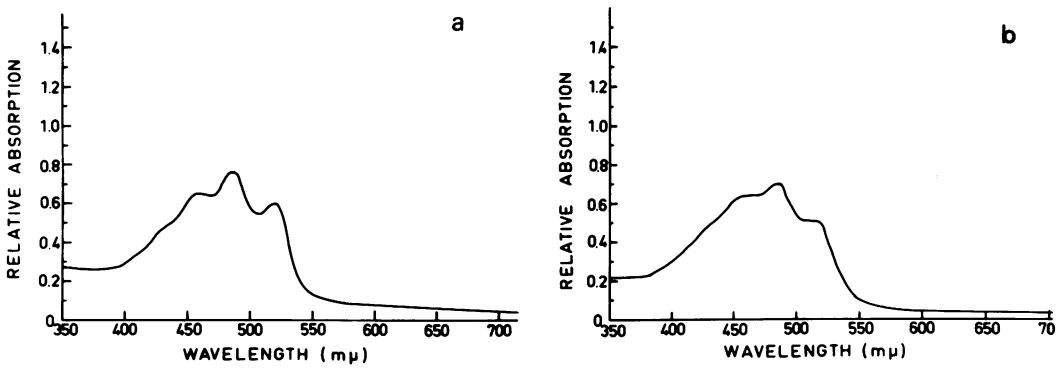

FIG. 5. *Absorption curve of pigment extract from myxobacter strain FP-1 in lower phase of Folch's solvent mixture (a) and n-hexane (b).*

light-scattering contaminants (such as polysaccharides). Determination of the GC content of strain FP-1, based on the ultraviolet absorbance temperature profile gave the value of 72.2% GC, and thus corroborated the %GC of 70 found by buoyant density estimation.

The %GC of ~70 found for strain FP-1 suggests a taxonomic relationship to the fruiting myxobacteria, in spite of superficial similarity to the *Cytophaga* group. Thus, it might be considered a nonfruiting variant related to the higher myxobacteria. In this sense, strain FP-1 may thus be similar to several myxobacterial strains, formerly considered to be related to *Cytophaga* (which were shown to contain a %GC of 67 to 69) and to be members of the *Myxococcus* and *Polyangium* genera.

Growth conditions. Growth of myxobacter FP-1 on modified Chu No. 10 medium was followed at different concentrations of Casitone (Fig. 6a) and at different temperatures (Fig. 6b). These figures show that optimal growth occurs in 0.2% Casitone and at 26 C. Myxobacter FP-1 did not grow on Nutrient Broth or Nutrient Agar or on unsupplemented modified Chu No. 10 medium. Addition to the latter of glucose (0.1%), Casamino Acids (0.05 to 0.2%), tryptone (0.1%), or Difco yeast extract (0.05 to 0.1%) did not allow significant growth. Although the unsupplemented modified Chu medium gave no growth, myxobacterial craters occurred on lawns of sensitive blue-green algae and bacteria on this medium.

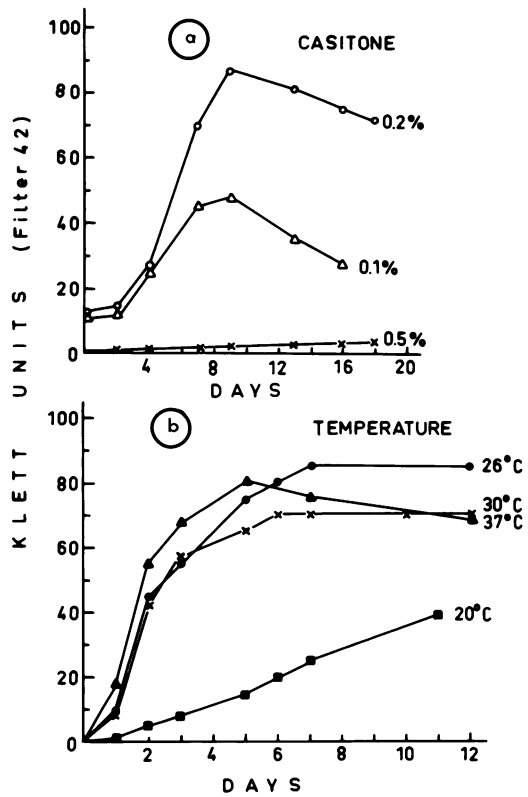

FIG. 6. *Growth of myxobacter strain FP-1 on Casitone-supplemented modified Chu No. 10 medium at different concentrations of Casitone (a) and at different temperatures (b).*

Spectrum of lytic activity. Myxobacter FP-1 showed a broad host range when tested for plaque-forming activity on blue-green algae (Table 1). In addition to the unicellular species, many, but not all, filamentous species (Fig. 7 and 8) were lysed rapidly. The chrysophyte *Prymnesium parvum* and the chlorophyte *Chlorella pyrenoidosa* were not lysed or killed, but some of the bacteria listed were lysed (Table 1).

Rapid lysis of sensitive blue-green algae by myxobacter FP-1 was characteristic on solid media. On the other hand, lysis was slow and irregular in two-membered cultures in liquid media in Erlenmeyer flasks. When such cultures

TABLE 1. *Sensitivity of algae and bacteria to lytic activity of myxobacter strain FP-1*

Organism	Lysed
Cyanophyta	
Unicellular	
Anacystis nidulans 6301	+
Coccochloris penyocystis 6307	+
Synechococcus cedorum	+
Filamentous	
Nostoc sp. 6305	+
Plectonema boryanum	+
Anabaena cylindrica 629[a]	−
A. cylindrica 381[a]	−
Oscillatoria amphibiae	−
O. prolifera 1270	+
Spirulina platensis[a]	+
S. tenuis[a]	+
Chlorophyta	
Chlorella pyrenoidosa	−
Chrysophyta	
Prymnesium parum	−
Eubacteriales	
Aerobacter aerogenes	+
Escherichia coli K-12	+
E. coli O111	+
Salmonella typhimurium G30C21	+
Bacillus subtilis	−
B. cereus	−
Pseudomonas fluorescens	+
Staphylococcus aureus	−

[a] Nonaxenic cultures. All other species were in axenic culture.

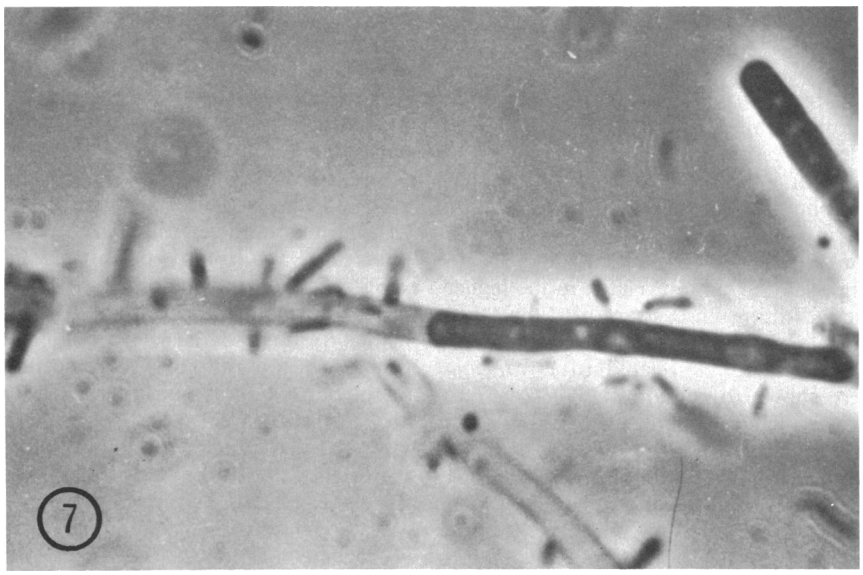

FIG. 7. *Lysis of Plectonema boryanum by myxobacter strain FP-1. Zeiss phase-contrast microscope.* × *4,000*

were shaken, lysis was prevented. In small volumes of liquid medium (2.5 ml) in test tubes with high concentrations of myxobacter FP-1, rapid lysis of all sensitive blue-green algae occurred.

Microscopic examination of myxobacterial plaques on *Nostoc* lawns showed that all of the vegetative cells in the algal filaments were completely lysed, whereas heterocysts remained unaffected (Fig. 8b). The gas vacuoles in the lysed cells also remained intact (Fig. 8c). A similar observation was reported by Singh and Singh (27) with cyanophages. This may provide a means for obtaining heterocysts and gas vacuoles in pure suspensions for study of their physiological capacities in isolation. Cells of *Nostoc* heated to 70, 100, and 120 C were all lysed by the myxobacter.

Mode of action of myxobacter FP-1 on blue-green algae. Direct continuous examination of the lytic process under a phase-contrast microscope on a thin agar film (1 mm thick) showed that lysis occurred only when there was direct contact between one of the polar ends of the myxobacter and the sensitive blue-green alga. Such a sequence is shown in Fig. 9. Here a single myxobacter (marked with arrow) was seen to cause lysis of the *Nostoc* cells within 20 min from the time of polar contact. After lysing the first blue-green cell (at 20 min), the same myxobacterial individual moved on, and attacked and lysed a neighboring cell (22 to 32 min). Figure 10 shows lysis of *Nostoc* cells upon multiple attack.

Experiments were undertaken to separate an extracellular lytic factor by use of filtered concentrated supernatants of two-membered cultures (myxobacter-*Nostoc*), as well as homogenates of the myxobacter (disintegrated in a Braun homogenizer). In all cases, none of the preparations lysed *Nostoc* in solid or liquid cultures. Since it appears that direct contact between the myxobacter and the blue-green alga is required for lysis, it is conceivable that the lysing enzyme is not excreted into the medium but that surface enzymes may be involved, in a manner resembling the digestion of cellulose fibers by *Cytophaga* (28, 29).

Lysis of the alga seems to require contact with the polar end of the myxobacterial cell for a certain period of time (10 to 20 min). This condition seems to rule out effective lysis in liquid media, as found above, and explains the inhibitory effect of shaking. The lack of special attachment organelles, like those found in *Bdellovibrio* (26), also indicates the special importance of suitable physical conditions allowing prolonged undisturbed contact.

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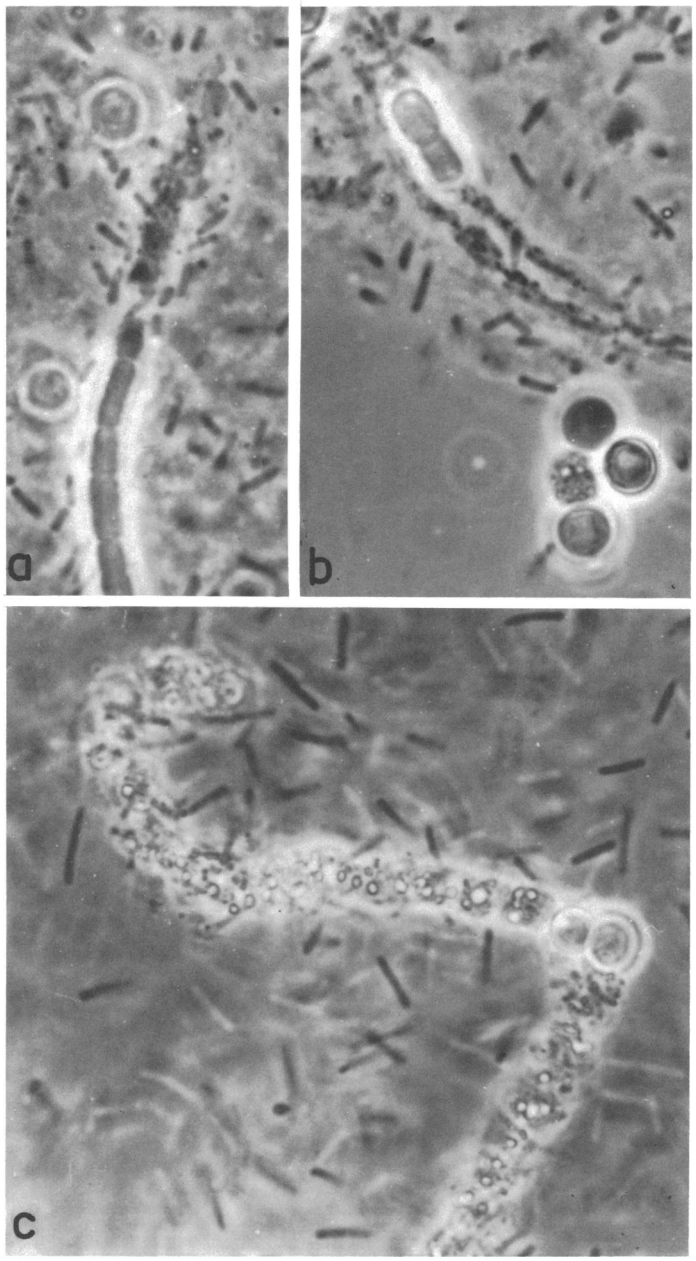

FIG. 8. *Lysis of Nostoc by myxobacter strain FP-1. Samples from different plaques on Nostoc lawns taken 5 to 7 days after inoculation from enriched myxobacterial culture. A partly lysed Nostoc filament is shown (a); the unlysed algal heterocysts are conspicious (b), and intact gas vacuoles appear at the site of the completely lysed filament (c). Zeiss phase-contrast microscope. × 4,000.*

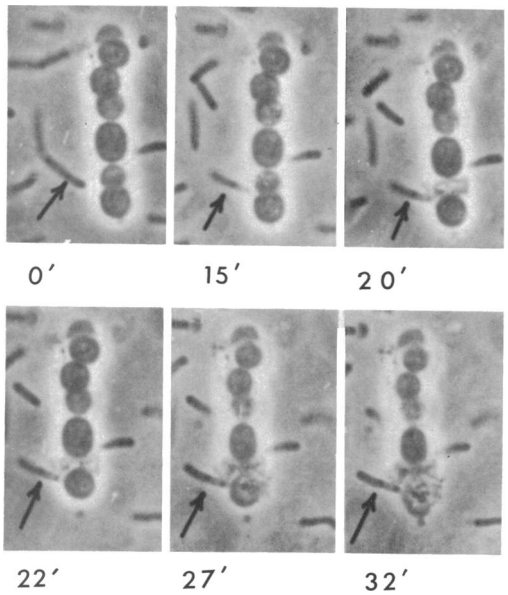

FIG. 9. *Sequence of lysis (in minutes) of Nostoc with low multiplicity of the myxobacters obtained by the thin-agar technique. Zeiss phase-contrast microscope. × 3,150.*

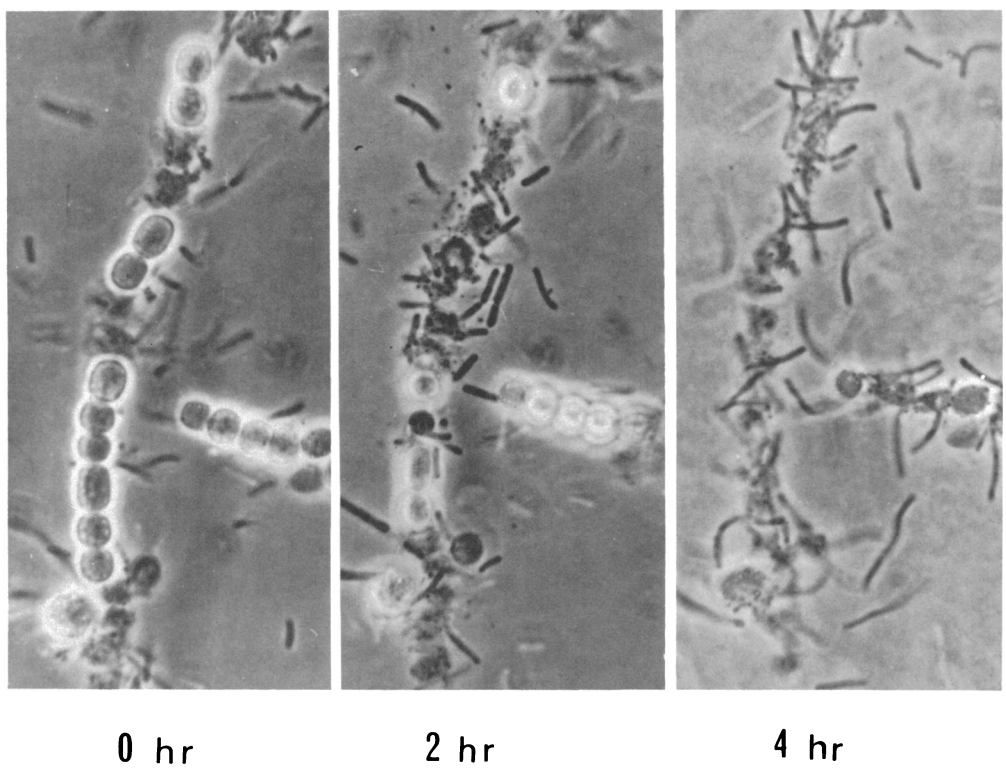

FIG. 10. *Sequence of lysis (in hours) of Nostoc filament with high multiplicity of the myxobacters obtained by the thin-agar technique. Zeiss phase-contrast microscope. × 3,150.*

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