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PREDATION OF AMOEBAE ON THE FILAMENTOUS BLUE-GREEN ALGAE

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Abstract

Amoebae grazing on filamentous blue-green algae are present in rice paddy field. A lobose and a filose amoebae were isolated and purified from the field and their properties were characterized. They have been maintained in axenic culture by feeding with ***Anabaena*** as sole prey. Their grazing behaviour observed by simplified microculture technique revealed that the amoeba approached to any part of the filamentous algae and engulfed a cell or a segment of the filament. In the presence of these amoebae, the population of *Anabaena* in culture was controlled to a very low level within a few days.

Introduction

Predation of protozoa on algae is one of important interactions between the primary producer and the secondary producer in aquatic ecosystem. This activity has been know to enhance nutrient regeneration (Joannes, 1965). It may also play a significant role in controlling the abundance of phytoplanktons (Canter & Lund, 1968). Protozoa, such as ciliates and flagellates, usually graze on the unicellular algae. The filamentous algae, because of their strandlike morphology, are not a good prey for the protozoa.

Blue-green algae (BGA) inoculated into rice paddy field was found to be attacked by amoebae. Predation of amoebae on algae under culture condition has been reported (Ho & Alexander, 1974; Surek & Melkonian, 1980). But information about the predatory activity of amoebae on BGA in rice paddy field was still limited. So a work was initiated to isolate, purify, and characterize the BGA-grazing amoebae present in rice field. Effect of these isolates on the algal population under laboratory condition was also observed in this study.

Materials and Methods

An axenic *Anabaena* HA101 which forming filamentous trichome with no mucilayer or gelatinous capsule was used as the prey for the isolation and cultivation of amoebae. This BGA was isolated from the local rice field and

purified in this laboratory. Free of bacterial contamination of the BGA was routinely checked by inoculating the algae in nutrient broth at 30°C. The BGA was maintained and cultured in a synthetic nitrogen-free medium (SM) containing 0.1 g of K_2HPO_4; 0.02 g of $MgSO_4 \cdot 7H_2O$; 0.01 g of Na_2CO_3; 0.01 g of $CaCl_2$; 20 mg of $FeSO_4$ (as Fe-EDTA); 0.62 mg of H_3BO_3; 0.223 mg of $MnSO_4 \cdot H_2O$; 0.029 mg of $ZnSO_4 \cdot 7H_2O$; 0.008 mg of $Na_2MoO_4 \cdot 2H_2O$; 0.013 mg of $CuSO_4 \cdot 5H_2O$; 0.01 mg of $CoCl_2$; 0.012 mg of KB and 0.008 mg of KI in 1,000 ml of distilled water, pH 7.4.

Amoebae were isolated from rice paddy field. Sample (0.1 ml/plate) taken from field was put on the algal lawn growing on the SM medium. The sample and algae were mixed and spread evenly on the plate and then incubated under 4,000 lux of white fluorescent light at 28°C. Plaques caused by the predation of amoebae would appeared after about three days of incubation. Because plaques due to the presence of bacteria, fungi or other agents could appear in the same plate, the plaques caused by the grazing of amoebae has to be confirmed microscopically (100× to 200×). If it was caused by amoeboid predation, crowded amoebae around the edge of each plaque should be seem.

Purification of amoebae were begun with single plaque isolation. This procedure was repeated at least three times in order to obtain single type of amoeba in the culture. The filose amoeba was further purified to be an axenic culture by treating with antibiotics and repeated single clone isolation; The amoebae growing on the algal lawn were transfered into sterile water which containing 20 μg/ml of chloramphenicol and incubated at 30°C for 12 hours. After incubation, the amoeboid suspension was properly diluted and spread on an algal lawn. After plaques were formed, amoebae in the plaque were resuspended in sterile water containing 5,000 unit of penicillin per ml, and incubated at 30°C for 20 hours. After that, the amoebae were re-incubated on algal lawn for plaques development. Free of bacterial contamination in the plaque was checked by transfering the amoebae into nutrient broth. The lobose amoebae was purified by similar procedures except that a heating treatment (53°C, 15 min.) was performed after it was treated with antibiotics. Amoebae in bacteria-free plaque were isolated and cultivated on SM agar slant with the pure *Anabaena* as sole prey.

Morphology of the amoebae cultivated either in aquatic or on agar surface were examined under microscope. Predation of the amoebae on filamentous BGA was studied with a simplified microculture technique; two point five ml of melted SM agar was smeared on a sterile slide. After hardening, 0.1 ml of algal suspension was evenly spread on the surface and then incubated at 30°C under light (about 4,000 lux) for overnight. Amoebae growing on slant was then transfered to the slide by loop. The slide was

incubated for about two hours for the amoebae to move toward the algae. Feeding behaviour was then observed with Zeiss phase-contrast microscope.

To test the effect of amoebae on algal population, *Anabaena* HA101 were cultivated in liquid SM medium. The amoebae were then added to the culture and incubated under white fluorescent light (about 4,000 lux) at 25°C without shaking. Samples were taken from this mixed culture at regular intervals and the population of algae and amoebae were determined. Concentration of algae was assayed by the chlorophyll absorption at 660 nm after it was extracted with methanol at 50°C for ten minutes. The concentration of amoebae were assayed by the method of "most probable number" with the *Anabaena* as prey.

Results

Several types of BGA-grazing amoebae were found to be present in paddy field. Among them, two have been purified and maintained as axenic cultures. One of them belongs to a lobose amoeba, and the other a filose amoeba. As shown in Fig. 1, clear plaques were formed on plate by the grazing of amoebae on algal lawn. Both isolates have grown normally with the *Anabaena* HA101 as sole prey since they were purified half year ago.

The lobose amoeba has a size of about 25 μm. It has a spherical nucleus and multiplys by binary fission. It forms round cyst with a diameter about 12 μm. The pseudopodia move in one direction, and the body moves forward through the pseudopodia. The lobose amoeba was identified as a species of *Amoeba* (Fig. 2) according to the taxonomic keys of Jahn *et al.* (1979). The filose amoeba has a size of 20 to 30 μm. It is an amoeba with long thread-like pseudopodia instead of ordinary pseudopodia. As shown in Fig. 3, the filopodia may in single or in branching group. They protruded more oftenly from the anterion region which was to lead the moving direction of the body. When the amoeba moving on solid surface, it does not have a defined shape, changing from round, oval, elongate to irregular. But when suspended in water, it usually maintained almost spherical with an average diameter about 15 μm (Fig. 4). The body has a central nucleus, reproduces by binary fission and forms cyst. The filose amoeba was identified as a species of *Nuclearia* based on the the paper of Cann & Page (1979) and Surek & Melkonian (1980).

The filose amoeba engulfed vegetative cell, heterocyst and akinete of *Anabaena* HA101. The vegetative cell was digested within the body, but the other two kinds were excreated out lately. It approached to the filamentous BGA and ingested cell either locating at the terminal or within the filament. The cell was drawn off from the filament one by one by the amoeba within

a short time. As shown in Fig. 5, an amoeba consumed nine cells within 15 minutes was seen. When the cell locating within the filament was attacked, it caused the breaking of the filament. And then the amoeba continued its predation from one of the newly created terminal end. The lobose amoeba attacked the filamentous BGA by adhering to any part of the filament, and then engulfed a cell or a segment of the filament. Both isolates were found to be able to attack certain species of *Anabaena, Nostic, Osillatoria, Tolypothrix* and some unidentified BGA. But the algae with distinct mucilaginous sheath or heavy gelatinous capsule were resistent to their predation. They do not prey on *Chlorella.* Effect of temperature and pH on the predatory activity of amoebae has been tested. It revealed that their predatory activity were normal at temperature ranged from 20°C to 40°C and pH from 5.0 to 9.0. As shown in Fig. 6 & 7, population of *Anabaena* suspended in water was heavily lost by the predation of amoebae, and the amoeboid population developed rapidly when they consumed the prey. Since these two types of amoebae would form cyst in this culture system lately, the BGA usually floushed again after the amoebae formed cysts and got into dormant state.

Discussion

Nutrient requirement of nitrogen-fixing BGA is relatively simple. They grow in the environment either containing combined nitrogen source or not. However, their population in nature usually is limited or varied wildly from place to place. Factors involved in the control of their population are complicated, but the biotic factor may play a very significant role. Certain viruses, bacteria, and fungi have been shown to be pathogenic to BGA (Fogg *et al.*, 1973) and the water-born invertebrates are also known to be grazers of algae (Wilson *et al.*, 1980). Based on our observation, the presence of BGA-grazing amoebae in rice paddy field is very common. So amoebae could be an important agent in controlling the fluctuation and population of BGA in rice field.

Different types of BGA-grazing amoebae are present in rice field. In this paper only two of them were purified as axenic culture and studied. These two isolates formed cyst and so could be easily dispersed by air. During making mass culture of *Anabaena* HA101 in open container here, contaminated by amoeba was oftenly encountered. Because adhering of amoeba to BGA is required for its predation, proper bubbling or stirring of the culture could prevent the BGA from to be attacked by amoebae.

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變形蟲對鏈狀藍綠藻之捕食

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在水田中含有多種捕食鏈狀藍綠藻之變形蟲，自其中分離、純化出具葉狀僞足與線狀僞足之變形蟲各一種，並對其性狀加以探討。它們已被純化至無菌狀態，然後以純化之念珠藻做爲唯一的食物，加以純種培養。利用簡化之袖珍培養法對它們捕食藍綠藻之行爲加以觀察，發現變形蟲首先接近藻體之任何部位，然後將鏈狀排列中之任一細胞或一段細胞加以吞噬。藍綠藻在培養液中的族羣因受這些變形蟲的捕食，於數天內就降至很低的密度。

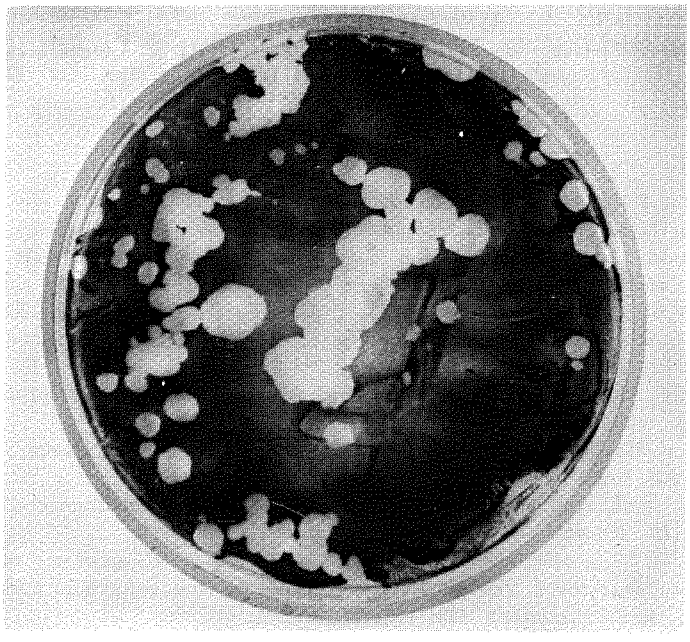


Fig. 1. Amoeboid plaques formed on the lawn of *Anabaena*.

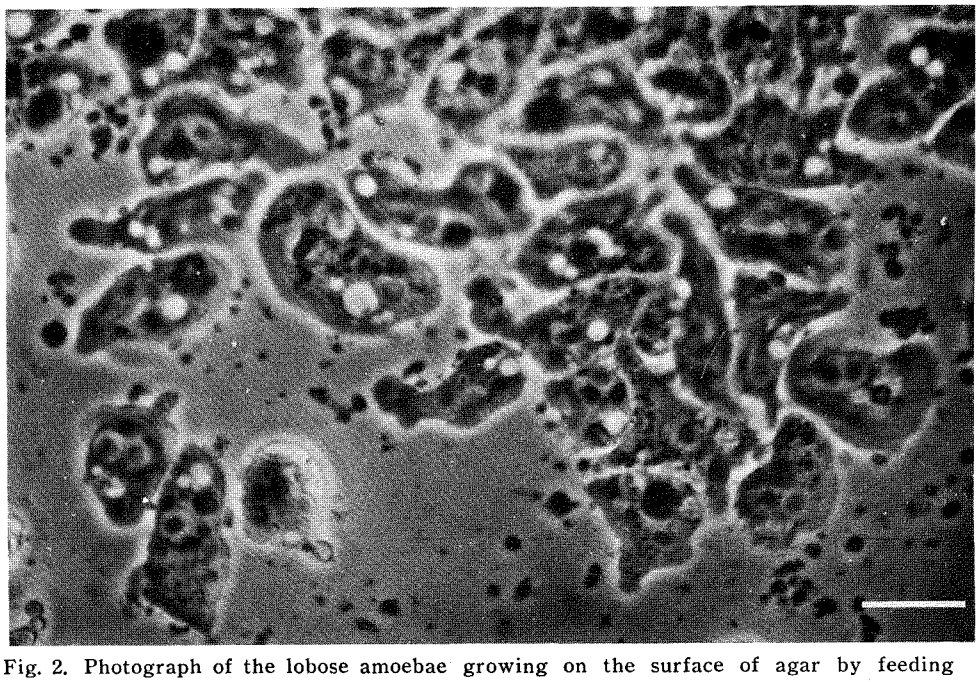


Fig. 2. Photograph of the lobose amoebae growing on the surface of agar by feeding with blue-green algae. The particles outside the amoebae are debris of the algae. The picture was taken by Zeiss phase-contrast microscope. The scale represents 20 μm.

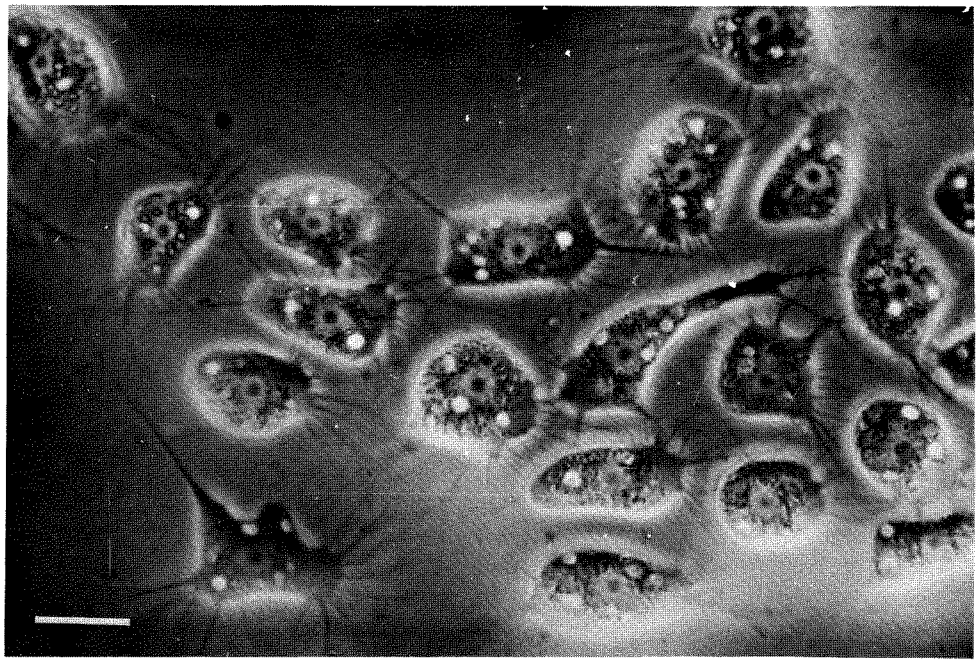
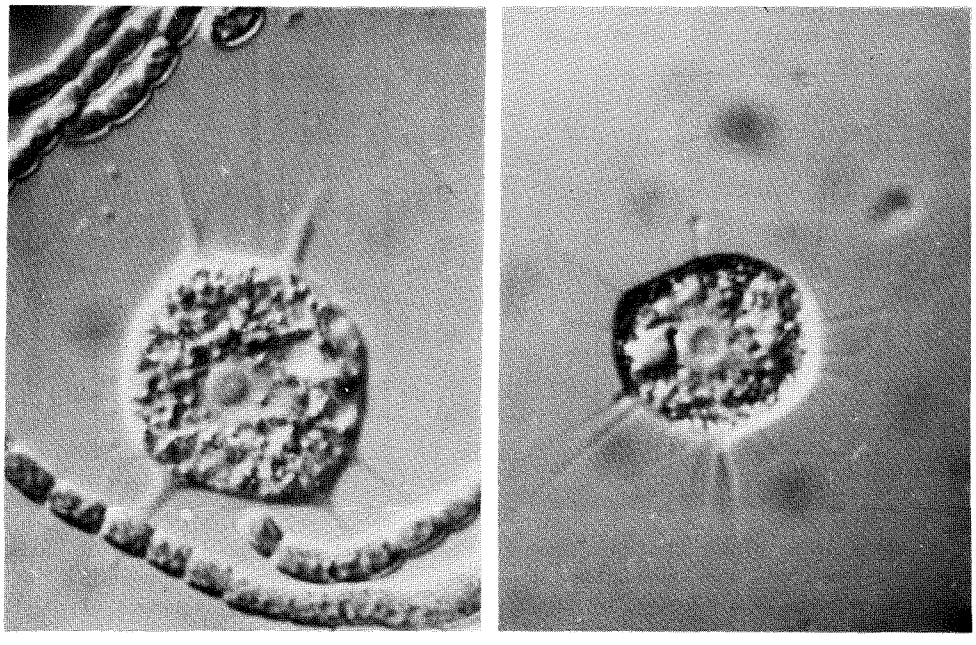


Fig. 3. Photograph of the filose amoebae moving on the agar surface. Zeiss phase-contrast microscope. The scale represents 20 μm.



A B

Fig. 4. Photograph of the filose amoebae suspended in water. (A) A amoeba approached to its prey, ×1,125; (B) A amoeba free in water, ×900. The pictures were taken by Zeiss interference microscope.

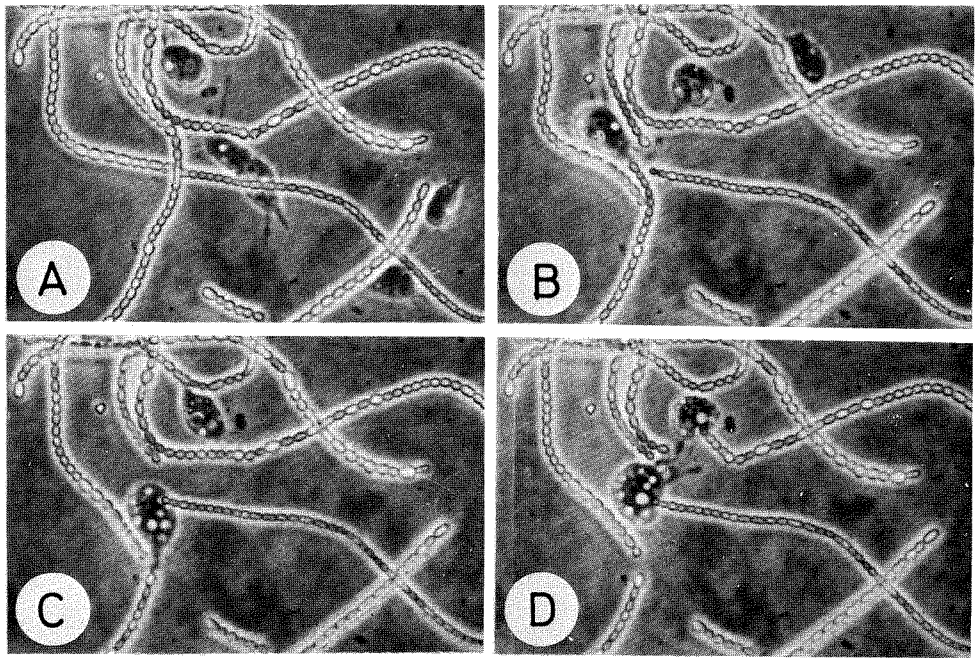


Fig. 5. Sequence of the attacking of filamentous *Anabaena* by the filose amoebae. (A) At zero time; (B) At 15 min., a filament was broken by the grazing of amoeba; (C) At 30 min., nine vegetative cells were ingested by an amoeba; (D) At 56 min., a second filament was attacked and broken.

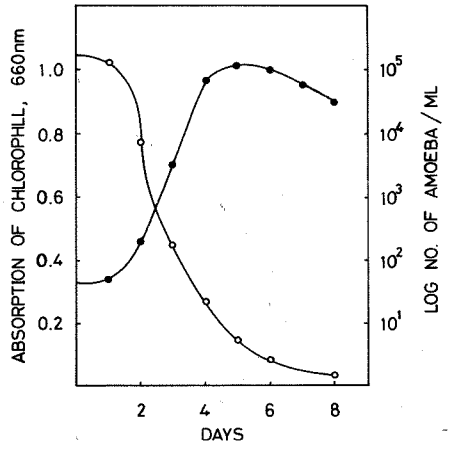


Fig. 6. Effect of the predation of the lobose amoebae on the population of *Anabaena*. (○) Changes of algal population; (●) Changes of amoeboid population.

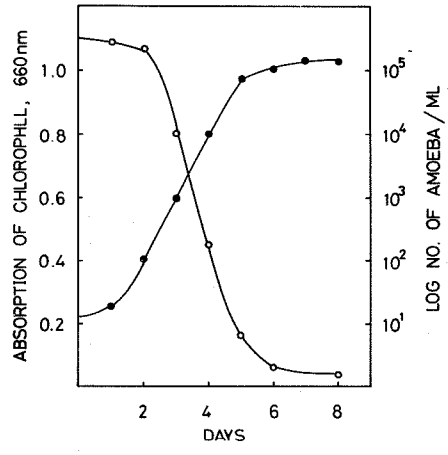


Fig. 7. Effect of the predation of the filose amoebae on the population of *Anabaena*. (○) Changes of algal population; (●) Changes of amoeboid population.