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Interaction Between Mixotrophic Flagellates and Bacteria in Aquatic Ecosystems

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Abstract

A small (3–5 μm) mixotrophic flagellate was isolated from Lake Kinneret and brought into a monoculture. The relationship between these flagellates and the accompanying bacteria were examined experimentally. When the mixotrophs were transferred to fresh medium and incubated in the dark they survived only if additions of bacteria were made. In the light, the protozoans increased after a lag of several weeks, concomitantly with the accompanying bacteria. When additional bacteria were given to the flagelates in the light at the start of the experiment, protozoan growth responded immediately and was accompanied by increasing numbers of bacteria. These results suggest that a "symbiotic" relationship may exist between this mixotrophic flagellate and bacteria in the light.

Keywords: phagotrophic phytoflagellates, Chrysophytes, phagotrophy, mixotrophy, microbial food web

1. Introduction

In the last decade there have been many studies concerning the role of the microbial assemblage in aquatic ecosystems (Berman, 1988; Sherr et al.,

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1986, 1988). From these investigations the perception of two major pathways of matter flux in planktonic systems has emerged: the classical route from primary producers to fish via metazoan zooplankton, and the path involving the "microbial loop" (Pomeroy, 1980; Azam et al., 1983). In the latter, primary produced material passes to bacteria that are subsequently grazed, mainly by protozoans which, in turn, may serve as food sources for zooplankton or larval fish (Wiadnyana and Rassoulzadegan, 1989; Sanders and Porter, 1990; Stoecker and Capuzzo, 1990).

For energy and growth, bacteria require organic carbon, nitrogen, and phosphorous. In natural waters the main source for these nutrients is often from phytoplankton, either through the release of dissolved organic carbon (DOC) from healthy or from dead algal cells, or from zooplankton releasing DOC following digestion of phytoplankton (Azam et al., 1983). Relatively large amounts of the primary produced carbon, nitrogen and phosphorous may be incorporated into bacterial biomass in some environments (Scavia and Laird, 1987; Pomeroy and Wiebe, 1988). In many cases, picophytoplankton (cell diameter < 2 μm) are a major component of the phytoplankton biomass and are responsible for more than 90% of the primary production (Pomeroy, 1974; Murphy and Haugen, 1985; Platt et al., 1983; Berman et al., 1984).

Despite high bacterial and picoplankton growth rates (doubling times 0.5 to 2 days) the numbers of these organisms in the trophogenic zones of natural waters tend to remain more or less constant due to heavy grazing by protozoa (Fenchel, 1987; Azam et al., 1983; Williams, 1984; Wright, 1988; Nagata, 1988).

The major consumers of picophytoplankton and bacteria are small (2–10 μm) heterotrophic flagellates (Sherr et al., 1984; Nagata, 1988; Sanders et al., 1989). The bacterivorous flagellates also serve as remineralisers of nitrogen and phosphorus (Caron and Goldman, 1988; Caron et al., 1988; Berman et al., 1987; 1991; Hadas et al., 1989) and as food for metazoan zooplankton (Wiadnyana and Rassoulzadegan, 1989; Stoecker and Capuzzo, 1990). These flagellates rapidly digest bacteria and other food sources and have rapid grazing and growth rates as well as high release rates of ammonia and phosphorous (Fenchel, 1987; Sherr and Sherr, 1984; Sherr et al., 1988).

We have been investigating the role of planktonic mixotrophic flagellates within the microbial loop. Mixotrophs can play a double role in the microbial food web. As autotrophs they may contribute to primary production, whereas as phagotrophs they act to recycle nutrients. They may also serve as a link in material flux between bacteria and higher trophic levels. Mixotrophic flagellates graze on both bacteria and picoplankton. Some measurements indicate that their grazing rates are as fast as those of nonpigmented flagellates (Bird

and Kalff, 1986, 1989; Porter, 1988; Sanders and Porter, 1988; Sanders et al., 1989). The ecological importance of mixotrophic flagellates in various aquatic environments has yet to be evaluated, but there is accumulating evidence of a high abundance of these organisms in oligotrophic oceanic water (Estep et al., 1986) and in some lakes (Bird and Kalff, 1987, 1989). At present, few details are known about the cellular physiology of these organisms.

In this paper we describe experiments in which the growth patterns of a mixotrophic flagellate isolated from Lake Kinneret were examined in light and dark with or without addition of bacteria. These studies revealed that there is a quasi-symbiotic relationship between the protozoans and accompanying bacteria under some conditions.

2. Materials and Methods

Phytoflagellate cultures

A small (3–5 μm) unidentified phytoflagellate was isolated from the euphotic zone of Lake Kinneret and maintained as monoculture in an inorganic standard culture medium, SCM (Moss, 1972) at 20°C, either in the dark with addition of T10 bacteria, or with no bacterial supplements at a light intensity of 100 μEin$\cdot$m$^{-2}\cdot$s^{-1}.

Bacterial cultures

A bacterial isolate (T10) from Lake Kinneret was grown in nutrient broth (Difco), at 37°C. The cells were harvested and washed twice with SCM medium, centrifuged and kept in the refrigerator. These bacteria, which are gram negative rods approximately 1 μm in length, were routinely used as a food source for the mixotrophic flagellates. Also fluorescently labelled bacteria (FLB) were prepared from T10 using the method of Sherr et al. (1987).

Scanning electron microscopy (SEM)

Samples of phytoflagellates were prepared according to the GTGO procedure (Gamliel et al., 1983) and examined using a JEOL 840 microscope.

Transmission electron microscopy (TEM)

Sample fixation was carried out by mixing equal volumes of suspended phytoflagellates in SCM medium and Karnowsky's double strength solution in 0.2 M phosphate buffer. After 2 hr the samples were centrifuged and the pellet

was placed in 2% agar. The agar cubes were post-fixed with osmium tetroxide, stained with uranyl acetate following serial dehydration in ethanol and propylene oxide and then embedded in Spurr's low viscosity medium (Spurr, 1963). Sections which were post-stained with uranyl acetate and lead citrate were observed in a JEOL 1200× Electron microscope operating at 80 kv.

Grazing experiments

Mixotrophic flagellates were cultured in SCM under a light intensity of 100 μEin·m^{-2}s^{-1} at 20°C. Fluorescently labelled bacteria (FLB) were used to estimate protozoan grazing rates following the methods of Sherr et al. (1987). Samples taken at various time intervals after the start of each experiment were fixed in a mixture of glutaraldehyde and paraformaldehyde according to Tsuji and Yanagita (1981) and examined under a Zeiss Axioscope epifluorescent microscope.

Relationship between flagellates and bacteria

Three flasks each containing 100 ml culture volume were prepared for each experiment:

Flask A: Monocultures of flagellates which were transferred to fresh medium SCM without any added bacteria. The transfer dilution was 1 to 20.

Flask B: Monocultures of flagellates were transferred as above, but were supplemented with T10 bacteria (8.3×10^6 cells·ml^{-1}).

Flask C: Bacteria T10 that were kept in the cold were transferred to fresh SCM (2.3×10^6 cells·ml^{-1}).

The three flasks were incubated for three weeks at 20°C at a constant light intensity of 200 μEin·m^{-2}s^{-1}. Parallel treatments were incubated in the dark. Samples were taken from each flash at time intervals from 0 to 650 hr for counting by epifluorescent microscopy. Bacteria and flagellate samples were fixed and filtered onto 0.2 μm and 0.8 μm black nucleopore filters respectively, and stained with DAPI (Porter and Feig, 1980).

3. Results

a. Characterization of the mixotrophic flagellates

As yet we have no definitive taxonomic identification of the mixotrophic flagellate which appears to be a Chrysophyte but is not an *Ochromonas*, and may be a new species. The cells are free swimming, and contain chlorophyll *a* and *c* as shown by HPLC and TLC chromatography.

Scanning electron micrographs show 3–6 μm diameter cells, with one short and one long hairy flagellum. The protoplast is naked, there is no cell wall and there are many cell membrane projections (Fig. 1). A single chloroplast

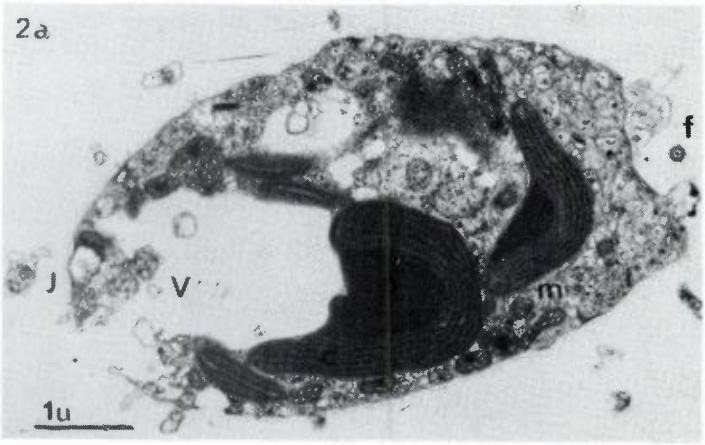
Figure 1. Scanning electron micrograph showing the flagella, and numerous cell membrane projections of a mixotrophic flagellate isolated from Lake Kinneret.

with thylakoids and pyrenoids is enclosed within the chloroplast endoplasmic reticulum which is continuous with the nuclear membrane (Fig. 2 arrows). At the cell periphery there are many mitochondria (Fig. 2a,b). The cell chloroplast was also visible as a large red spot under epifluorescent microscopy.

b. Relationship between bacteria and mixotrophic flagellates

Previous grazing experiments (not detailed here) using fluorescently labelled bacteria had clearly demonstrated bacterivory in these phytoflagellates (Fig. 3). The present series of experiments was designed to investigate the effect of light and dark conditions on the growth patterns of the flagellates and the accompanying bacteria.

When non-axenic cultures of flagellates containing low numbers of bacteria ($< 1 \times 10^5$ cells·ml^{-1}) were transferred to SCM and incubated in the dark, most of the protozoa disappeared within several days due to death or encystation. Addition of T10 bacteria to these cultures resulted in an increase of flagellate numbers from 1×10^3 to 1×10^5 cells·ml^{-1} within 48 hr. In order to maintain



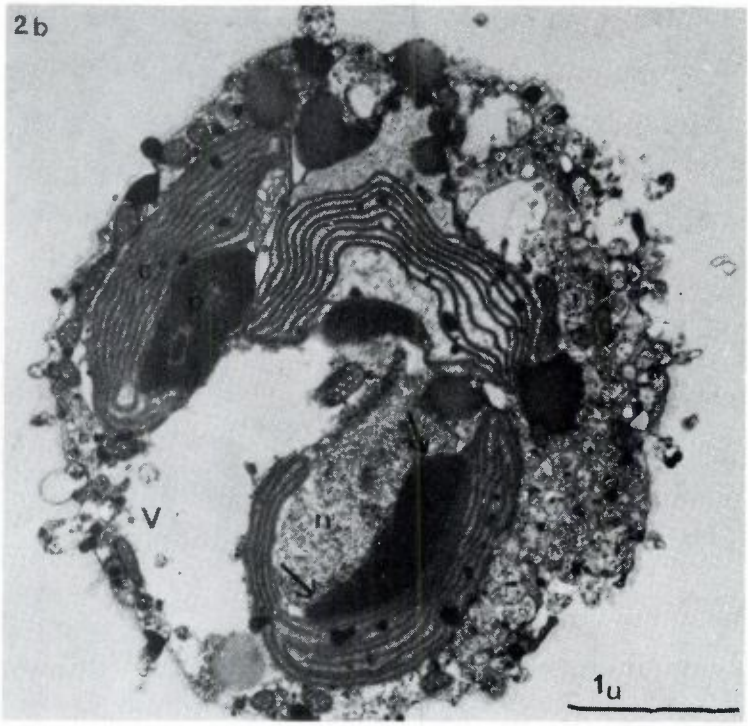


Figure 2.a,b Transmission electron micrographs of a mixotrophic flagellate showing the vacuole (v) opposite to the flagellum (f), the chloroplast (c) with thylakoids and pyrenoid (p) enclosed within the chloroplast endoplasmic reticulum (arrows), with many mitochondria (m) at the cell periphery.

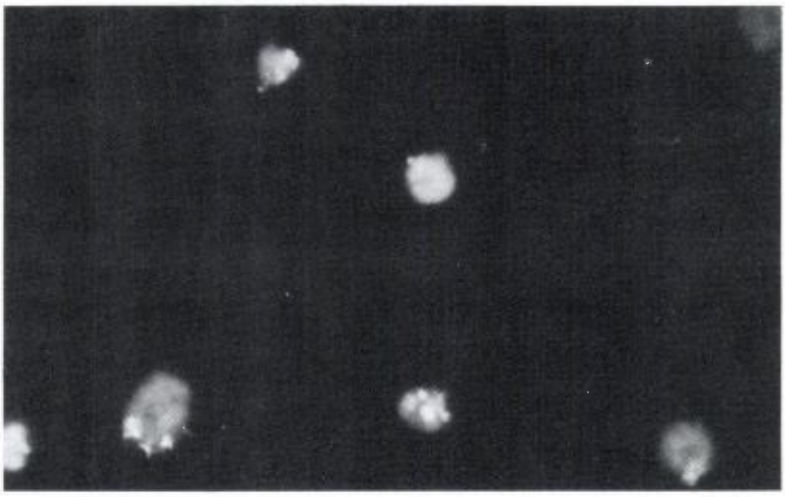


Figure 3. Uptake of fluorescently labeled bacteria (FLB) by phytoflagellates

this abundance of protozoans in dark incubations, weekly additions of bacteria were required.

A different pattern was observed in cultures incubated in the light. When cultures were transferred to fresh SCM (**Flask A**) phytoflagellate numbers declined in the first day from 2×10^5 to 1×10^5 cells·ml^{-1} while bacterial counts increased ten-fold to 5×10^6 cells·ml^{-1}. Subsequently we observed an

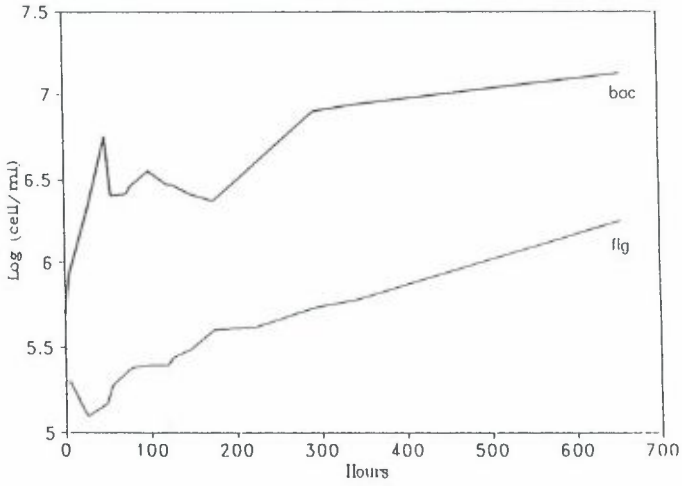


Figure 4. Time course of phytoflagellate and bacterial growth in the light without added bacteria (**Flask A**). Flagellates – flg, Bacteria – bac.

increase in the abundance of flagellates. The system behaved like the classical predator-prey model for the next few days as flagellate numbers increased and bacteria declined. After this phase, both the bacteria and protozoan populations steadily increased in numbers throughout the rest of the experiment.

In **Flask B**, when mixotrophic flagellates were transferred and immediately supplemented with bacteria, the initial number of flagellates was the same as in the previous case (2×10^5 cells·ml^{-1}), but their numbers increased within 2 hr. The initial concentration of bacteria was 9×10^6 cells·ml^{-1} and during the first day, declined to 5×10^6 cells·ml^{-1} due to intensive grazing. After this, bacterial numbers started to rise. Subsequently there was a continuous, overall increase in both bacterial and flagellate numbers throughout the three week duration of the incubation (Fig. 5).

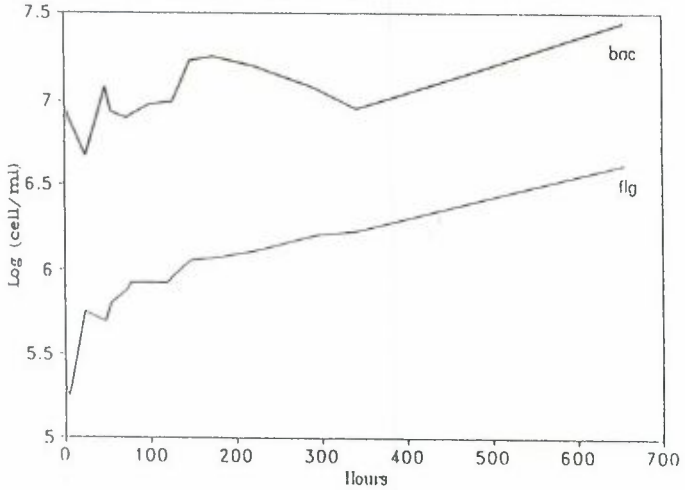


Figure 5. As in Fig. 4 but with bacteria added initially (**Flash B**). Flagellates – flg. Bacteria – bac.

In **Flask C**, with no flagellates, bacterial numbers increased from 2.3×10^6 to 1.7×10^7 cells·ml^{-1} within the first 4 days and then declined steadily to 1×10^6 cells·ml^{-1} (Fig. 6).

In a separate experiment, phytoflagellates were transferred into SCM in the light without additional bacteria but at a lower initial number than in the previous experiments, (1×10^4 instead of 2×10^5 cells·ml^{-1}). In this case, the number of protozoans remained constant for 3 weeks and only then started to rise. Bacterial counts were not made in this experiment.

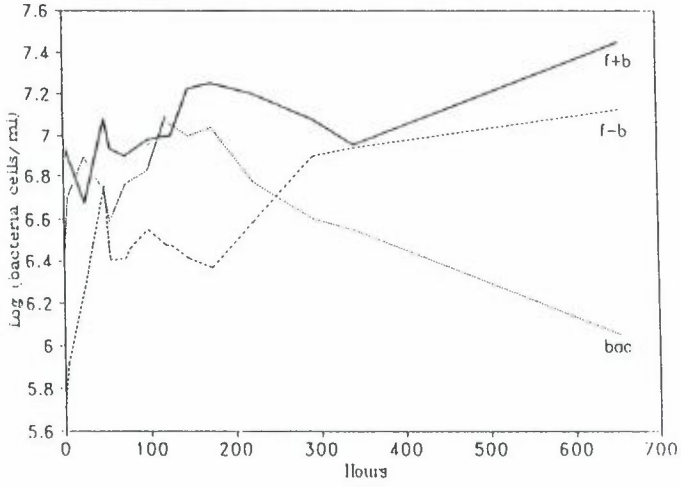


Figure 6. Time course of bacterial growth. **Flask A**: f–b, **Flask B**: f + b, **Flask C** – (bacteria only) – bac.

4. Discussion

Fenchel (1986) suggested that a minimal bacterial threshold density of $\sim 0.5 - 1.5 \times 10^6$ cells·ml^{-1} is needed to sustain phagotrophic flagellates, below this concentration grazing is energetically unfavorable. The requirements for this mixotrophic flagellate appear to be similar. In our experiments, even in the light, the protozoans started to increase only when bacterial counts exceeded 1×10^6 cells·ml^{-1}. For this mixotroph, it seems that photoautotrophy is sufficient only for maintenance, but not enough for growth. *Ochromonas* sp. exhibited the same behavior (Anderson et al., 1989). In order to grow in the dark these phytoflagellates require an external food supply, while in the light they can survive for relatively long periods without supplemental nutrition.

When heterotrophic flagellates are cultured together with bacteria they show typical predator-prey relationships (Fenchel, 1986). Initially there is an increase in bacterial numbers. This is followed after some time by a rise in the heterotrophic flagellate abundance with a concomitant decrease in the bacteria. Batch cultures of non-pigmented flagellates isolated from Lake Kinneret showed similar patterns and ceased growth after 4 days when the bacterial density fell below 1×10^6 cells·ml^{-1} (Hadas, personal communication).

The mixotrophic flagellates in our experiments behaved differently. In the light, after the first few days there was an increase in the total numbers *both* of bacteria and of flagellates even without any added bacteria. Addition of extra bacteria at the beginning of the experiment stimulated immediate growth of flagellates. These observations would suggest that a kind of "symbiosis" exists

between mixotrophic flagellates and bacteria, with the bacteria being able to derive some benefit from the flagellates while at the same time serving as prey. This relationship only occurs in the light, perhaps because of energetic constraints.

From an ecological viewpoint, mixotrophic flagellates act trophically both as algae and as heterotrophic flagellates. Bacterivorous flagellates in culture recycle soluble phosphorous and ammonia to the medium (Berman et al., 1987, Berman, 1991; Caron and Goldman, 1988; Hadas et al, 1990) while algae not only carry out photosynthesis but also release dissolved organic compounds. Presumably mixotrophic flagellates act similarly to algae and unpigmented flagellates.

From our experiments we can infer that although the mixotrophs were grazing on the bacteria they were also releasing materials which enhanced bacterial growth. Thus, the bacterial population may have obtained substrates for energy and growth from the protozoans while, in turn, the bacteria served as food and probably supplied essential compounds such as vitamins to the mixotrophs. This scenario has some basis in studies (reviewed by Aaronson, 1980) which indicate that phagotrophic chrysophytes have an obligatory requirement for several B vitamins, and also that these organisms may release dissolved organic compounds in the form of amino and other organic acids, sugars, lipids and proteins.

This "symbiosis" exists only under two conditions: light to drive photosynthesis in the mixotrophs is necessary, and a minimal concentration of bacterial cells to sustain the flagellate population is required.

In some aquatic ecosystems, mixotrophic flagellates comprise about 50% of the phytoplankton population (Bird and Kalff, 1987, 1988; Sanders et al., 1989). These organisms contribute both to the primary production and to the flow of organic carbon from bacteria to higher trophic levels. We suggest that in nature there may be a "symbiosis" on the population level between bacteria and mixotrophic flagellates. The mixotrophs obtain most of their energy and growth requirements by grazing on bacteria and by doing so regulate the abundance of the latter. In the light, the mixotrophs photosynthesise and release compounds which stimulate the heterotrophic bacteria. Thus, the bacteria may benefit from the nutrient cycling activity of the mixotrophic flagellates in a manner similar to that which we observed in our cultures.

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