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Observation on *Poterioochromonas* sp. (Chrysophyte)

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Abstract

Poterrioochromonas sp., isolated from *Microcystis* cultures in 2002, was described with LM, SEM, TEM. The grazing characteristics of this strain were also observed in laboratory experiments. The results showed that this strain has the representative features of the genus except for the lorica, and the most conspicuous feature of *Poterioochromonas* sp. was about the chromatophores.

Keywords: Microstructure, Ultrastructure, *Poterioochromonas* sp., Microcystis Aeruginosa

1. Introduction

Golden alga is an important component of plankton in the water ecology. Some species have characteristics of plant nutrition, such as chromatophore (chloroplast) or special product of assimilation (chrysolaminaran, *et al.*). So they are called as "chrysophytes" by botanists. However, some species have two flagella and can swim, and depend predominantly on phagotrophic nutrition without chromatophore which characterizes the phagotrophic nutrition. Thus some zoologists term them as "chrysomonads", which is an important part of protozoa. Additionally, some species have both the characteristics of the plant nutrition (photosynthesis) and the phagotrophic nutrition, *i.e.*, they are mixotrophy. Although osmotrophy (dissolved) and phagotrophy (particulate), is observed in many algae, it is the use of particulate food that has generated most interest and to which the term mixotrophy usually applies [1].

Since Pringsheim [2] first reported that a *Ochromonas* ingested small algae, the phenomenon of ingestion of phytoplankters by chrysomonads has been widely recognized. Some genus of chrysomonads are capable of grazing blue-green algae (*Anacystis* and *Microcystis*) and green algae (*Chlorella*, *Chlamydomonas* and *Carteria*), and diatom (*Achnanthes*) [3-6]. These observations suggest that the ingestion of algae by mixotrophic chrysomonads is common. Our research group got a species of golden alga, isolated from *Microcystis* cultures in 2002. This alga could grow not only by ingestion and digestion of *Microcystis*, but also in phototrophic condition at the same time, which is described as mixotrophy. Mixotrophic algae are common in most aquatic ecosystems and, when numerically dominant, they depend significantly on phagotrophy [7,8]. Because of their small size and high metabolic rate they may also be important in the regeneration of nutrients [9], and thus an understanding of their nutritional characteristics is significant.

The golden alga we got was identified as *Poterioochromonas* sp., a strain that is phylogenetically close to *Poterioochromonas malhamensis* (99% similarity) by 18s rDNA (GenBank Accession No.AY699607) [10]. In this paper, we investigated the growth and ingestion characteristics of this strain, including the biological morphological features under different growth conditions.

2. Materials and Methods

Poterioochromonas sp. was isolated from the mass culture of *Microcystis aeruginosa* in our lab in 2002. A clone culture was established by picking up single cells with micropipettes.

1) Cultures were grown in a flask with sterile BG-11 medium at 22℃ under an illumination of *ca.* 25 μmol photons·m^{-2}·s^{-1} with a photoperiod of 12 h : 12 h (Light: Dark) from daylight fluorescent lamps.

2) Feeding *Poterioochromonas* sp. in a relative low ratio of 3:1 (prey : predator) every two days for a week, the initial condition of the predator is 10^6 mL^{-1}.

3) Inoculating low densities (ca. 10^3 mL^{-1}) of *Poterioochromonas* sp. into the cultures containing approximately 10^6 or 10^7 mL^{-1} M. *aeruginosa* FACHB469.

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We often sampled and observed the organism with an olympus CX 41 light microscope (LM).

2.1. Surface and Ultrastructure of *Poterioochromonas* sp.

For SEM (Scanning electron microscope), cells were fixed for 2 hours in 2.5% glutaraldehyde at room temperature, one drop of cells were placed on little glass slides, which coated with 0.1% poly-L-Lysine, dried for 30 min, and subsequently washed three times (10 min each) in 0.1 M phosphate buffer, pH 7.0. After three 10-min rinse in ultrapure water, samples were dehydrated through 50%, 70%, 80%, 90%, 95% and 100% ethanol (5 min each stage) and then 1:1 (ethanol : isoamyl acetate) for 10 min at room temperature, and used the critical point drying in a HITACHI HCP-2 apparatus after replaced by pure isoamyl acetate. The slides containing the algae were then mounted on stubs and coated with gold in a GIKO ID-3 sputter coater. Coated specimens were examined with HITACHI S-3000N SEM.

For TEM (Transmission Electron Microscopy), *Poterioochromonas* cells were harvested by gentle centrifugation. Cells were washed 2 times with PBS (pH = 7.0, 0.1 M), fixed with 2.5% glutaraldehyde, and then put in 1% OsO_4 for 2 hours at room temperature. After graded ethanol dehydration, samples were embedded in EPOXY epon-812 and polymerized at 70℃ for 8 hours. Sections were cut, stained with uranyl acetate and lead citrate, and then examined with a HITACHI H-600 TEM.

3. Results

The general appearance of *Poterioochromonas* sp., as observed with LM, is illustrated in **Figure 1** and **Figure 2**.

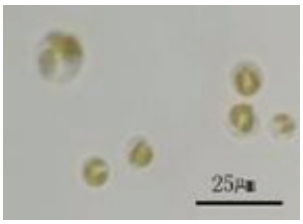


Figure 1. Photomicrographs of *Poterioochromonas* sp. in autotrophy.

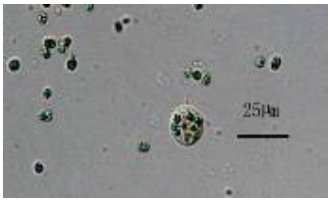


Figure 2. Photomicrographs of *Poterioochromonas* sp. in mixotrophy.

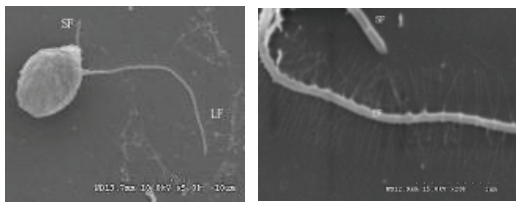
We found that the autotrophic cells, with two unequal long flagella and two yellow-green chromatophores, are spherical to elongate, and approximately 5-10 μm in di--ameter. We did not see any eye-spot. They could not only photosynthesize with chromatophores, but also efficiently digest the preys. And the volume of the predator could become larger when there were prey(s) in the cell. The diameter of the largest cells can reach 25 μm. The size and color of the chromatophores would change a lot during the *Poterioochromonas* cell ingested and digested preys. We only noted that the cells reproduce asexually by binary division, remaining motile, although it is reported that some chrysophyte can reproduce sexually. We did not see any lorica or scale outside the cell with LM.

From the pictures of the SEM, we noticed two kinds of cells: sphere or ellipse, as **Figure 3** shown.

The *Poterioochromonas* cells bear two heterodynamic flagella: pleuronematic flagellum with hair-like appendages (mastigonemes, 1.25 μm) and acronematic flagellum without any appendage (**Figure 4**). The two flagella are unequal in length: the longer is almost two times the body length; the shorter is half of the body's.

From the pictures of TEM, we observed that the cells are uninucleate, with a fine periplast but no rigid cell wall or spines or scales outside. The two chromatophores were oriented around the nucleus and lacked a pyrenoid. The single Golgi body is anterior to the nucleus and close to the flagellar bases. Mitochondrial cristae are tubular (**Figure 5**).

When added to the cultures of the *Poterioocromonas* sp., *M. aeruginosa* FACHB469 could be swallowed, and transported to a single membrane-bound food vacuole and digested there (**Figures 6**, **7** and **8**) by the predators. The



Figures 3-4. Scanning electron micrograph of *Poterioochromonas* sp.; SF: short flagellum; LF: long flagellum.

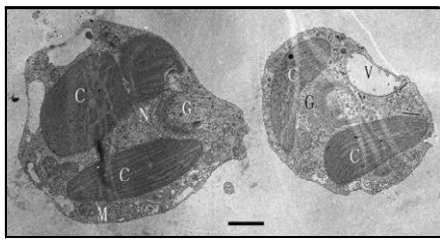
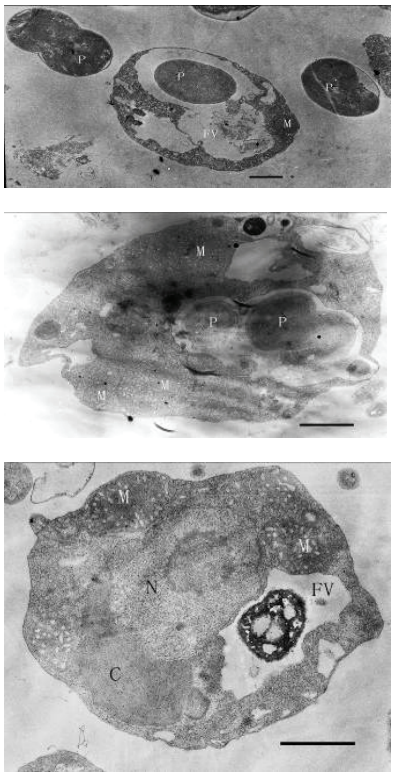


Figure 5. Transmission electron micrograph of *Poterioochromonas* sp. under autotrophy. C: chromatophore, G: golgi body, M: mitochondrion, N: nucleus, V : vacuole. Bar = 1 μm.



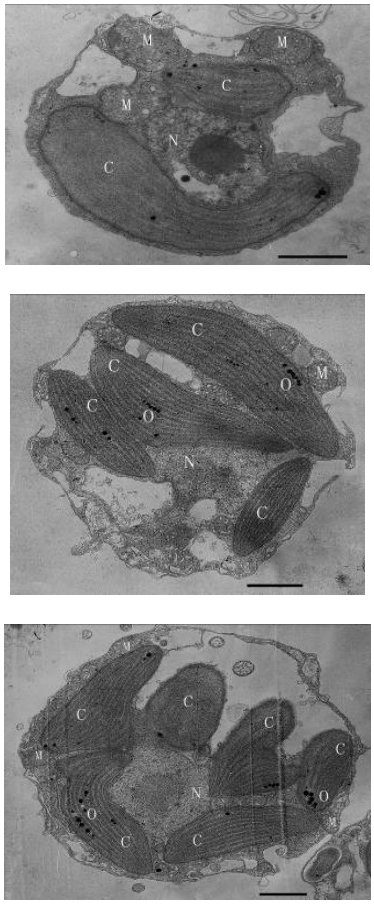
Figures 6-8. Transmission electron micrograph of *Poterioochromonas* sp.with prey(s). P : prey. Bar = 1 μm.

shape of the predator could be elongated, and the ultrastructure would change a lot. The most conspicuous feature of the predators was about the chromatophore.

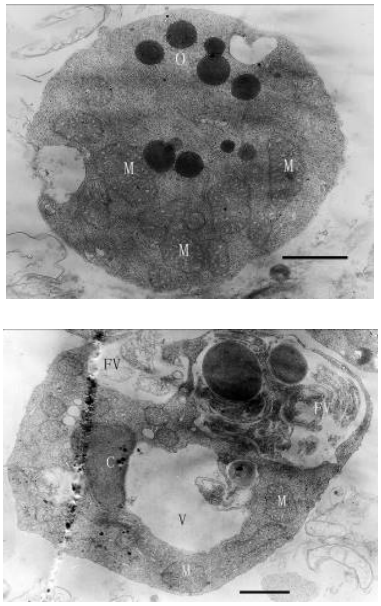
When feeding *Poterioochromonas* sp. in a relative low ratio of 3:1 (prey: predator) every two days for a month, we could see no less than two swelling chromatophores with blurry or clear lamina around the nucleus (**Figures 9**, **10** and **11**). Some times the number of the chromatophores could reach six. At the same time there are many little osmiophilic globules in the interthylakoid spaces of the chromatophores. The predators could still move well and ingest preys.

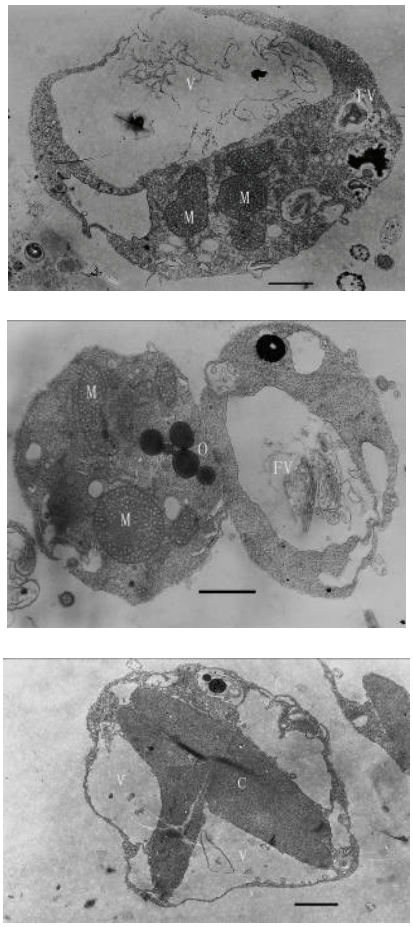
When inoculating low densities (ca. 10^3 mL^{-1}) of *Poterioochromonas* sp. into the cultures containing approximately 10^6 or 10^7 mL^{-1} M. *aeruginosa* FACHB469, the predators could ingest preys very quickly and grow exponential rapidly. Meanwhile the chromatophores of most predators might become shrunken or missing in the first few days (**Figures 12**, **13** and **14**), and there were also many large osmiophilic globules located in the cytoplasm at the same time (**Figure 15**). Later when the most preys were nearly eaten off, the predators entered a "stationary growth phase", and chromatophores of *Poterioochromonas* sp. could appear again (**Figure 16**), and the large osmiophilic globules would disappear.

During the process of mixotrophic growth, the volumes of mitochondria become much larger (**Figures 8** and **12**) than those under autotrophy. We also observed a cell under the division (**Figure 17**).



Figures 9-11. Transmission electron micrograph of *Poterioochromonas* sp. growth with low concentration of preys. O: osmiophilic globule. Bar = 1 μm.





Figures 12-16. Transmission electron micrograph of *Poterioochromonas* sp. when adding with high concentration of preys. O: osmiophilic globule. Figures 12-15. Sampled within 3 days; Figure 16. Sampled after 7 days. Bar = 1 μm.

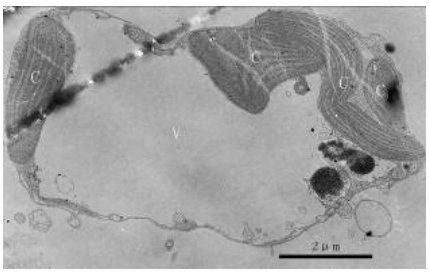


Figure 17. Transmission electron micrograph of *Poterioochromonas* sp. in division. Bar = 2 μm.

4. Discussion

Mixotrophy in algal flagellates is an interesting phenomenon from both cytological and ecological viewpoints [6]. However, as we know, there are few reports about such chrysomonas fed with algae in China. We isolated a strain Poterioochromonas and got the clone culture in 2002 [10]. We studied many morphological features and characteristics of this golden alga though we are not sure which species it was.

First, the most conspicuous feature of Poterioochromonas sp. was about the chromatophores. The morphology and number of chromatophore changed a lot during the mixotrophic growth. When the concentration of preys was relatively low, the number of chromatophores would increased obviously, and when much plentiful, the chromatophores would be shrunken or disappeared in the "exponential growth phase", while they would be observed again when the predators entered a "stationary growth phase" (**Figures 9-14** and **16**). Based on references [1,11], the reason for the shrunken and disappearance of chromotaphore appears to be the result of rapid mixotrophic growth as the flagellate division rate exceeds the assemblage of the cellular organelle, such as chromotaphore. The recovery of chromotaphore may be attributed to the effects of preys limitation and subsequent decrease in the predator growth rate and gave enough time for the assemblage of chromotaphore.

During the changes of chromatophores, osmiophilic globules changed similarly. The globules apparently formed from breakdown products of the chromophore membranes as well as from pigments synthesized during growth, and they are a reservoir of energy-rich components in the cell [12,13]. The appearance of osmiophilic materials seems to be the product of the digesting preys and the disorganized chromophore of the predator.

The giant mitochondria might indicate the metabolic utilization of the preys, which might provide more energy for the predators.

Additionally, from the preys' view, after being swallowed for a period of time, the prey(s) in the *Poterioochromonas* disappeared, it suggested that the predators not only ingest but also digest the prey organisms.

We could not observe any lorica outside *Poterioochromonas* sp. with TEM and LM, which was the special structure for the genus and difficult to identify [14]. More information should be explored to conclude whether this strain has lorica or not.

5. Conclusions

In conclusions, we investigated the morphological features and growth characteristics of *Poterioochromonas* sp., the results showed that the strain has the representative features of the genus except for the lorica. Mixotrophic chrysomonads can be found in many kinds of water bodied, including oligotrophic environments [15,16], mesotrophic and eutrophic waters [11,17]. It is an ecology strategy to control the harmful algae by using mixotrophy. Although we have done some research in the laboratory, there are many problems required further investigation before putting *Poterrioochromonas* sp. in the field.

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