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Binary fission patterns of heterotrophic nano-flagellates, *Actinomonas mirabilis* Kent and *Cafeteria roenbergensis* Fenchel and Patterson, observed with a CCD camera

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Abstract: Patterns of binary fission of the heterotrophic nano-flagellates *Actinomonas mirabilis* and *Cafeteria roenbergensis*, which were isolated by a stepwise dilution method from the water taken in inner part of Tokyo Bay, Japan, were recorded with a microscopic CCD camera. By analysis of the video records, the entire course of the fission could be divided into 7 phases for *Actinomonas* and 6 phases for *Cafeteria*. *Actinomonas* performed a longitudinal binary fission in 10–20minutes, whereas *Cafeteria* performed an apparently transverse binary fission in 5–10 minutes.

1. Introduction

Heterotrophic nano-flagellates (HNF) have been recognized as bacterial feeders and play an important role in marine habitats (e.g. AZAM *et al.*, 1983; GOLDMAN *et al.*, 1985; CARON *et al.*, 1985; ECCLESTON-PARRY and LEADBEATER, 1995). Therefore, studies have been carried out in order to estimate their quantitative impact on energy flow in microbial food webs, for example, measurement of their growth (FENCHEL, 1982) and grazing rate (SHERR *et al.*, 1987; NYGAARD *et al.*, 1988; NYGAARD and HESSEN, 1990). These studies, however, treated HNF as a group without paying attention to species compostion. Such studies assume that the modes, frequency and time duration of grazing and fission of the HNF assemblages are homogeneous, but it is not clear that they are. We observed the grazing pattern of *Actinomonas mirabilis* Kent and *Cafeteria roenbergensis* Fenchel and Patterson (ISHIGAKI and TERAZAKI, 1998). The doubling time for both species at 20℃ was about 2 hours (unpublished data). We consider that correct information about these physiological rates is necessary to determine adequate sampling design to investigate the ecology of the assemblages.

In this paper, we repoort the modes and time duration of binary fission in two HNF, *Actinomonas* and *Cafeteria* determined using a microscopic VTR system.

2. Material and method

HNF were isolated in March 1994 from water collected from the landing stage at the Tokyo University of Fisheries, located at the inner part of Tokyo Bay, using a stepwise dilution method. Two species of HNF, *Actinomonas mirabilis* Kent and *Cafeteria roenbergensis* Fenchel and Patterson (Fig. 1), were established in rice grain culture, where they fed on bacteria derived from the original seawater. Stock cultures were subcultured with fresh rice grains once per month and maintained at 20℃ at a salinity of 36 psu in the dark.

These flagellates were injected into a spermatozoon counting chamber (Sefi-madical Instruments, Haifa, Israel: MAKLER COUNTING CHAMBER) which has a depth of 10 μm between cover-slip and slide-glass. This chamber was placed under an inverted phase contrast microscope (Nikon Diaphot 300, 12V 100W Halogen Lamp, Tokyo, Japan) equipped wiht a

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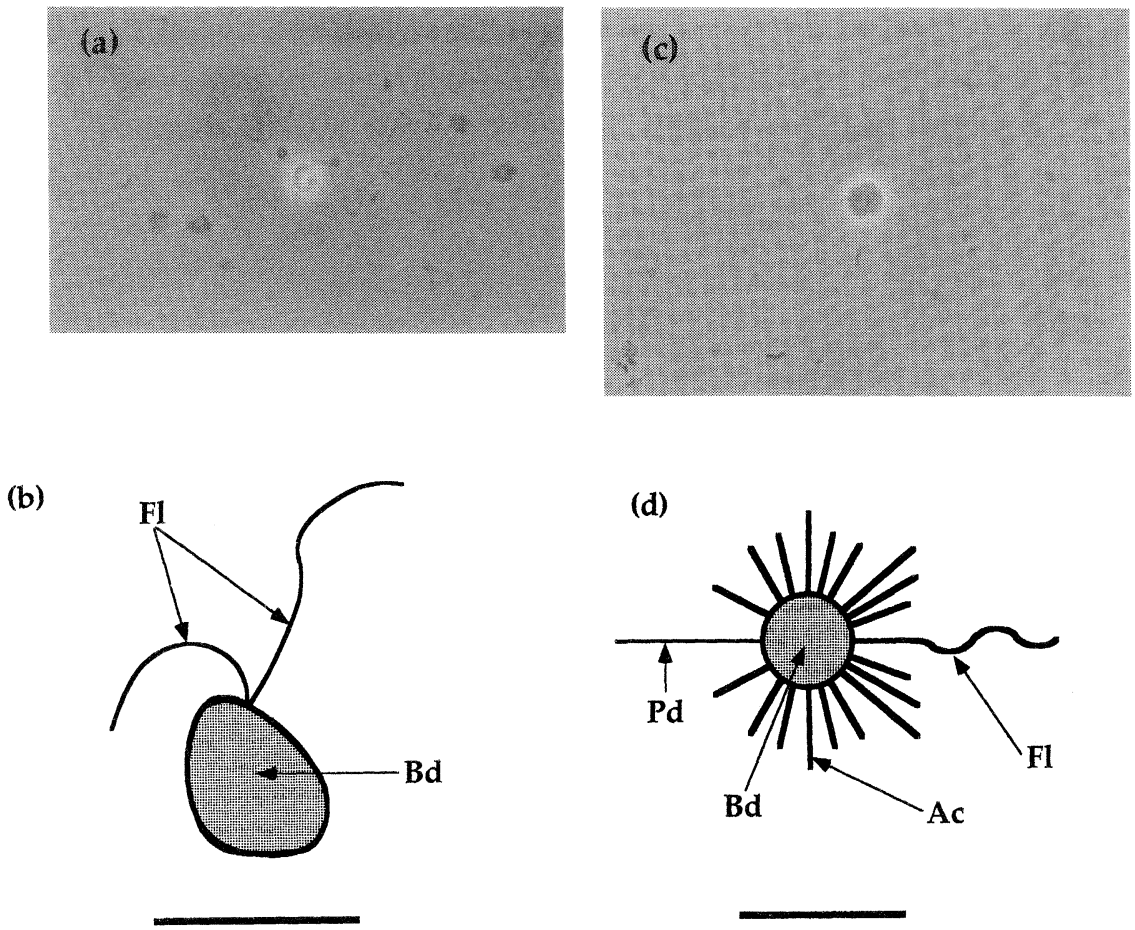

Fig. 1. *Cafeteria roenbergensis* Fenchel and Patterson (a), (b) and *Actinomonas mirabilis* Kent (c), (d). Phase contrast micrograph of living cell (a), (c) and schematic diagram (b), (d). Bars indicate 5 μm for (a), (b), and 10 μm for (c), (d). Ac: Actinopodia. Bd: Body. Fl: Flagella. Pd: Pedicel.

CCD camera (Nikon, Tokyo, Japan). The microscope was installed in an incubator box (TLTG -200 Low temperature incubator gloves box, Technica Co. Ltd., Tokyo, Japan). Binary fission of the cells at the early exponential growth phase of *Actinomonas* and *Cafeteria* which had been incubated at 20 ℃ and 36 psu was recorded by VTR (Victor, HR-V3, Tokyo, Japan). The records were made at 17 ±0.3 ℃ in the incubator box, and no specimen was observed for longer than 30 minutes since the surface temperature of the chamber could increase by 3 ℃ in 30 minutes. A new sample was placed in the chamber every 30 minutes, and samples from the same culture were observed throughout a period of 24 hours. During each observation, if a flagellate moved, the stage of the microscope was moved to bring it back to the centre of the field.

3. Results

The continuous process of binary fission of *Actinomonas* cells was divided into 7 phases as follows. Phase 1: two short flagella were observed in all dividing cells and appeared one on each side of the basal portion of the mother flagellum (Fig. 2 ①). Phase 2: daughter flagella extend in length and the mother cell body

expanded (Fig. 2 ②). Phase 3: the mother flagellum stopped moving, shrank to the body and was absorbed into or was divided from the body, and daughter flagella extend to a length equal to 1.5 times the cell body length (Fig. 2 ③). Phase 4: the mother cell body became narrow in the middle (Fig. 2 ④). Phase 5: the part between the daughter cell bodies was gradually stretched forming a bridge (Fig. 2 ⑤). Phase 6: this bridge extended and became thread-like (Fig. 2 ⑥). Phase 7: the mother cell separated into two daughter cells (Fig. 2⑦). This binary fission pattern was longitudinal and was observed in 25 examples of *Actinomonas*. The time duration required from phase 3 to 7 of the fission in *Actinomonas* was about 20 minutes maximally, and about 10 minutes minimally. All cells which subsequently divided first developed the two new short flagella on both sides of the basal portion of the mother flagellum. However, some cells on which the two new flagella appeared on both sides of the mother flagellum did not proceed to divide into two cells, and their two new flagella shrank and disappeared without division of the cell.

In contrast, the binary fission pattern of *Cafeteria* was divided into 6 phases. Phase 1: the movement of the long flagellum stopped (Fig. 3①). Phase 2: the cell body elongated (Fig. 3②). Phase 3: the part between anterior (daughter cell) and posterior (mother cell) hemisphere cell became constricted, and a pair of new flagella appeared at the opposite end of the cell, on the future daughter cell (Fig. 3③). The new flagella were diagonally symmetric around the centre of cell body with respect to the mother flagella. Phase 4: the distance between the mother cell and daughter cell was gradually increased and bridged as occurred in *Actinomonas* (Fig. 3④). Phase 5: this bridge extended to become thread-like (Fig. 3⑤). Phase 6: the cell finally separated (Fig. 3⑥). This binary fission pattern is seemed to transverse and was observed in 13 samples. The time duration required from phase 2 to 6 of the fission in *Cafeteria* was maximally about 10 minutes and minimally about 5 minutes. During binary fission, *Actinomonas* and *Cafeteria* always stopped swimming and held their body stationary on the glass surface by using actinopodia or the short flagellum.

4. Discussion

Protists are unicelluar and have asexual and sexual modes of reproduction (e.g. FARMER, 1980). This study concerned asexual reproduction and involved the observation of binary fission. Cell binary fission is divided into three types depending on the plane in which fission occurs; these are longitudinal, transverse and oblique (or pseudo-transverse) binary fission. Flagellates divide almost exclusively by longitudinal binary fission (FARMER, 1980: LAYBOURN-PARRY, 1984). The binary fission pattern of *Pteridomonas*, which is very similar to *Actinomonas*, shows the same fission pattern as found for *Actinomonas* (PATTERSON and FENCHEL, 1985). The shortening of the original mother tinsel flagellum is consistent with the view that each new basal body forms first an anterior tinsel flagellum and in the daughter cell a former anterior basal body becomes a posterior basal body (SLEIGH, 1988). Before cell division each basal body replicates and two new long tinsel flagella would be expected to appear on the two new anterior basal bodies. In our observations, *Actinomonas* divided by longtitudinal binary fission. *Cafeteria* showed transverse-like binary fission. We did not observe that daughter cell receives one old flagellum and newly formed flagellum during cell division in this case. For this we consider that it is necessary more clearly to observe fission pattern of *Cafeteria*.

It was observed that a sign for binary fission of *Actinomonas* was the appearance of two new short flagella on both sides of the basal portion of the mother flagellum. However, this was not observed as a clear sign for binary fission of *Cafeteria* (Figs. 2, 3). For *Actinomonas*, in all dividing cell the new short flagella appeared, however, all the cell in which the new two flagella appeared did not divide. Cell division of protists is attribute to their cell cycle. The appearance of the new short flagella was recognized a state of preparation for binary fission.

In the future, it is necessary to observe the life cycle for *Actinomonas* and *Cafeteria* and also to analyse the relationship between fission

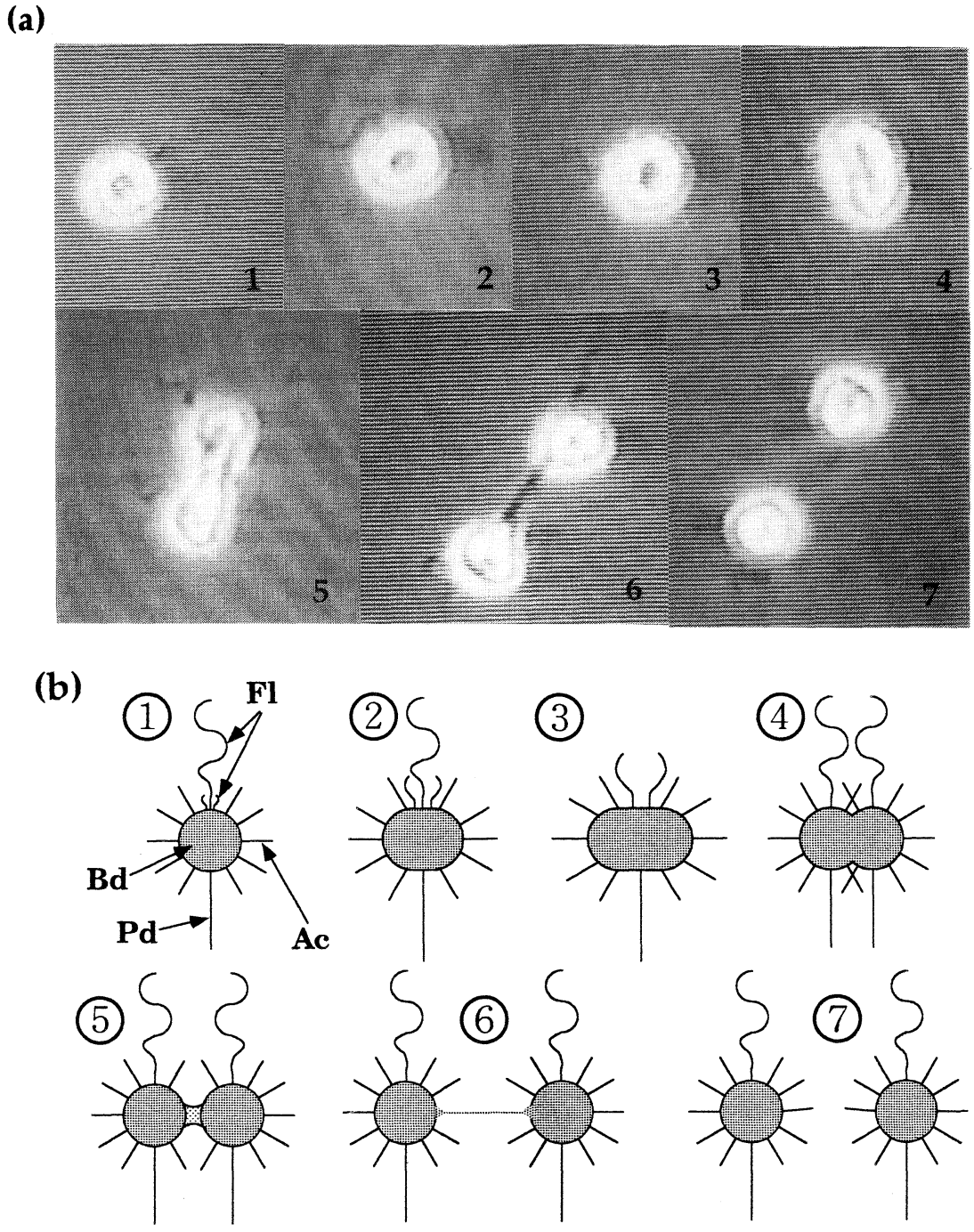

Fig. 2. Fission pattern of *Actinomonas* . (a): photograph from video tape. (b): schematic diagram. Numbers correspond to the phases described in the text. Abbreviations follow Fig. 1.

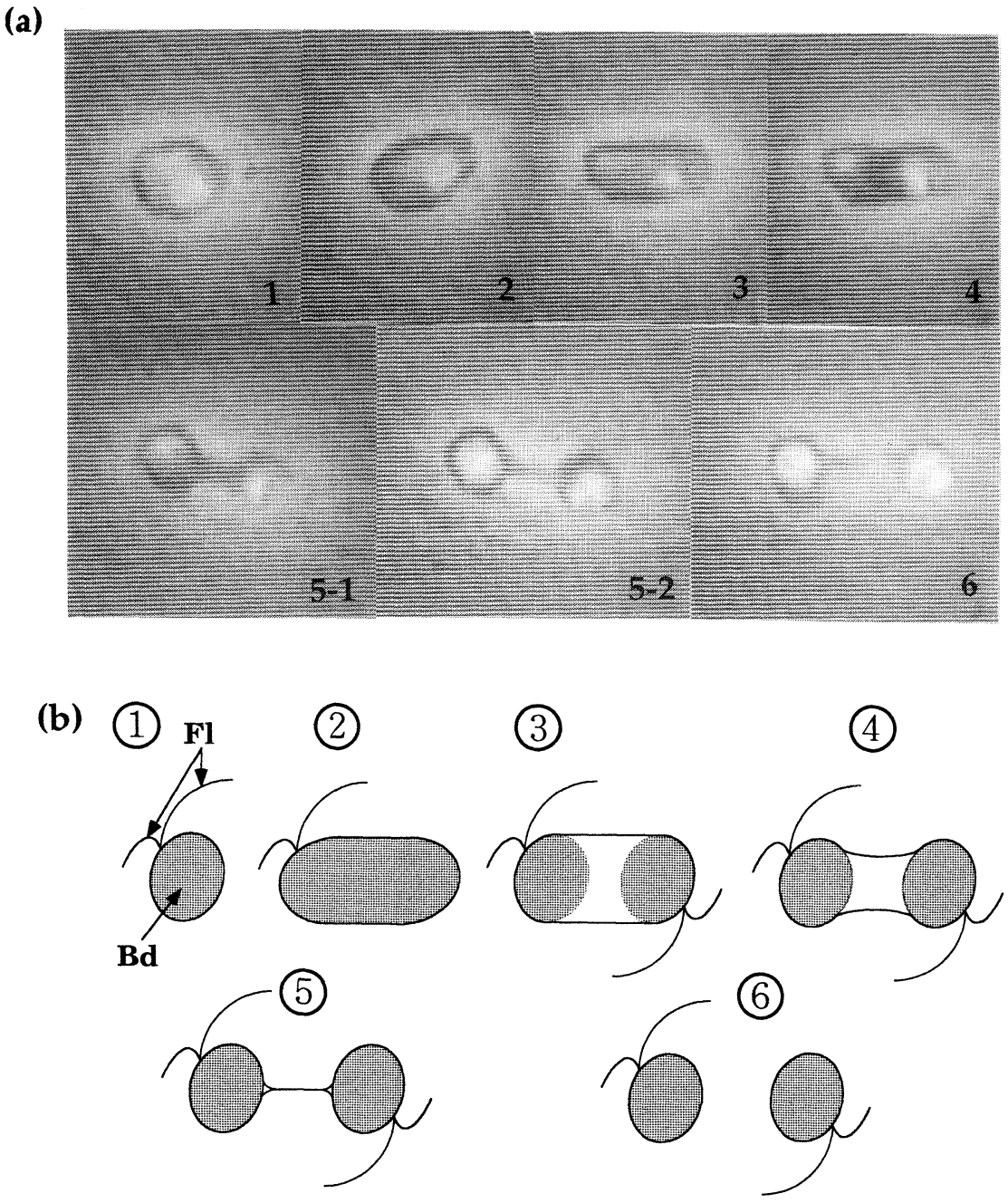

Fig. 3. Fission pattern of *Cafeteria*. (a): photograph from video tape. (b): schematic diagram. Numbers correspond to the phases described in the text. Abbreviations follow Fig. 1.

and natured environmental conditions.

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