Two Staining Methods in the Study of Ciliates

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Abstract — Ciliates have two kinds of nuclei, somatic macrocnulei and germinal micronuclei. For the study of nuclear behaviour during their conjugation (sexual reproduction mode), nuclear staining techniques are very important. In the last decade, we studied nuclear behaviour of several species of Paramecium, and several nuclear staining methods were used. Here carbol fuchsin and ''apofluor'' (a double vital fluorescent dyes of acridine orange and Hoechst 33342) will be introduced.

Keywords — Ciliates; Paramecium; Carbol fuchsin; Acridine orange; Hoechst 33342

I. INTRODUCTION

Paramecium caudatum is the representative of ciliates. As all other ciliates, it has two types of nuclei, a germinal micronucleus and a somatic macronucleus[1]. During the last decade, some new findings of nuclear behaviour during conjugation of several species of *Paramecium* were reported[2-8], all of which benefited from the improvement of several experimental techniquesl[9-12] and adopting several staining methods, such as protargol[13,14], immunofluorescence[15,16], carbol fuchsin[17,18] and "apofluor"[19,20]. Recently, we have introduced these methods in detail. One is volume-fixing method suitable for the observation of living cells and photographing[21]. The second is a method how to collect large number of synchronized conjugating pairs[22], and the third is a technique to get large amount of magnetic nanoparticles by ultrasonic treatment[23]. Here, we will mainly introduce two convenient staining methods, carbol fuchsin and "apofluor".

II. MATERIALS AND METHODS

Main Equipments and Materials

Fluorescent microscope (Nikon 50*i*), acridine orange (AO, Shanghai Chemical Reagent Company, China), Hoechst 33342 (HO, Beyotime Institute of Biotechnology, China), carbol fuchsin solution (self-made), the exconjugant suspension of *P. caudatum*.

Methods

Carbol fuchsin staining

According to Carr & Walker[17], the protocol for the preparation of carbol fuchsin solution was summarized as following, which is scale down for its easy preparation. 1. Weigh 30 mg basic fuchsin and dissolve in 1 mL of 75% ethanol to make it completely dissolved. 2. Add 9 mL of 5% phenol aqueous solution to the above solution and mix well. 3. Add 1.08 mL acetic acid and formalin each into the above solution, respectively, and mix well. 4. Add 28 mL of 45% acetic acid aqueous solution into the above solution and mix well. 5. Finally, 40 mL carbol fuchsin staining solution would be obtained, which can be stored at room temperature for at least one year. 6. Before cell staining, the staining solution could be diluted with 45% acetic acid aqueous solution properly. 7. A temporary slide is made according to volume-fixing method (usually half of the cells moving slowly)[10,21], then the staining solution is added on one side of the cover glass, and a piece of absorbent paper is used to suck the staining solution on the other side making the staining solution spread over the whole cover glass. 8. After 3-5 min, cells can be observed under the bright field microscope and photographed.

"Apofluor" staining

"Apofluor" is the combination of two vital fluorescent dyes, AO and HO[19]. The stock solutions (50x) of AO (1 μg/mL) and HO (10 μg/mL) prepared with autoclaved H_2O should be kept at 4°C[20]. When it is ready for staining, 10 μl each is added to 480 μl exconjugant suspension of *P. caudatum*. About 10 min later, temporary

preparations are made by volume-fixing method[10,21]. Usually, 13 μL cell suspension is corresponding to the cover glass of 22 ㎜*22 ㎜, when about 50% of cells moves very slowly being convenient for observation and photographing under Nikon 50*i* fluorescent microscopes with UV filters.

III. RESULTS AND DISCUSSIONS

Concerning carbol fuchsin solution, nuclei on all developing stages are stained the same colour (Figure 1A-C). As to the "apofluor", contrast to the normal developing nuclei stained blue (Figure 1A′-C′), the apoptotic nuclei are stained yellow green (red arrows in Figure 1A′).

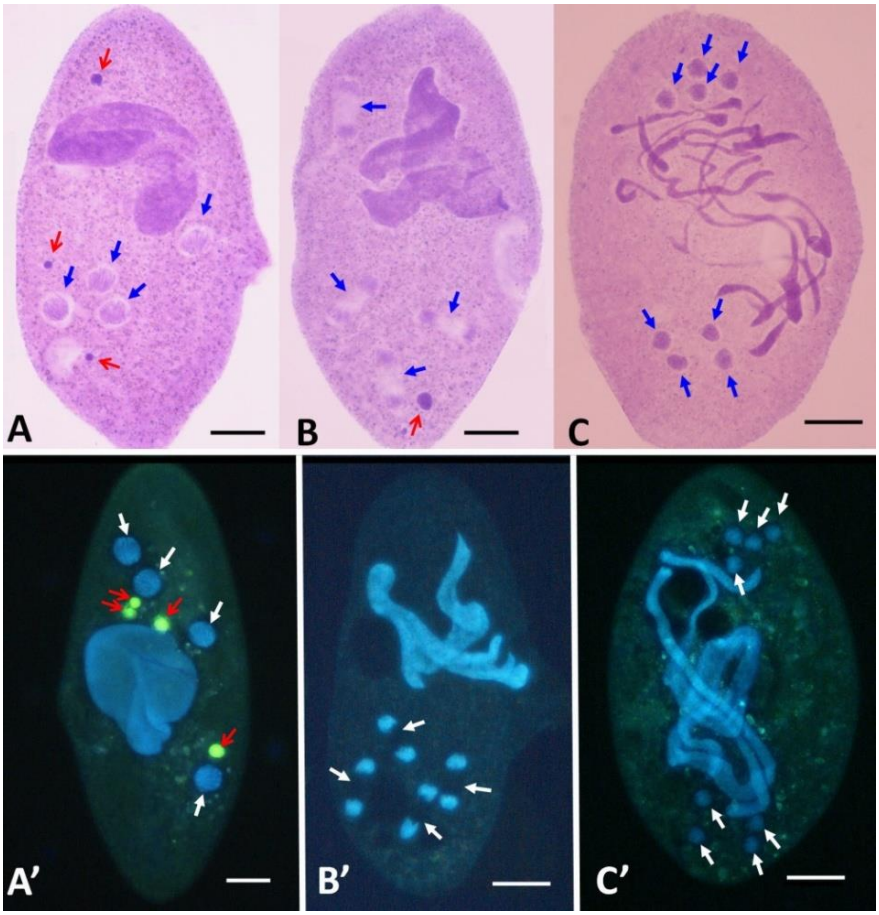

Fig. 1 Exconjugants of *P. caudatum* stained by carbol fuchsin or "apofluor"
A-C: Carbol fuchsin staining; A′-C′: "Apofluor" staining. A, A′: Cells on the metaphase of the third postzygotic division; B, B′: Cells on the anaphase of the third postzygotic division; C, C′: Cells on the telophase of the third postzygotic division. Red arrows: Apoptotic meiotic nuclei; Blue/white arrows: Non-degenerating normal nuclei. Bars: 20 μm.

These above two staining methods have one common feature: speedy. Therefore, they are easy to be used. Of course, fluorescent microscope is indispensible for "apofluor" staining. In this sense, carbol fuchsin staining is the most convenient method, which is applicable to both the research and the student experiment of *P. caudatum*. However, "apofluor" is able to distinguish the apoptotic nuclei from normal/developing nuclei, which is convenient for studying apoptosis[2,20].

In fact, we have tried these two staining methods to other ciliates, both work well[24]. Concerning protargol[13,14] and immunofluorescence[15,16], they have their own advantages. Unique nuclear division of the third prezygotic division was indicated by protargol in *P. caudatum*[3] and *P. polycaryum*[14]. Cytoplasmic microtubules surrounding the surviving meiotic nuclei and the nuclei on-going the third postzygotic division were indicated by the immunofluorescence with anti-alpha tubulin antibody[7]. However, experiments with protargol and immunofluorescence could not always get ideal results.

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