Introduction of an Effective Method for Separating Conjugating Pairs of *Paramecium*

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Abstract *— For the studies on the ciliate conjugation, it is indispensable to prepare a large number of conjugating (mating) pairs. In the last twenty years, we have done some studies on the conjugation of several species of Paramecium, and an effective method for separating mating pairs of Paramecium has been established, which will be introduced here.*

Keywords *— Ciliate, Paramecium, Conjugating pairs, Pair separation, Iron-dextran particles*

I. INTRODUCTION

Ciliates have two important characteristics, nuclear dimorphism and conjugation, the former means that two types of nuclei (micronuclei and macronuclei) co-exist in a cell, and the latter is the sexual reproduction mode of ciliate[1]. *P. caudatum*, the representative of ciliates, has one micronucleus and one macronucleus[2], whose classical conjugation study was reported in 1907[3]. During the last 20 years, we have had some new findings[4-9], such as the unique patterns of the third prezygotic division[6] and the third postzygotic division[8]. All these were closely related with the sufficient number of mating pairs, which were separated with the method of feeding iron-dextran particles (referred as iron particles later)[4]. The iron particles are nanoscale magnetic aggregates of Fe_3O_4[10], which were used in ciliates for the first time for collecting food vacuoles of *Tetrahymena* in 1993[10], and for isolating cells of *Tetrahymena* with abnormal oral structures in 1995[11], then for separating mating pairs to study the nuclear behaviour during the conjugation of *Paramecium* in 1999[4]. However, the reported method for the preparation of the iron particles[10] has a disadvantage of low productivity, which makes the iron particles hard to be popularized in the ciliate studies. In 2010, the protocol was optimized by ultrasonic treatment to the large aggregates of Fe_3O_4[12], which usually are discarded during the preparation. This improvement made the production of the nanoscale iron particles increased greatly, and makes it possible to separate a large number of mating pairs insuring the studies on the conjugation of *P. caudatum* as well as other *Paramecium* species[13,14].

As all other free-living ciliates, *P. caudatum* has an oral apparatus to swallow the liquid of its surroundings as food vacuoles[2]. While their conjugating pairs have no ability to form food vacuoles due to the membrane fusion between 2 cells of a mating pair on their ventral-sides enclosing the oral apparatus[2]. If the iron particles added to the mixtures of un-conjugated single cells and conjugating pairs, the single cells can form food vacuoles containing iron particles, the mating pairs cannot. This phenomenon provides the researchers a chance to separate the mating pairs from the single cells by application of the magnets, which attract the single cells and loose pairs both having iron particle-containing food

vacuoles. In fact, we have introduced a method for the observation of living *Paramecium* previously[15]. Here, we will introduce a method for collection of a large number of mating pairs of *Paramecium* according to our previous reports[4,16].

II. MATERIALS AND METHODS

Equipments and Materials

Centrifuges, NdFeB Magnets, Petri-dish, Pasteur pipette, iron-dextran particles, mixtures of conjugating pairs and un-conjugated single cells of *P. caudatum*.

Methods

Step 1: Discard the upper part of the mixtures, which mostly contains single cells and loose pairs to reduce the operating volume of the cell suspension. In fact, most cells accumulate together and remain on the bottom of the Petri dish.

Step 2: Add several drops of iron particles to the remaining mixtures. After 10-15 min, put the Petri-dish on the NdFeB Magnets for 5 min or more, when the single cells and loose pairs are attracted to the magnets, while the pairs swim freely in the medium.

Step 3: Suck the cell suspension containing free-swimming mating pairs with a Pasteur pipette slightly to a new dish avoiding disturb the single cells attracted to the magnets. Repeat this operation 3 times to remove the unneeded cells to the most extend.

Step 4: Concentrate mating pairs by centrifugation (1 000 rpm, 2 min, room temperature), which are then suspended in K-DS (buffer solution for *Paramecium* culture)[17]. Repeat this operation 5 times to remove the extra-iron particles remained in the mating pair suspension.

Step 5: After the last centrifugation, suspend the mating pairs in a suitable volume of K-DS and remove to a new Petri dish for the following experiments.

III. RESULTS

Most mating pairs were removed from Petri dish (Fig. 1A), while the single cells and loose pairs containing iron particles were remained on the bottom of the container (Fig. 1B).

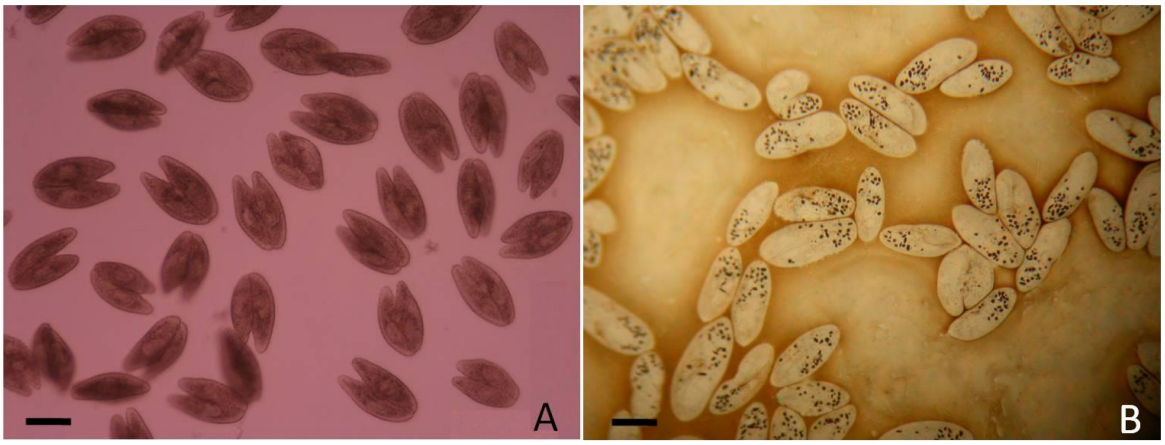

Fig. 1 Mating pair separation of *P. caudatum* by feeding of the iron-dextran particles
A: Separated mating pairs. B: Single cells with iron-dextran particle-containing food vacuoles. Bars: 100 μm.

IV. ADVANTAGES

1. This method is simple, fast and effective for collecting a large number of mating pairs of *P. caudatum* as well as other *Paramecium*[13,14].

2. This method can be used for getting both of synchronized and non-synchronized mating pairs. If the synchronized mating pairs are wanted, add iron particles 2-2.5 h after the induction of mating reaction; otherwise, add iron particles later according to the demands.

3. This method can be expanded to collecting conjugating pairs of other ciliates by adjusting the centrifugation parameters. Neither too fast centrifugation nor too slow centrifugation will be good for getting high quality conjugating pairs.

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