Sains Indonesia Vol. 9, No.2 (2004) 8-11

Light Microscope Examination on the Sexual Reproduction of *Closterium navicula*

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Abstract

It was studied a process of a sexual reproduction in homothallic alga *Closterium navicula*. Conjugation in *C. navicula* (Brebisson) Lutkemuller results in production of single zygospore. Pairing symmetrical cells occurs prior to papillae formation. Papilla from one gametangial cell frequently rises out faster and larger than the other one. Previous to fusion of gametic protoplasms, papillae fuse to form one broad canal within the gametic protoplasm fuse and form one zygospore. The zygospore is slightly rounded with smooth wall.

Keywords: Closterium navicula, conjugation, papillae, conjugation tube, zygopores.

1. INTRODUCTION

Sexual reproduction in desmids, especially in the genus *Closterium*, is reported to be different in each species [1-6]. Investigation of sexual reproduction should be thoroughly carried out since characteristic of zygospore, the product of reproduction, hold an important role for species identification. Formation of zygospore in *Closterium* produces one (single) or two (twin) zygospore (s). Although the number of zygospore produced in every mating cannot be referred as taxonomic relationship among species of *Closterium*, the process itself was proved to be taxonomic character in *C. moniliferum-C. ehrenbergii* species complex [5]. Hendrayanti *et al.* [3] reported that some processes of sexual reproduction in *C. wallichii* Turner were similar with *C. moniliferum* and *C. ehrenbergii*. Phylogenetic studies based on nuclear small sub unit ribosomal DNA in the genus *Closterium* showed that *C. wallichii* had a closed relationship with *C. moniliferum-C. ehrenbergii* species complex [7].

In the present study we examined the process of sexual reproduction in homothallic alga *C. navicula*. The shape of cell is not lunar like most *Closterium* but elongate with rounded apices. *Closterium navicula* is one of the small *Closterium* with 51 µm length and 13 µm width. Mating in this species produces one zygospore [2] but the process itself has not been reported yet. Through the examination with light microscope we describe the process of sexual reproduction in *C. navicula*. The study is important to collect information of mating studies in *Closterium*.

2. MATERIAL & METHODS

The culture of *C. navicula* (strain NIES-178) used in this study was obtained from The NIES Collection. The cultures were grown in the test tubes (18x150 mm) containing 10 ml of C Medium [4] and maintained at 20^{0}C, under a 16:8 L:D cycle at about 25 µmol photons $^{-2}s^{-1}$ light from fluorescence lamps. The cultures were subcultured periodically to maintain good growth condition.

Formation of spores was induced by mating-induced (MI) medium [4]. Three drops of exponentially growing culture were inoculated into each well of a six-well plate containing 3 ml MI medium per well. The plate was placed in a 25^{0}C incubator and provided with 35 umol photons $^{-2}s^{-1}$ light from fluorescence lamps. Observation was carried out within a week after inoculation.

3. RESULTS

Conjugation in *C. navicula* resulted in the production of single zygospore, as reported by Hashizume [2]. Prior to conjugation, two mature symmetrical cells came close to each other, confronting each other's mid region (Fig. 1). The pairing gametangial cells kept this position until papillae slowly rose out from each mid region of the gametangial cells (Fig. 2). Usually, the papilla from one of the gametangial cells grew larger than the other one. As the papilla walls of the gametangial cells enlarged against each other, the cell bent to one side (Fig. 3).

The protoplast began to migrate away from the cell wall of cell apices along the fusion of papillae to become a conjugation tube (Fig. 4). From each gametangial cells, a gametic protoplast moved out synchronously through the conjugation tube. The gametic protoplasts fused (Fig. 5) and formed a zygote at the midway between now empty two gametangial cells. Mature zygote had three layers and frequently it was released from the gametangial cells (Fig. 6).

Figure 1. Pairing gametangial cells of C. navicula. The mid region of the cells is confronting to each other.

Figure 2. Papilla (P) from one gametangial cell develops faster than the mating partner.

Figure 3. Enlarging papillae, pushing the 2 pairs of gametangial cells to opposite directions.

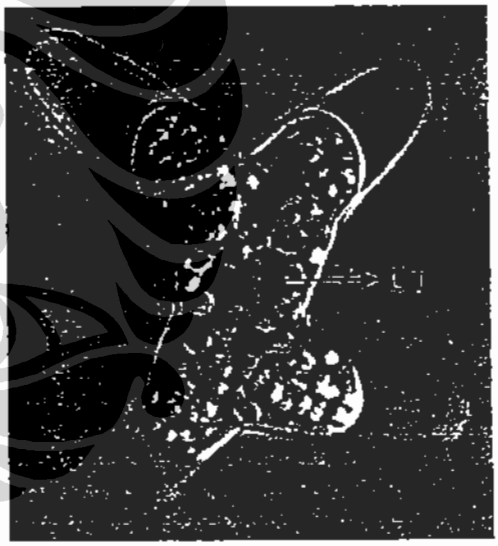

Figure 4. Synchronously moving gametic protoplasts. The papillae fuse to form a conjugation tube (CT) within in the protoplasts fuse.

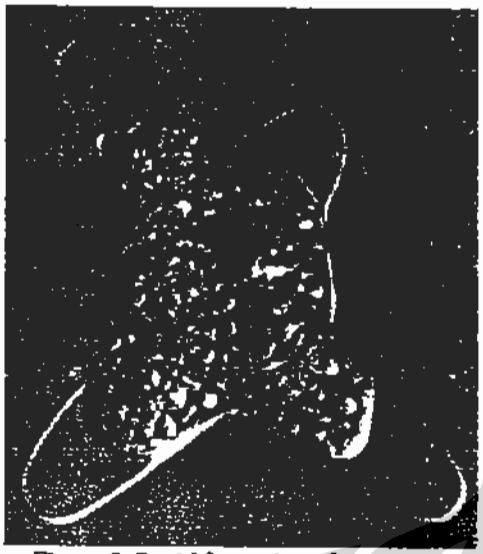

Figure 5. Rapid formation of zygospore.

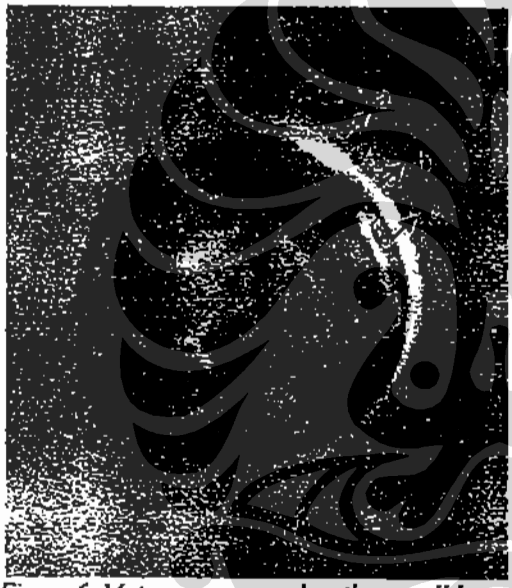

Figure 6. Mature zygospore has three wall layers. a: The outermost layer that thin and lay separately from the other layers. b: middle layer. c: the thick inner layer.

4. DISCUSSION & CONCLUSION

The first common phase of the process of sexual reproduction in conjugation algae, is pairing between compatible cells, followed by papilla formation and fusion of gametic protoplast (zygospore formation) [8,9,10]. However, since every species has their own unique cell characteristic, the mating course among species became quite different.

Pairing position looks similar in all *Closterium*. Most species of *Closterium* has lunar shape so that when two cells mate, they cling to confront each mid region of the cell [3,11,5]. However, cling position is not found in the mating reaction of non-lunar shape *C. navicula* (this study). The mating cells approach to each other and adjust their mid region before the formation of papilla.

So far, the shape and the size of papilla is not consider as a taxonomic character in conjugating algae. Extensive studies in *Netrium* concluded that instead of the shape and the size of papilla, the thickness of cell walls at the fusing region of two conjugation papillae is considered to be taxonomically important [12]. In *C. navicula*, papilla is formed at the mid region of gametangial cell. This is different with *C. moniliferum*, *C. ehrenbergii*, or *C. wallichii* (all produces twin zygospores when mate) where papilla rises out at the tip of gametangial cell [3,11,5]. The difference mostly due to the process of mating itself and so far is not species specific [13].

Zygospore is formed by fusion of gametic protoplasts, which have migrated equal distances in enlarged papilla. This study shows that the papilla fused to form a typical conjugation tube. Hendrayanti *et al.* [3] had been discussed about the conjugation tube, which in some species may form like a hyaline bladder or a conjugation vesicle. Conjugation tube in the mating of *C. navicula* is like a broad canal within in the gametic protoplast fuse to form zygospore.

One zygospore is formed at the end of the mating process. Studies of sexual reproduction in *Closterium* which form two zygospores, such as *C. moniliferum* and *C. ehrenbergii* [5], it is clear from the observation that two gametangial mother cells are first activated sexually to form a conjugating pair and then divide to produce four gametangial cells. Each pair of gametangial cells then produces one zygospore so that the result of the process is two zygospores. The presence of the sexual cell division in most species of *Closterium* that form single zygospore, such as *C. navicula*, is rather difficult because the morphology of gametangial cell looks like vegetative cell. But, thrifty observation in the mating of *C. navicula* showed that the gametangial cells always darker in color and shorter in size than vegetative cell. Thus, the sexual cell division must occur prior to the gametangial cell formation. The formation of gametangial cell was considered to be controlled by internal factors such as gametangiogenic substances of the alga [4]. Lately, Hogetsu & Yokoyama [14] and Fukumoto *et al.* [15] reported activity of pheromone that induced the sexual cell division in *C. ehrenbergii* (twin zygospores) while Tsuchikane *et al.* [16] found two pheromones responsible for inducing sexual cell division in *C. peracerosum-strigosum-littorale*

complex. The wall of the produced zygospore is smooth and the size is 42 µm in length and 40 µm in width.

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