满江红鱼腥藻在羽叶满江红大孢子果内的去向及其超微结构的变化

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摘　要　用激光共聚焦扫描图象系统(LCSIS)和透射电镜追踪和表征满江红鱼腥藻在发育中的满江红孢子果内的去向及其超微结构的变化。进入满江红孢子果的鱼腥藻营养细胞连锁体与孢子果的长轴大体呈平行状态，并活跃排列，最后形成鱼腥藻营养细胞群体。该群体占据了整个孢子果果腔(由囊群盖围合而成)和大孢子囊(或小孢子囊)周围一切可用的间隙。当果腔开口闭合时，部分营养细胞开始了向厚垣孢子分化的进程。在孢子果发育中期，果腔内的营养细胞连锁体都变成了外部形态类似于厚垣孢子的细胞链。从营养细胞向厚垣孢子的分化经历了细胞质重组和膜系统重排等一系列超微结构的变化。诸如多角体(Carboxysome)、藻蓝素颗粒、核糖体和类囊体之类的细胞内含物在数量、大小、形状和分布等方面随着厚垣孢子本身及其宿主孢子果发育进程而改变。文章还讨论了共生藻与自生蓝藻在厚垣孢子分化模式与超微结构特征的异同及其意义。

关键词　满江红；孢子果；鱼腥藻；共生；超微结构
中图分类号　Q 942

The Fate and Ultrastructural Changes of *Anabaena azollae* Within the Debeloping Sporocarps of *Azolla*

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Abstract　The fate and ultrastructural changes of *Anabaena azollae* entrapped into the developing sporocarps of *Azolla pinnata* were followed and characterized by laser confocal scanning image system (LCSIS) and transmission electron microscopy. The entrapped vegetative *Anabaena* hormogonia tended to run parallelly to the long axis of the young sporocarps and underwent an active division, forming eventually an *Anabaena* colony, which occupied the entire indusial chamber and all the space available around the megasporangium or microsporangia. Some of the vegetative cells initiated differentiation to akinetes while the opening of the chamber was closed. At an intermediate stage of the sporocarp development, all the vegetative cells on the hormogonia gradually transformed into the akinete-like chains. Ultrastructurally the preakinetes underwent the cytoplasm

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reorganization and the membrane systems rearrangement. The inclusions, such as carboxysomes, cyanophycin granules, ribosomes and thylakoids, varied in number, size, and their distribution in the cell along with the differentiation course of the akinete. The similarities and differences in differentiation patterns and ultrastructural feature between the endophytic and free-living cyanobacteria as well as their possible significance were discussed.

Key Words *Azolla*; Sporocarp; *Anabaena*; Symbiosis; Ultrastructure

Azolla is a genus of aquatic fern, which is also only the fern to be symbiotic with nitrogen-fixing cyanobacterium, commonly know as *Anabaena azollae*. Some of *Azolla* species regularly have sexual stage producing mega- and micro-sporocarp, housing the mega- and micro-spore, respectively. The fertilization of egg cells within the megaspores occurs inside the megaspocarps and the young sporophytes initiate their development here also (Lumpkin and Plucknett, 1980). This arrangement is coupled with the retention of some *Anabaena* filaments, which initiate to differentiate into akinetes as they became enclosed in the developing sporocarp pair, leading to the continuity of the fern-*Anabaena* symbiosis through the life cycle. The mechanism by which the vegetative cells of *Anabaena* were partitioned into the developing sporocarps has been suggested by Calvert *et al.* (1985); Zheng *et al.* (1988, 1994); Perkins and Peters (1993). Morphology and structure of *Anabaena* cells within the sporocarps of *Azolla* were described (Moore 1969; Herd *et al.* 1985; Becking 1987; Braun-Howland and Nierzwicki-Bauer, 1990). There has been, however, a limited knowledge about the fate and ultrastructural changes of the vegetative cells packaged into the young sporocarps although the changes have been simply illustrated in a report (Perkins and Peters, 1993). As a further contribution toward to an understanding of the nature of *Azolla-Anabaena* symbiosis, we give a detailed account of the fate, as observed by using laser confocal scanning image system (LCSIS), and changes, as revealed by using transmission electron microscopy.

1 Materials and Methods

The fronds of *Azolla pinnata* examined in this study were collected from the pond of the campus of the University of Sydney. The sporulating fronds were washed several times with tap water and dissected carefully with fine needle and scalpel under Olympus stereomicroscopy. The sporocarps at various developmental phases separated from the fronds were kept, respectively in 1 ml Eppendorf tubes containing distilled water. The specimens for LCSIS observation were transferred to the tube containing 2% Tween-80 and shook for 2 min in order to precipitate them into the bottom of the tube. The samples for TEM examination were pre-fixed in 2.5% glutaradehyde in PBS (pH 7.4) for at least 2 days at 4℃ and washed three times in PBS again. Then the individual sporocarps were post-fixed in 1% osimum tetroxide in PBS for 90 min, washed three times in PBS, and dehydrated through an ethanol

series. Finally they were incubated in propylene oxide and embedded in Epon 812. The samples were sectioned with a diamond or glass knives under an LKB Ultratome III ultramicrotome, and mounted on copper grids and were stained for 10 min in uranyl acetate in 50% ethanol followed by 2 min lead citrate. The sporocarps were examined with emphasis on the symbionts under BioRad MRC 600 LCSIS. All sections were examined with JEOL JEM-100CX transmission electron microscopy at accelerating potential of 80 kv.

2 Results and discussion

2.1 LCSIS observations

The morphological characteristics of the young sporocarps of *Azolla pinnata* were similar to those observed by light microscopy in earlier studies (Konar and Kapoor, 1974). At the early stage (shown in Fig. 1) the *Anabaena* were found to be hormogonia, which were short, roughly straight, and perhaps motile filaments lacking heterocysts. The vegetative hormogonia were entrapped into a chamber enclosed by two-cell-layer-thick indusium, which surrounds the entire megasporangium or microsporangia. The vegetative hormogonia entering the chamber through the pore at the top of young sporocarp tended to run roughly parallel with the long axis of the sporocarp and to one another (Fig. 1). The entrapped hormogonia usually consisted of 10–20 rounded vegetative cells approximately 2.0–2.5 μm wide and 3.0–3.5 μm long (data not shown). At this stage the vegetative cells seemed to undergo an active division. The cells formed gradually an *Anabaena* colony, occupying eventually the entire chamber and all the space available around the megasporangium or microsporangia (Figs. 1, 2).

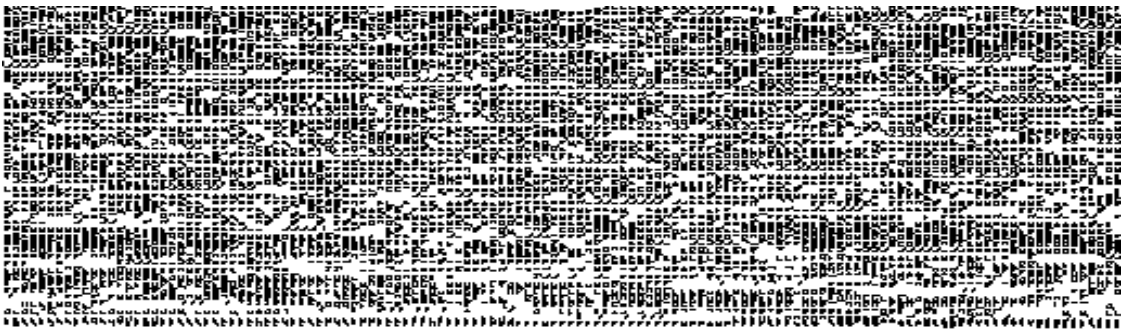

Fig. 1　A longitudinal section of the young sporocarp at the first node of the main stem branches from the stem apex, showing the *Anabaena* hormogonia entraped into the chamber of the young sporocarp. a= *Anabaena*; mas megasporangium. Bar= 100 μm.

Fig. 2　A longitudinal section of the developing sporocarp at the third node of the main stem branches, showing the *Anabaena* hormogonia in the chamber of the developing sporocarp. mis= micrisporangium, Bar= 100 μm.

It has been previously reported that the *Anabaena* cells actually multiplied within the sporocarp cavity of *Azolla*, and it occurred, however, only after the pore of the indusium was closed (Becking, 1987; Zheng and Huan 1994). Our data shown that enlongation and enlargement of a few vegetative cells of the hormogonia occurred as the pore was closed, indicating that the vegetative cells morphologically initiated their differentiation into akinetes. The extension of the differentiation occurred from one cell to neighboring cells and from one hormogonium to another. At an intermediate stage of the sporocarp development, all the hormogonia within the sporocarps became transformed to the akinete-like chains. The length of the cell increased from 3.5 μm to 10–15 μm. According to our observations on *Azolla pinnata*, the akinetes only appeared within the chamber of the developing sporocarps. There was not any preakinete outside the indusium. This was different from the results on *Azolla* mexicana Presl (Perkins & Peters, 1993) in which some of the vegetative cells began to differentiate into akinete-like structure prior to the pore complete closure.

2.2 Ultrastructural examination

A typical vegetative cell packaged into the forming chamber was shown in Fig. 3. The cell wall was characteristic of gram negative bacteria with peptideglycan. Between the plasma membrane and tiylakoids there were electron-transparent granules of glycogen. Numerous carboxysomes (polyheadral bodies) were also apparent. The ultrastructural characteristics of the vegetative cell seen in the present study were similar, to some extent, to those reported by Neumuller and Bergman (1981). As the onset of the differentiation of the vegetative cell into akinete, the morphological changes mentioned above were accompanied by several notable changes in ultrastructural characteristics. In the present data the cytoplasmic organization and membrane system arrangement of presumptive akinete was suggested to divide to six stages as follows: (1) The accumulation of cyanophycin granules in cytoplasm was companied with the formation of irregular electron transparent and electron dense intermixed areas, being similar to the observations on *Azolla maxicana* by Perkins and Peters (1993). Trace of fibrillar material, which seems to be an envelope precursor secreted from cytoplasm through the cell wall, appeared around the cell (Fig. 4). (2) Thylakoids and carboxysomes disappeared. More fibrillar material deposited surround the cell, and subsequently formed a discontinues fibrous envelope layer, separating from the cell wall by an electron transparent space (Figs. 5, 6). (3) The cytoplasm was more electron dense and disorganization. The deposition of the material surrounded the cell was extensive, becoming a continuous and undulate zone (Fig. 5, 6). (4) Cyanophycin granules seemed to fuse each other and associate with carboxysomes, and membrane-like structures appeared at the periphery of the cytoplasm, which was mostly like to be reappearing of thylakoids (Figs. 5, 7, 9). Widening of the electron transparent space and thickening of the peptideglacan layer in the outer membrane system continued (Fig. 9, 10). (5) The association of cyanophycin granules leaded to formation of a large body (up to 2 μm), which sometimes occupied nearly a half of the volume of cyto-

plasm. The body was surrounded by an unit membrane. Thylakoid membranes were scattered throughout the cytoplasm, which was accompanied by an increase in ribosome content (Fig. 6, 8). (6) The thickness of the peptideglycan reached a maximum level, which was about twice wider than that in vegetative cell. The profile of the uniform lamella of the envelope layer was visible in a mature akinete. When akinete became mature the envelope consisted the two layers with different electron densities (Fig. 10).

Fig. 3　A logitudinal section through the vegetative cell entrapped into the chamber of the young sporocarp at the first node of the main stem branches. cs= carboxysome; t= thylakoid, Bar= 0.5 μm.

Fig. 4　A presumptive akinete. Note the dense, disorganized cytoplasm, the accumulation of the cyanophycin granules in cytoplasm and trace of fibrillar material (arrow) around the cell wall. cy= cyanophycin granular, Bar= 0.5 μm.

These stages occurring at the differentiation process from the vegetative cell into akinete indicated that *Anabaena* inside the developing sporocarps underwent membrane rearrangement (Perkins and Peters, 1993) and cytoplasm reorganization. The inclusions, such as cyanophycine granules, carboxysomes, glycogen granules, ribosomes, and thilakoids varied in size, shape and number with various differentiation phases of both *Anabaena* cells and sporocarps. However, polyphosphate granule, lipid body and gas vesicles were rarely found within akinete in our materials. An accumulation of large quantities of cyanophycin granules seemed to be one of remarkable features, which occured within akinete of both symbiotic (Perkins and Peters, 1993; Becking, 1987) and free-living cyanobacteria (Leak and Wilson, 1965; Meller and Lang, 1968; Clark and Jensen, 1969; Wildman *et al.*, 1975; Sutherland *et al.*, 1979). In the present study the mature akinete showed membrane systems, to some extent, similar to those of free-living cyanobacteria. The two-layered envelope, for example,

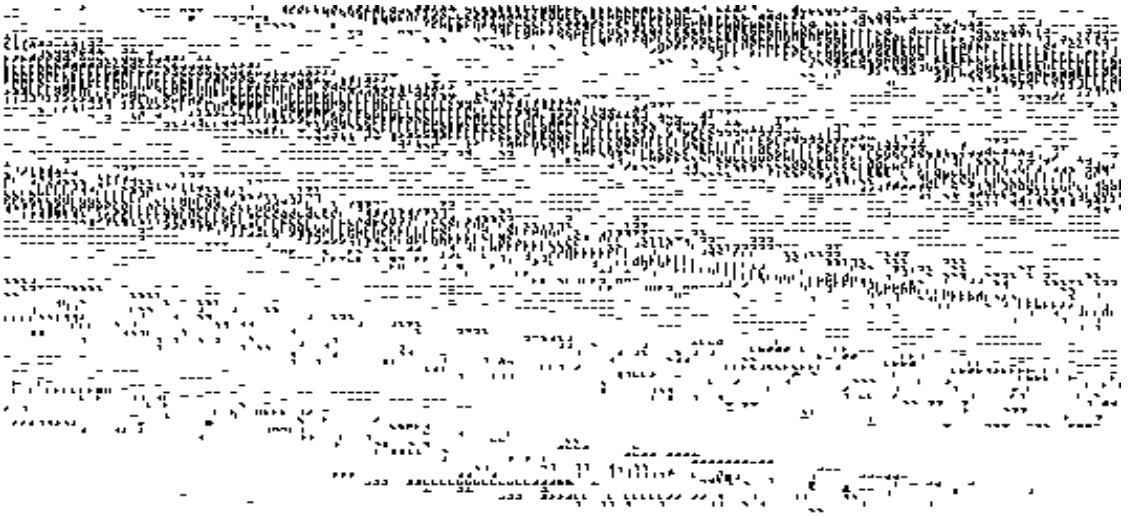

Fig. 5 A portion of two developing akinetes showing a cyanophycin granule associated with a carboxysome. en= envolope, Bar= 0.5 μm.

Fig. 6 A preakinete, showing the cyanophycin granule surrouded by an unit membrance and the deposition of fibrous materials in the granular. Note the thylakoid membranes reappeared within the dence cytoplasm and in negative contrast. Bar= 0.5 μm.

is common to the mature *Anabaena* akinete in our material and *Nostoc* PCC 7524 (Sutherland *et al.*, 1985). However, the cell wall of the later akinete differs from that of the former cell in having a greatly thickened and less electron dense peptideglycan layer (Sutherland *et al.* 1979, 1985). The thylakoid arrangements within advanced akinete appeared very similar to that of vegetative cell described by Numuller and Bergman (1981) who investigated the ultrastructure of *Anabaena azollae* in *Azolla pinnata* and was the same as that of blue-green algae reported by Whitton (1971, text-Fig. 1).

Grilli Caiola and de Vecchi (1980) suggested there were two types of akinetes in *Nostoc* isolated from cycas coralloid root. The second type, which contained large and numerous cyanophycin granules, ribosomes, few polyheadral granules, represented the mature, quiescent akinete, since it was able to survive for a long time in unfavorable growth conditions. Based on the ultrastructural characteristics the akinete of the symbiont found in the present study is, in a large extent, similar to the second type. This was confirmed by the fact that the megasporocarps, which had been kept for four years at low temperature, together with the akinetes could germinated and reestablished a mutualistic symbiosis.

Interestingly, during the sporulation of *Azolla* the differentiation and development of both the sporocarps and the akinetes were sychronised. The akinete at the sixth stage mentioned above could only be found in a mature megasporocarp, which frequently located at the

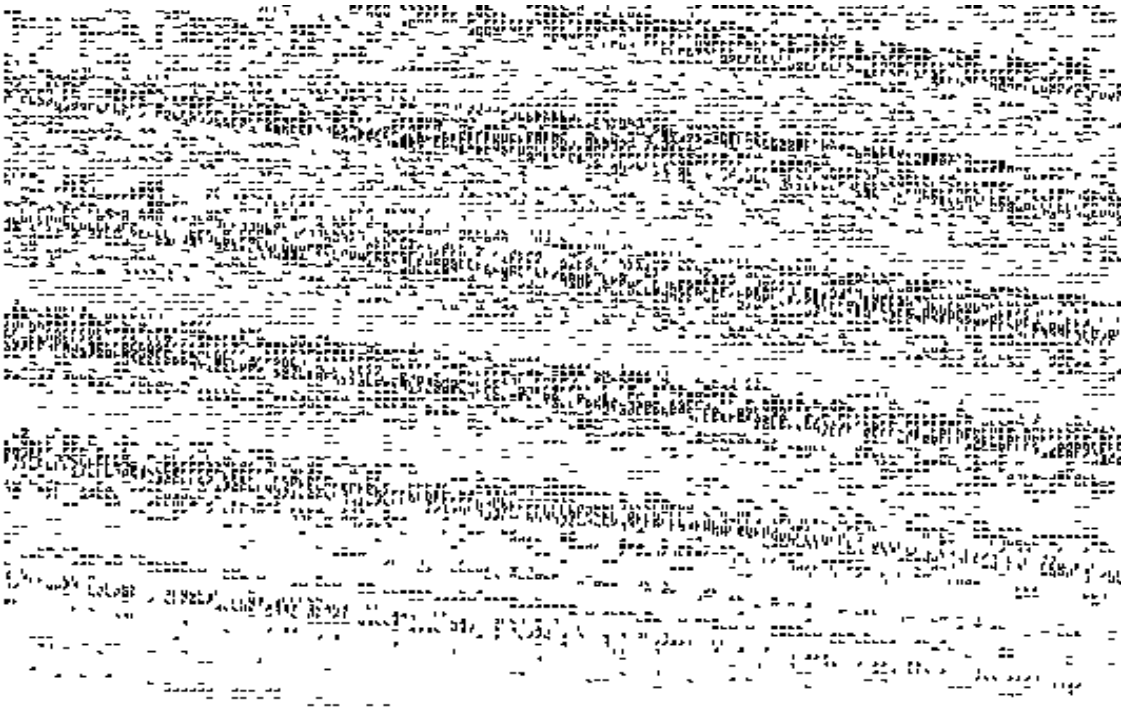

Fig. 7　A advanced akinete, showing two carboxysomes associated each other. Bar= 0.1 μm.

Fig. 8　A nearly mature akinete within the nearly mature megasporocarp at the fifth node of the main stem branches. Note a big cyanophycin body, scattered thylakoids in cytoplasm and the wide electron transparent area between the cell wall and the envolope. Bar= 0.5 μm.

Fig. 9– 10　A comparission of the thickness of peptidoglycan layer in the cell outer membrane systems betweem the developing (Fig. 9) and mature (Fig. 10) akinetes. Note the envolope (Fig. 10) consisted of the two layers with different electron densities. Bar= 0.1 μm.

sixth or seventh branch node of the main stem. However, the factors controlling the parallel development between the sporocarps and the akinetes have so far been poorly understood. Nevertheless, an attempt to know signal exchange between *Azolla* and *Anabaena* should be made besides physical factors. This is currently investigation in our lab.

3　Acknowledgments

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