

## CHAPTER 5

### Observations and Comparison of Septal Structures

#### 5.1 INTRODUCTION

The septal apparatus is considered to be significant in fungal taxonomy owing to its evolutionary conservativeness (Alexopoulos et al. 1996), and thus utilized for defining each fungal phylum. For example, the Ascomycota has the Woronin bodies or membrane-bound septal pore organelles around the septal pore, whereas the Basidiomycota is characterized by the dolipore septum accompanied by septal pore caps (Alexopoulos et al. 1996).

In the case of the phylum Zygomycota, the septal structure is employed for the classification of the orders (Benjamin 1959, 1979; Benny et al. 2001). The kickxellid orders [Kickxellales and Dimargaritales (Zygomycetes), and Harpellales and Asellariales (Trichomycetes)] regularly produce primary septa, while other orders do not form such septa (Benjamin 1979). These orders share a specific type of septa characterized by a large central pore occluded by a plug. Functionally, the plug seals the septal pore and prevents the entire loss of cytoplasm when the mycelium is ruptured naturally or forcibly (Jeffries & Young 1979, Brain et al. 1982).

Except in the Kickxellales, the septal structures of the kickxellids have been relatively well examined using a transmission electron microscope (TEM). On the septal ultrastructure of the Harpellales and the Asellariales, many reports have been published (for example, Reichle & Lichtwardt 1972, Manier 1973), as compiled by Sato (2001). These works have revealed that the septal structures of these orders are almost identical to those of the Kickxellales including the plug that lacks protuberances, although the cross wall margin around the septal pore is not so much conspicuous as those of the Kickxellales and the Dimargaritales (Saikawa 1977).

In the Dimargaritales, the septum of each representative of the three genera has already been studied with TEM (Saikawa 1977; Jeffries & Young 1979, 1981; Brain et al. 1982). *Dimargaris verticillata* R. K. Benj. 1959 (Saikawa 1977), *Dimargaris cristalligena* (Jeffries & Young 1979, 1981), *Dispira cornuta* Tiegh. 1875 (Saikawa 1977), and *Tieghemiomyces californicus* (Brain et al. 1982) are the species already detected. Dimargaritalean septa are typically characterized by the upper and lower protuberances associated with the plug.

Compared with other orders of the kickxellids, the septal ultrastructure

is poorly investigated in the Kickxellales. Among the known 28 species, only 3 species, *Linderina pennispora* (Young 1969, Benny & Aldrich 1975), *Kickxella alabastrina* (Saikawa 1989), and *Ramicandelaber longisporus* (Ogawa et al. 2001) have been examined ultrastructurally. The former two species belong to the *Coemansia* group, and the latter belongs to the *Ramicandelaber* group of the chapter 4. Septal ultrastructures of representatives of the *Spiromyces* group, however, have never been examined.

Because the septal apparatus typifies the Kickxellales and it is evolutionary stable than other morphologies (Raper & Fennell 1952, Moss & Young 1978), observations of the septa of the order, especially those of the *Spiromyces* group, would be helpful to evaluate the morphology-based groups proposed in the chapter 4 and refine the taxonomy of the Kickxellales. Then, the septal structures of the Kickxellales were examined with a TEM.

From the preliminary survey using an optical microscope, all kickxellalean species appeared to produce the septal structure like *L. pennispora* (Young 1969, Benny & Aldrich 1975) and *K. alabastrina* (Saikawa 1989), and their plugs were stable in both acidic stains and 3% KOH. However, among them, the septa of three species require more precise examinations. That is, the plug of *Myconymphaea yatsukahoi* had protuberances exceptionally, and the details of the septa of *Mycoemilia scoparia* (tentative name) and *Ramicandelaber brevisporus* (tentative name) were unclear due to their small size. Furthermore, each of these species also represents the morphological group of the chapter 4. Consequently, they are selected as the materials for the observation. Taking into account the close relationships within the kickxellids, the septal structure was discussed comparing with that of other kickxellids.

## 5.2 MATERIALS AND METHODS

Septal structures of *M. yatsukahoi*, *M. scoparia* (tentative name), and *R. brevisporus* (tentative name) were investigated using a TEM. For the observation of septal ultrastructure, ultrathin sections of vegetative hyphae were prepared by the following procedures: Mycelia of the fungi were obtained from submerged cultures within one-tenth strength and agar free ME-YE medium at room temperature, and these were fixed in the Karnovsky's fixative [an aqueous solution of paraformaldehyde (2%) and glutaraldehyde (2.5%) buffered at pH 7.2 with HCl-sodium cacodylate] for 3 hr, and then postfixed in 1% Osmium tetroxide for 2 hr. After the dehydration through an ethanol series and acetone, samples were embedded in

the Quetol 651 resin (Nissin EM Co., Ltd.). From the samples, ultrathin sections were cut with a diamtome knife using a LKB NOVA ultramicrotome, and then these were stained with uranyl acetate and Reynolds' lead citrate. These sections were examined with LEM-2000 (Topcon Co., Ltd.) operating at 80 kV.

### 5.3 RESULTS

Some differences in septal structures were recognized between the species detected, especially in the shape of the plugs, although all the species essentially shared the kickxellalean type septa (Fig. 5-1).

#### 5.3.1 *Myconymphaea yatsukahoi*

Septal structures of *M. yatsukahoi* were unique in possessing the plug associated with a protuberance. In the species, the bifurcate cross wall surrounded the central pore and an electron dense septal plug subsisted in the cavity (Fig. 5-2). The plug was essentially lenticular and continuous with a minute protuberance that located the upper side of the plug (Figs 5-3, 5-4). The protuberance appeared to be composed of the same material of the plug. The protuberance was not so conspicuous in TEM micrographs than in optical microscopic figures. This might result from a fixation artifact. Such artifacts that cause the flattening of protuberances are known in *Tieghemomyces californicus* (Brain et al. 1982).

Two differently stained layers were observed in the cell wall. The outer layer was the thin and electron dense, while the inner layer was thick, electron lucent, and continued to the cross wall. The electron lucent cross wall was stratified (Fig. 5-3) as known in aerial hyphae and the base of the pseudophialides of *Linderina pennisporea* (Young 1969, Benny & Aldrich 1975). Within a cell, a constant cell membrane lined the cross wall, the cavity, and the cell wall, and the cell membrane and cytoplasm were continuous through the septal pore.

In this species, membranous organelles were frequently observed around the septa (Fig. 5-3). This would be endoplasmic reticulum as described in vegetative hyphae of *Dimargaris cristalligena* (Jeffries & Young 1979). A similar organelle, however, was interpreted as the cell membrane bulged on one side of the septum through the cavity in aerial hyphae of *L. pennisporea* (Young 1969).

#### 5.3.2 *Mycoemilia scoparia* (tentative name)

Septal structures of *M. scoparia* (tentative name) were basically similar

to those of *L. pennispora* (Young 1969, Benny & Aldrich 1975). The cross wall was composed of electron lucent material and bifurcate around the septal pore to form lenticular cavity (Fig. 5-5). Septal plugs consisted of electron dense material occupied the cavity (Fig. 5-6).

In the cell wall of the species, two differently stained layers were indicated. The inner layer was thick and electron lucent and the outer layer was thin and electron dense, and the inner layer was continuous with the cross wall (Fig. 5-5). A continuous cell membrane lined the inner surface of a cell, the cross wall, and the cavity.

### 5.3.3 *Ramicandelaber brevisporus* (tentative name)

Septal structures of *R. brevisporus* (tentative name) were almost identical with those of the congener, *R. longisporus* (Ogawa et al. 2001). The cross wall had a central pore that was surrounded by the bifurcate margin of the wall and occupied with an electron dense plug (Fig. 5-7). The plug was thinner and accounted for the larger ratio in the cross wall than that of other kickxellaleans, and its margin and center were partially thick (Fig. 5-8). The shape of the plug in electron micrographs appears to be rather flatter than the light microscopic figure. The flattening under TEM may be a fixation artifact as elucidated in the protuberances of *T. californicus* (Brain et al. 1982).

Two-layered nature of the cell wall was also recognized in *R. brevisporus* (tentative name). The inner and electron lucent layer of the cell wall was continuous with the cross wall, and a cell membrane lined these walls and the central cavity.

The stratified nature of the cross wall and the continuity of the cell membrane between the adjoining cells were uncertain. Cytoplasm was highly vacuolated.

## 5.4 DISCUSSION

The results of the present observations largely agreed with the precedent observations by Young (1969), Benny & Aldrich (1975), Saikawa (1989), and Ogawa et al. (2001); the members of the Kickxellales share a type of septa. Thus, total septal structure appears to have little value for the infra-ordinal taxonomy of the Kickxellales, probably because it is too conservative for the taxonomy at that level. However, the shape of septal plugs shows disparities between the morphological groups in the chapter 4 and supports the grouping. The plug is

essentially lenticular in the *Coemansia* group (Young 1969, Benny & Aldrich 1975, Saikawa 1989), flat and disk-shaped in the *Spiromyces* group, and quite thinner in the *Ramicandelaber* group (Ogawa et al. 2001). Lenticular plugs of the *Coemansia* group rather resemble those of the Harpellales than those of the *Spiromyces* group and the *Ramicandelaber* group, and such differences between the groups may be phylogenetically important.

For distinguishing the Kickxellales and the Dimargaritales, the presence of septal plug protuberances has been used as a significant characteristic as well as the solubility of the plug (Benjamin 1979). The Kickxellales lacks the protuberance, while the Dimargaritales produces it on the upper and the lower sides of the plug (Benjamin 1959). However, the plug of *M. yatsukahoi* exceptionally bears a protuberance on the upper side that is not so prominent as that of the Dimargaritales. The plug of *M. yatsukahoi* superficially resembles that in sporangiophore of *D. cristalligena* in being accompanied by a single protuberance, but *M. yatsukahoi* was accommodated into the Kickxellales since its plug was stable in both acidic stains and 3% KOH the same as other kickxellalean species. The presence of the protuberance found in the species appears to be a morphological variety in kickxellalean septal plug.

In the Dimargaritales, such variety has already been known: the presence and the shape of the protuberance vary depending on organs or species. For example, in *D. cristalligena*, the plug in vegetative hyphae possesses globose protuberances on both sides like other species of the Dimargaritales (Benjamin 1959, Saikawa 1977, Brain et al. 1982). However, the lower protuberance is obconical in sporangiophores and sporiferous branchlets of the species, and the plug of infection pegs lacks protuberance or has a reduced protuberance only (Benjamin 1959; Jeffries & Young 1979, 1981). A similar organ-dependent variation of the protuberances occurs in *T. californicus* (Jeffries & Young 1981, Brain et al. 1982). In conclusion, the existence of protuberances appears to be not absolute in the Dimargaritales.

These facts suggest that the existence of protuberances would be insufficient as the diagnosis to differentiate the Dimargaritales from the Kickxellales, although that has been regarded as a key character (Benjamin 1979). This finding in *M. yatsukahoi* would be significant to evaluate phylogenetic relationships of the Kickxellales and other kickxellids, because the absence of protuberances has been one of the bases of the hypothesis that the Kickxellales

more closely relates to the Harpellales and the Asellariales than to the Dimargaritales (Lichtwardt 1973, Moss & Young 1978).

The bilayered nature of the cell wall observed in all the three species examined here is shared among the kickxellids including the Asellariales (Reichle & Lichtwardt 1972, Manier 1973, Brain et al. 1982). The chemical components of the layers have been identified in the selected species of the Kickxellales (*Goemansia mojavensis*, *L. pennispora*, and *Martensiomycetes pterosporus*) and the Dimargaritales (*Dimargaris bacillispora*, *D. cristalligena*, *D. cornuta*, and *T. californicus*) (Benny 1972). While the outer layer of the wall and the septal plug contain lipofuscin pigments and other lipids, the inner layer and cross wall mainly consist of chitin (Benny 1972). Differing from the outer layer, the plug lacks unsaturated lipids, and this may cause the difference in electron density between them (Benny 1972). Cross walls additionally contain hyaluronic acid, but no difference is detected between the cross wall and the inner layer in electromicrographs. Although the difference between the layers is not identified, the wall of the Harpellales has a similar composition (Whisler 1963, Sangar & Dugan 1973).

The cross wall was stratous in *M. yatsukahoi* and *M. scoparia* (tentative name). Such stratification has been observed in *L. pennispora* (Young 1968) and *D. cristalligena* and *T. californicus* (Dimargaritales) (Brain et al. 1982, Jeffries & Young 1979). The structure was insecure in *R. brevisporus* (tentative name) because of the limit of the resolution. These structural and chemical similarities of the cell wall probably manifest the monophyly of the kickxellids.