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FULL PAPER

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Two ultrastructural aspects of the trichospore of *Pennella angustispora* (Harpellales): canals in the sporangiospore cell wall and appendage formation

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Abstract Ultrastructure of the trichospore (monosporous sporangium) and its generative cell of *Pennella angustispora* was observed by electron microscopy. In the sporangiospore, both poles of the cell wall were thickened in appearance with canals. Appendages developed longitudinally along the wall of generative cell before the formation of a septum that would delimit between the trichospore and its generative cell.

Key words Appendage · Harpellales · *Pennella angustispora* · Trichospore · Ultrastructure

Introduction

Species of Harpellales live in the digestive tracts of insect larvae living in water. The thalli of this group attach to gut linings by special appresoria called holdfasts. The spore in this order is called the trichospore, which is a sporangium containing a single sporangiospore, and the spore has one to several appendage(s) on the basal part (Lichtwardt 1986).

Prominent structures for holdfast production have been found in the trichospore, such as spore bodies containing a holdfast substance (Moss and Lichtwardt 1976; Horn 1989) and canals in the cell wall through which the substance is released (Horn 1989; Sato 1993). It is thought that rapid attachment of young thalli to the gut lumen was supported by these structures (Horn 1989; Sato 1993). Although spore bodies can be observed under the light microscope (Lichtwardt 1967, 1984; Frost and Manier 1971; Moss and Lichtwardt 1976; Williams 1983; Horn 1989; Sato and Aoki 1989), the canals can only be observed by electron microscopy. Ultrastructural investigations on the canals are limited so far (Manier 1973; Moss and Lichtwardt 1976; Horn 1989; Sato 1993).

The appendage is one of the key characters for identification of species in Harpellales (Lichtwardt 1986). Although the existence and number of appendages can be observed under the light microscope, the ontogeny of trichospores can be known only by electron microscopy. Moss and Lichtwardt (1976) stated that there are two types of appendage ontogeny in trichospores; however, no additional ultrastructural work on ontogeny has been reported up to the present. From ecological and taxonomic points of view, the accumulation of ultrastructural aspects is thought to be important, especially for groups that are difficult to culture such as those in Trichomycetes.

Pennella angustispora Lichtwardt is a species belonging to the Legeriomycetaceae in Harpellales. This fungus lives in the larval hindgut of black flies. To date, one ultrastructural study has been conducted on this fungus, but it only focused on the holdfast structure and thalli that contain the septal structure (Mayfield and Lichtwardt 1980). In this study, the existence of canals and the ontogeny of the type of appendages were examined at the ultrastructural level.

Materials and methods

Larvae of a species of *Simulium* (Diptera) were collected in a brook on Mt. Takao, Tokyo, in June 1989, and were dissected with sharp forceps and sharp blades to derive thalli of *Pennella angustispora* attached to the hindgut cuticle. The hindguts containing the fungus were fixed with 2.5% glutaraldehyde in a phosphate buffer solution at pH 7.3 after Mizuhira (1988). After rinsing in this buffer solution for 2 h, the specimens were postfixed with 2% osmium tetroxide (OsO_4) in the same buffer solution overnight at 5°C, dehydrated with an acetone series, and embedded in Spurr's resin (Spurr 1969). Ultrathin sections were stained by uranyl acetate and lead citrate and then observed by a JEOL-100C electron microscope at 80 kV.

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Results

Light microscopy

Unreleased mature trichospores contained a row of spore bodies (Horn 1989) near the apical part of the trichospore (Fig. 1). At least two appendages were found to have been formed longitudinally along the wall of the generative cell (Fig. 1). In the early stage of trichospore formation, the point between the spore and the generative cell was constricted in a necklike appearance (Fig. 2).

Electron microscopy

Unreleased trichospores forming sporangiospores on apical generative cells were observed. The sporangial wall consisted fundamentally of four layers (Fig. 3): the outermost thin electron-dense layer (L1), a second thin electron-translucent layer (L2), a third electron-dense layer (L3), and the fourth electron-translucent layer (L4) (Fig. 4). In part, a thin electron-dense layer (L7) making the layers into six was observed in the fourth layer (Fig. 5), or the outer two layers were not distinguishable around the basal part of the sporangial wall (Fig. 7). The sporangiospore wall was observed as a two- or three-layered structure. Along the lateral side of the sporangiospore wall, an electron-dense outer layer (L5) and electron-translucent inner layer (L6) were observed (Fig. 4). At the basal part of the sporangiospore, a moderate electron-dense layer (L8) was observed (Fig. 7).

The sporangiospore wall at the apical tip was thicker than the lateral wall and included several vertical canals in parallel (Fig. 3). Canals are tubelike structures open to the outside of the sporangiospore through the cell wall and face the plasma membrane at a right angle to the innermost part. An electron-dense deposition was observed between the tip of the invaginated sporangiospore wall with canals and sporangial wall (Fig. 3). This deposition was also observed between the canals (Fig. 3). Spore bodies were located behind the plasma membrane. At the basal part of the sporangiospore (Figs. 6, 8), thickening of the cell wall was also observed, which showed a domelike appearance. In a skipped serial section from Fig. 6, a vertical tubelike structure in parallel was observed in the thickened cell wall (Fig. 8).

The sporangiospore was delimited from the generative cell by a septum containing a single central pore occluded by an electron-opaque plug (Fig. 6). The septum originated from the electron-translucent inner layer of the cell wall flared around the central pore. Extension of the cell wall originating from the inner layer of the sporangial wall was observed immediately beneath the constricting part (Figs. 6, 8). In the area where the trichospore was connected with the generative cell, the translucent layer of the generative cell wall was thinner or barely visible (Figs. 6, 8). Immediately under the septum, appendages were located along the generative cell wall longitudinally and were contiguous with the extension of the cell wall (Figs. 6, 8). The appendages were less electron dense and were surrounded by the electron-dense matrix (Figs. 6, 8).

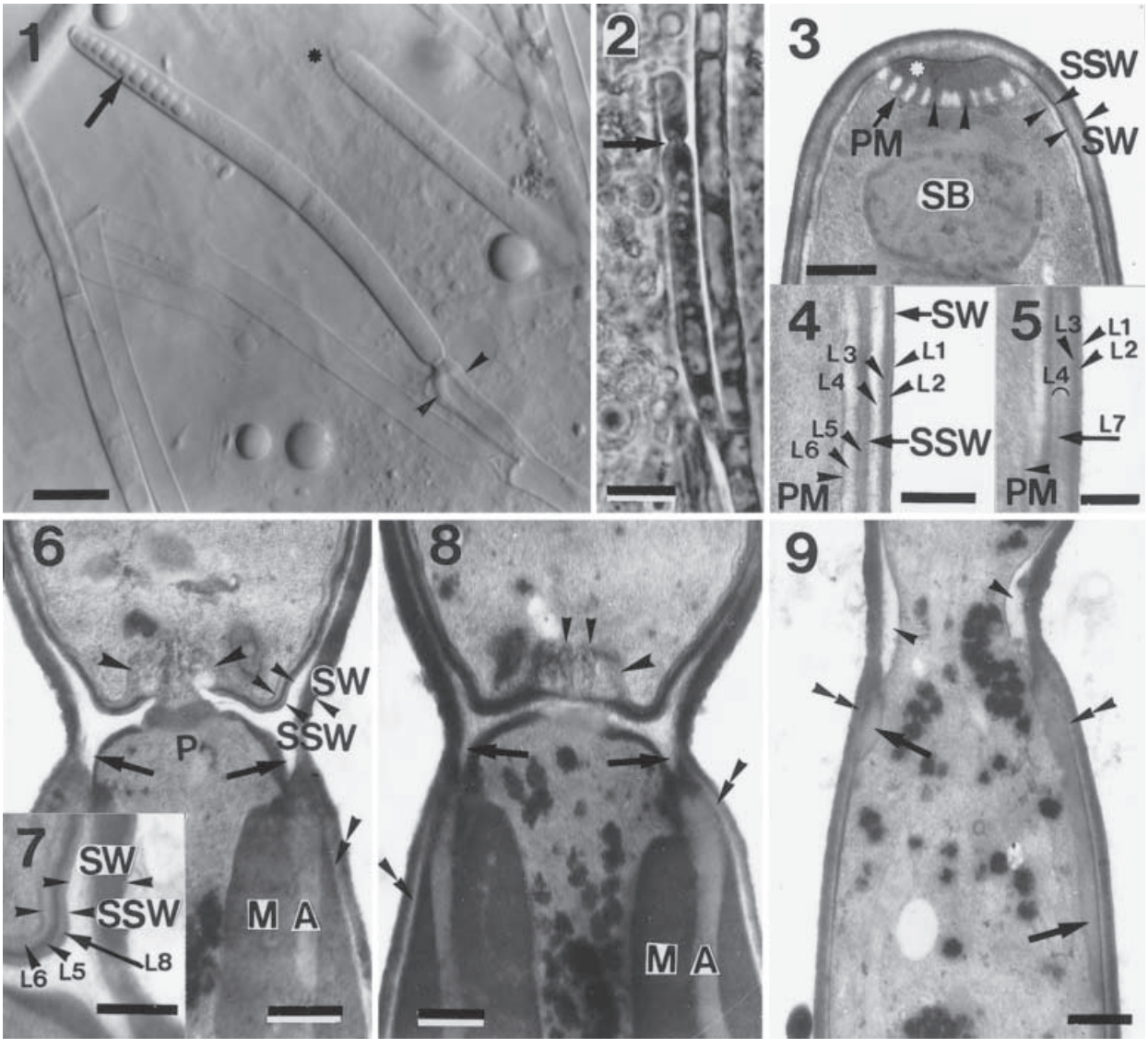
In the early stage of trichospore formation before a sporangiospore was produced inside, centripetal thickening of the translucent inner layer of the cell wall was observed at the necklike part, even though a septum between the spore and its generative cell had not formed (Fig. 9). Immediately below the necklike part, an electron-dense accumulation that was contiguous to the thickening cell wall was observed longitudinally along the cell wall of its generative cell, and the translucent inner layer of the generative cell wall was once again thinner or barely visible (Fig. 9).

Discussion

In *Pennella angustispora*, spore bodies were observed with a light microscope as in *P. arctica* Lichtwardt & Williams (Lichtwardt 1984), and electron microscope observations showed canals in the cell wall as in earlier studies in several genera (Manier 1973; Moss and Lichtwardt 1976; Horn 1989; Sato 1993). These observations suggest that *P. angustispora* and *Smittium* spp. (Horn 1989; Sato and Aoki 1989; Sato 1993) have a manner of attachment process very similar to each other.

Sporangiospore cell walls with canals have been recorded in the following species: *Legeriomyces ramosus* Pouzar (Manier 1973), *Genistellospora homothallica* Lichtwardt (Moss and Lichtwardt 1976), *Smittium culicis* Manier (Moss and Lichtwardt 1976; Horn 1989), and *S. culisetae* Lichtwardt (Horn 1989; Sato 1993). There are some differences in the existence of canals, as follows. *Genistellospora homothallica* has cell wall thickening only at the apical part of its sporangiospore (Moss and Lichtwardt 1976), although *S. culisetae* has cell wall thickening at both poles of its sporangiospore and only the apical thickening has canals (Sato 1993). *Smittium culicis* has canals on both poles of the sporangiospore wall and the holdfast substance is released at either end (Horn 1989). Vertical tubelike structures in the thickening basal cell wall observed in *P. angustispora* are thought to be the developing stage of canals, and this fungus may have canals at both poles of its sporangiospores, as in *S. culicis*. In *L. ramosus*, only the apical part of the sporangiospore has been observed (Manier 1973). Horn (1989) reported an interwall layer between the sporangiospore and sporangial wall in *Smittium* spp., which is located at the same position as the electron-dense deposition observed in *P. angustispora*. The electron-dense deposition in *P. angustispora* might be the precursor material of the interwall layer.

Sato et al. (1989) reported that the sporangial wall of *Smittium morbosum* Sweeney consisted of four layers. In this study, *P. angustispora* also showed the same structure. The sporangiospore wall of *S. morbosum* also consisted of four layers (Sato et al. 1989) and Horn (1989) reported that the sporangiospore wall of *S. culisetae* was multilayered. We observed two or three layers of sporangiospore wall in *P.*



Figs. 1, 2. Light photo micrographs of *Pennella angustispora*. **1** An unreleased mature trichospore. *Arrow*, a row of spore bodies; *arrowheads*, appendages; *asterisk*, a tip of a generative cell after the detachment of a trichospore. Nomarsky's interference apparatus. *Bar* 10 μm. **2** Young trichospore. *Arrow*, necklike constriction between a trichospore and its generative cell. Phase contrast. *Bar* 10 μm

Figs. 3–9. Ultrathin sections of *Pennella angustispora*. **3** Apical part of trichospore. *PM*, plasma membrane; *SB*, spore bodies; *SSW*, sporangiospore wall; *SW*, sporangial wall; *arrowheads*, canals; *asterisk*, electron-dense deposition. *Bar* 1 μm. **4** Higher magnification of sporangial and sporangiospore wall. *PM*, plasma membrane; *SW*, sporangial wall; *SSW*, sporangiospore wall. *Bar* 0.5 μm. **5** Higher magnification of sporangial wall. *Arrow*, an electron-dense layer in the fourth layer making the layers of sporangial wall into six; *PM*, plasma membrane. *Bar* 0.5 μm. **6** Basal part of trichospore and distal part of generative cell. *A*, appendage; *M*, matrix; *P*, septal plug; *SSW*, sporangiospore wall; *SW*, sporangial wall, *arrows*, extension of cell wall of the sporangial wall; *arrowheads*, thickening of cell wall; *double arrowheads*, thinner appearance of translucent cell wall layer. *Bar* 1 μm. **7** Higher magnification of lower part of sporangial and sporangiospore wall. *SW*, sporangial wall (the outer two layers are not distinguishable); *SSW*, sporangiospore wall. *Bar* 0.5 μm. **8** Basal part of trichospore and distal part of generative cell in a serial section of the cell shown in Fig. 6. *A*, appendage; *M*, matrix; *arrows*, extension of cell wall of the sporangial wall; *large arrowhead*, thickening of cell wall; *small arrowheads*, initial canals; *double arrowheads*, thinner appearance of translucent cell wall layer of the generative cell. *Bar* 1 μm. **9** Generative cell producing young trichospore. *Arrows*, electron-dense deposition in initial appendage formation; *arrowheads*, thickening of inner layer of cell wall; *double arrowheads*, thinner appearance of translucent cell wall layer. *Bar* 1 μm

angustispora. This difference is thought to result from the immaturity of the specimens.

Light microscopic observations of appendages that formed longitudinally along the generative cell wall in the generative cell have been made in *P. angustispora* (Lichtwardt 1972). Such an appearance was also observed in this study with both light and electron microscopes. Appendages were thought to be formed after the accumulation of electron-dense matrix. Moss and Lichtwardt (1976) identified two basic types of appendage formation of trichospores: type A, in which appendages develop at the late stage of trichosporogenesis after septal formation; and type B, in which appendages develop at the early stage of trichosporogenesis before septal formation. The timing of

appendage formation of *P. angustispora* is recognized as type B. Moreover, the longitudinal location of appendages in their generative cell, the position of each appendage in the center of the matrix and in parallel to the generative cell wall, and the position of the appendage attachment are very similar to the appendages of *G. homothallica*, except for electron density. Moss and Lichtwardt (1976) reported that the electron density of the matrix was low and that of appendages was high. In this study, however, the electron densities were exactly the opposite. This difference might have been caused by the difference in fixatives: $KMnO_4$ was used by Moss and Lichtwardt (1976) and glutaraldehyde-OsO_4 was used in the present study.

A septum observed at the base of the trichospore was the type of Harpellales (Lichtwardt 1986) as well as the septa formerly reported in the thalli of this species (Moss and Lichtwardt 1976). Detachment of trichospores from their generative cells appears to occur around the translucent layer wall area immediately below the septum.

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