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The septal pore apparatus of Rhizoctonia solani was studied in hyphae of different ages by scanning electron microscopy. The formation of the septal pore apparatus begins with an annular swelling followed by the development of a dome-shaped perforated pore cap, which usually has three apertures. In older hyphae, the septal pore apparatus is absent, and the septal pore may be plugged. In some cases, four apertures are observed in the pore cap.

Introduction

Since Girbardt (7, 8) first presented his micrograph of the septum of the basidiomycete Polystictus versicolor and Moore and McAlar (13) described the components and distribution of the so-called dolipore septum, several other reports have been published on this subject (5, 6, 10, 12, 14, 15, 17, 18). This structure has also been studied in Rhizoctonia solani with the transmission electron microscope (1, 2, 4, 15) and was reported to consist of an annular swelling that forms the bounds of the septal pore near the center of the septum and of a perforated septal pore cap consisting of a dome-shaped structure found on each side of the septum (1, 4). A similar apparatus has also been observed in other Basidiomycetes (5, 6, 10, 14, 15, 17, 18). The importance of the septal pore apparatus has been emphasized in physiological and genetic studies involving protoplasmic streaming (2, 3, 4, 18) and nuclear migration (4, 6, 16), respectively. The nature of the septum of fungal cells has also been suggested as a basis for phylogenetic studies (1, 12, 18).

Transmission electron microscopic studies of ultrathin sections (which are usually longitudinal) did not permit detailed insight into the spatial arrangement of this structure. Scanning electron microscopic studies of the apparatus have not been carried out.

The purpose of this work was to study the structure and development of the septal pore apparatus of R. solani using the scanning electron microscope (SEM).

Materials and Methods

A virulent isolate of R. solani, which had been maintained on yeast extract medium was used (11). The fungus was grown at 27°C on a cellophane sheet that had been placed on yeast extract agar in Petri dishes. To observe the initial stages of the septal pore apparatus development, a 2-day-old colony, which had not yet reached the plate margin, was used. Later developmental stages were studied using a 7-day-old colony. The fungus was peeled from the cellophane and carefully rolled to attain mechanical support when gluing onto the SEM stub and to permit better fracturing at low temperature. The rolled fungus was fixed in 5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.0–7.4, for 2–3 h, washed twice with the buffer, and then dehydrated by immersing in increasing concentrations of ethanol (10% to 100%, in 10% increments), 10–15 min in each concentration. The rolled mycelium was dipped into liquid nitrogen (−196°C) for 20 min, and using two forceps, hyphae were fractured into small pieces. Only samples fractured at a distance smaller than 3 mm from the tips were used for examination of young hyphae. The fractured mycelia were attached with colloidion on stubs and immediately immersed again in liquid nitrogen for 20 min before coating with 200-Å-thick gold in a Sputtering Coating Unit, E 5000 (Polaron Equipment Ltd., England) under vacuum. The specimens were examined in a Cambridge Stereoscan electron microscope S4 at 20 kV. All micrographs represent cross sections of the mycelium. The terminology introduced by Bracker and Butler (1) is used here for description of the septal pore apparatus.

Results and Discussion

The septal swelling and pore cap are formed after development of the cross wall (4). In very young hyphae (Fig. 1), which have been fixed in glutaraldehyde before fracturing in liquid nitrogen, the cells are filled with cytoplasm and no septa could be detected. Figure 2 shows an early stage of cross wall formation in a young hypha. According to Buller (3) and Bracker and Butler (4), this process takes 10 min; however, no information has been available concerning the time of formation of the septal swelling and pore cap. In the young hyphae, only septal swellings can be seen (Fig. 3). At this stage the septal pore is
closed and only two or three perforations can be seen (Fig. 3). When the pore cap first appears in young hyphae (Fig. 4), it has no differentiated perforations and its surrounding septum has a wavy appearance. The pore cap has been reported to consist of a discontinuous membrane and appears to be modified endoplasmic reticulum (4). Butler and Bracker (4) have presented a micrograph (No. 17) of two adjacent *R. solani* cells showing a significantly smaller pore cap perforation in the nonvacuolated younger cell. Our micrographs of older hyphae (Fig. 5) show three highly differentiated perforations in the pore cap that have an approximately triangular

Figs. 1–10. Stages in development of the septal pore apparatus in *Rhizoctonia solani*. All magnifications are approximate. Scale lines represent 1 μm except for Fig. 1, where it indicates 10 μm. Young hyphae are shown in Figs. 1–4. Fig. 1. Fractured hyphae; most are filled with cytoplasm (C). × 2800. Fig. 2. Early stage in cross wall formation. × 15 000. Fig. 3. Annular swelling with three perforations (arrows). × 23 000. Fig. 4. Early stages in pore cap formation. Note the wavy septum and the initial perforations in the pore cap. × 16 500. Fig. 5. Dome-shaped pore cap with three apertures. One is partially hidden (arrow). × 16 500.
Fig. 6. Small septal pores (arrow) under the large perforations in the pore cap. × 28 000. Fig. 7. A stage in the pore cap disappearance showing three remnant protrusions (arrows) attached to the annular swelling. × 17 000. Fig. 8. Advanced stage of disappearance of the septal pore apparatus. × 15 000. Fig. 9. Plugged septal pore with annular swelling. × 15 000. Fig. 10. Pore cap with four perforations. × 20 000.
shape with a base larger than the apex. In Fig. 6, a possibly older pore cap with larger perforations and a partially closed septal pore is seen. The different diameters of the perforations may be due to their flexibility, which is required for the passage of cell organelles as suggested by Bracker and Butler (2) and Butler and Bracker (4). The perforation of the pore cap in Figs. 4–6 is varied in size, apparently enlarging during aging. Butler and Bracker (4) have indicated that the apertures of the cap are up to 1 μm in diameter. The capacity of organelles to pass from one cell to another is determined by the size of both septal and pore cap openings as well as by the plasticity of the organelles (2, 4).

After aging or hyphal injury the septal pore apparatus disappears (3, 4). Giesy and Day (6) stated that degradation of the primary dolipore in Coprinus lagopus occurs in connection with dikaryotization during nuclear migration. Janszen and Wessels (9) have reported enzymic dissolution of hyphal septa in Schizophyllum commune. Indeed, in older cells in which the septal swelling is still present, the remnants of three protrusions are seen (Fig. 7). In Fig. 8, the protrusions and the septal swellings have disappeared and only a slightly elevated circle remains around the pore.

Septal pore plugging occurs in aged cells or in cells under stress (3, 4). Plugging in R. solani is discussed by Butler and Bracker (4) and in other fungi by Moore and Marchant (13). Figure 9 probably shows such a plugging of the central pore.

Based on the size and arrangement of the perforations in the pore cap, Wilsenach and Kessel (18) described two types of septal pore apparatuses: (i) the “Polyporus type” with small pores of regular size and spacing, and (ii) the “Rhizoctonia type” with large pores of irregular size and spacing in the cap. Setliff et al. (15) described a third type with an apparently continuous pore cap in Polyporus tomentosus. However, our micrographs of R. solani show that the perforations of the cap are very regularly distributed, and in addition, in young hyphae these perforations are less developed. In older hyphae, four perforations in one pore cap can be seen in some cases (Fig. 10).

Inconsistencies in the number of perforations of the septal pore cap of R. solani could be due to differences in the physiological condition of, or to genetic variation in, the different hyphae examined.

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