Ultrastructure of Dormant and Germinated Sporangiospores of *Rhizopus arrhizus*

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Summary

Ultrastructural investigations of dormant and germinated sporangiospores of *Rhizopus arrhizus* were conducted by the use of thin sectioning and freeze-etching procedures. Dormant spores contained deep furrows and prominent ridges with swellings along the sides of the ridges. The furrows and ridges almost disappeared as spores swelled during germination. The plasma membranes contained protuberances or depressions (approximately 50 nm diam) depending upon the nature of the fracture. Mitochondria in dormant spores were spherical and contained few cristae compared to mitochondria of germinated spores which were larger, more diverse in shape and contained abundant cristae. Treatments of spores with 20-25% glycerol prior to freeze-etching or thin sectioning resulted in the production of artifacts between the cell wall and the plasma membrane. Vesicles were rarely observed at the apices of young germ tubes, but were present in abundance in growing hyphal tips. Surface views of young germ tubes revealed the presence of microfibrils.

1. Introduction

*Rhizopus arrhizus* is a common post-harvest pathogen on a number of fruits (Ogawa et al. 1961, Smith 1962), and has been reported to be pathogenic on humans and animals (Emmons et al. 1970). *Rhizopus* sporangiospores were studied at the ultrastructural level by Hawker and McV. Abbott (1963) and Nečas et al. (1963). These investigations indicated that sporangiospores of *R. nigricans* and *R. sexualis* contained several nuclei and characteristic organelles and that during germination a new wall forms which extends around developing hyphal cells. Buckley et al. (1968) investigated germinating sporangiospores of *R. stolonifer* and *R. arrhizus* and reported the presence of ribosomes which were visualized by special staining procedures and cytosomes which contained 6 nm particles. They suggested that the
cytosomes contained protein and lipid and possibly participated in membrane elaboration during germination.

The only reported investigation of *Rhizopus* sporangiospores which utilized freeze-etching was by Hess and Weber (1972). The purpose of the present investigation was to study dormant sporangiospores with freeze-etching and to utilize both freeze-etching and thin sectioning to investigate germinated sporangiospores of *R. arrhizus*.

2. Materials and Methods

The isolate of *Rhizopus arrhizus* Fischer used in this investigation was obtained from infected peaches in California (Weber and Ogawa 1965 a). Cultures were maintained on potato dextrose agar. Mycelium and sporangia were separated and then the sporangiospores were transferred to 0.1 M phosphate buffer, pH 6.5, containing 0.01 M proline. The spores were shaken in this medium at room temperature to induce synchronous germination (Weber and Ogawa 1965 b). For ultrastructural investigations 20 ml of spores were placed in 100 ml of the germination medium in 250 ml flasks which were shaken 50 shaking per minute at 25 °C for 8 hours. Germinated spores were then concentrated by centrifugation prior to fixation for ultrastructural investigations. Spores were also germinated on agar medium which was prepared from the phosphate buffer-proline medium by addition of 2% agar. The spores were fixed for ultrastructural investigations by flooding the agar surfaces with buffered fixative. For thin-sectioning studies the spores were collected as described above and fixation and embedding was done according to the glutaraldehyde-acrolein-osmium procedures of Hess (1966).

Sporangiospores were germinated in the phosphate buffer-proline medium. Spores were separated from mycelium by use of glass wool filters and processed for freeze-etching according to the methods described by Sassen et al. (1967), except that 5.25% sodium hypochlorite (Chlorox) was used in place of Eau de Javelle to dissolve spores from frozen-etched replicas and replicas were left overnight at 52 °C in 70% H2SO4. The spores which were treated with glycerol were left for 1–2 hours in 20–25% freshly mixed glycerol prior to freeze-etching.

3. Results

Dormant sporangiospores of *Rhizopus arrhizus* characteristically exhibit deep furrows and prominent ridges which sometimes contain localized swellings along the sides of the ridges (Figs. 1 and 2). Various methods were used in attempts to fix dormant sporangiospores for thin sectioning investigations. These attempts were not successful. However, when dormant spores are frozen-etched, it is possible to characterize the wall layers and organelles. Fig. 2 shows the plasma membranes of sporangiospores after the walls and cytoplasm have been fractured away. When the cytoplasm is fractured away the plasma membrane contains prominent protrusions (Figs. 2 and 3), however, when only the cell wall is fractured away the plasma membrane contains prominent depressions (Figs. 2–4). In some instances large depressions in the plasma membrane are evident after the cell wall has been fractured away (Fig. 5). Commonly the protrusions and depressions are approximately 50 nm in size (Figs. 3 and 4). The large depressions which are also seen occasionally