Enzymic dissolution of hyphal septa in a Basidiomycete

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Treatment of hyphal-wall preparations of *Schizophyllum commune* with chitinase and R-glucanase results in the dissolution of septa but the walls retain their structural integrity.

Extensive heterokaryotization in Basidiomycetes depends on the dissolution of septa which normally prevent the migration of nuclei (Giesy and Day, 1965). Previous studies (Wessels, 1969*a*) have shown a correlation between the activity of a β-1,6 glucanase (R-glucanase) and the occurrence of nuclear migration in *Schizophyllum commune*. This suggests that the enzyme functions in the removal of the septal barrier prior to nuclear migration. Because R-glucan is one of the major hyphal-wall components of this fungus, this notion obviously requires that R-glucanase more easily destroys the structural integrity of the septa than that of the hyphal walls. To obtain evidence for such a difference in structural stability, isolated hyphal walls, which still contain intact septa, were treated with various enzymes and alkali. Thin sections of the extracted wall preparations were then examined in the electron microscope for the effect of the treatment on septa and hyphal walls.

Hyphal-wall preparations were obtained from the homokaryotic strain 699 using the procedure of Manocha and Colvin (1967). Subsequent treatments removed distinct wall polysaccharides from these wall preparations. Alkali-soluble glucan (S-glucan) was removed by extracting the walls with 1 N KOH at 25 C for 18 hr. Alkali-insoluble glucan (R-glucan) was removed by incubating the walls with an R-glucanase preparation obtained from the culture fluid of *S. commune* (Wessels, 1969*b*). Virtually complete removal of R-glucan was achieved by incubating the walls (1 mg) with a solution of 1 mg R-glucanase in 1 ml 0.05 M McIlvain buffer (pH 5.5) at 30 C for 24 hr. Chitin was only partially removed by incubating the walls with chitinase in McIlvain buffer at 30 C for 3 hr. The chitinase was obtained by purifying a commercial chitinase preparation (Koch and Light, England). After washing with water, untreated walls and extracted residues were embedded in 2% agar and fixed in glutaral-

dehyde (30 min) followed by potassium permanganate (20 min). After removal of excess fixative and dehydration in an ethyl alcohol series, the agar blocks were infiltrated and embedded in Epon. Thin sections (700–900 Å) were post-stained with uranyl acetate followed by lead citrate.

Treatment of the walls with alkali, R-glucanase, or chitinase alone does not visibly affect the structural integrity of the walls and the septa; only the septal swellings are removed by alkali or R-glucanase. Also, septa are still present after treatment with alkali followed by chitinase or R-glucanase. However, when chitinase-treated walls, which still contain septa, are incubated with R-glucanase, the septa disappear completely (Fig. 1). The R-glucanase treatment

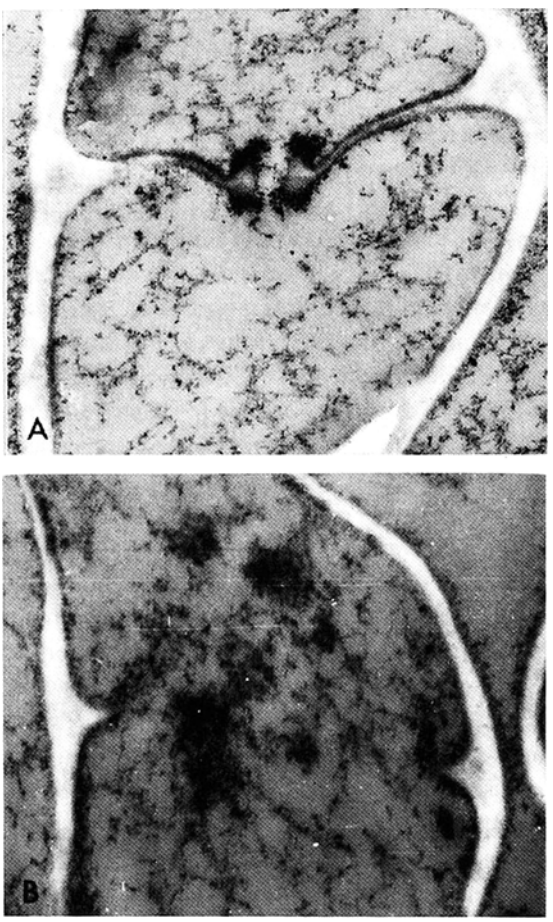

Fig. 1. Isolated hyphal walls of *Schizophyllum commune* treated with chitinase (A) and with both chitinase and R-glucanase (B). The dark material inside and outside the walls is agar used for embedding the walls.

removes all the R-glucan, yet the walls remain entire probably due to the presence of S-glucan. Since the septa are dissolved, S-glucan appears to be absent from these structures or, if present, it does not contribute to the maintenance of septal structure. Such differences in structure of septa and walls may explain why in a common-*A* heterokaryon and a *B*-factor mutant of *S. commune*, two instances in which high R-glucanase activities have been encountered (Wessels, 1969*a*), the septa are frequently disorganized while the hyphal walls remain intact and only assume an irregular shape.

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