Comparative Inhibitory Effects of Cytochalasins on the Gametangial Differentiation in *Allomyces arbuscula*

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**Abstract**

In contrast to cytochalasin D which selectively prevents differentiation of female gametangia, cytochalasins A, B and E also masculinize *Allomyces arbuscula* by preventing septation between female and male gametangia, thereby allowing the dominant expression of the male characteristics.

Reference


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Comparative Inhibitory Effects of Cytochalasins on the Gametangial Differentiation in *Allomyces arbuscula*

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**ABSTRACT.** In contrast to cytochalasin D which selectively prevents differentiation of female gametangia, cytochalasins A, B and E also masculinize *Allomyces arbuscula* by preventing septation between female and male gametangia, thereby allowing the dominant expression of the male characteristics.

Sexual differentiation in the *Allomyces* species involves the segregation of two gametangial compartments of opposite sex arranged either with the orange male above the colorless female in the so-called epigynous species *A. macrognus* or with the male below its female partner in the hypogynous *A. arbuscula* (Emerson 1941; Olson 1984).

We have recently observed an accumulation of actin in the apical female gametangia of *A. arbuscula* (Turian et al. 1992). This differential localization of actin has now been extended to the epigynous *A. macrognus* (Nguyen Thi and Turian 1992a,b). It was therefore of great interest to investigate further whether this difference is dictated by the sex rather than by the usual pattern of apical localization of actin as is now well known in vegetative hyphae (Heath 1990). In contrast, still little is known on the possible involvement of cytoskeleton in the subapical growth and the differentiation of intermediate, asexual or sexual, fungal compartments such as gametangia or sporangia (Hyde and Hardham 1992). An intercalary accumulation of actin has now been found in the subapical gametangia of the epigynous species *A. macrognus*. This actin-enriched female type of differentiation could be inhibited by the anti-actin drug cytochalasin D (Nguyen Thi and Turian 1992b). It was then interesting to compare the presumed masculinizing effects of other cytochalasins among the wide range of such anti-actin compounds available (Betina 1989).

**MATERIALS AND METHODS**

The Bali strain of *Allomyces arbuscula* BUTL. (gift Dr. C. Stumm, University of Utrecht, The Netherlands) was grown and maintained on the standard *Difco* YpSs agar medium (Emerson 1941). Meiopores liberated from resistant sporangia were grown at 25 °C for 4 h on dialysis membranes (15 x 15 mm) placed on this medium. Individual membrane-bound colonies were then transferred onto the same medium supplemented with the cytochalasin or in 1 mL of DS (Machlis and Ossia 1953) solution containing different concentrations (10-100 mg/L) of cytochalasins A (CA), B (CB) or E (CE). Controls were mycelia treated with Me2SO, at the working concentrations, as well as nontreated mycelia. An Olympus microscope coupled with an Olympus camera C-35 AD-4 was used and micrographs were taken on Kodak Ektachrome 64T.

**RESULTS AND DISCUSSION**

CE (10 mg/L), CA (20 mg/L) and CB (20 mg/L) were minimal concentrations to slow down extension growth of *Allomyces* hyphae. During the 1st hour of incubation in the presence of the drugs, their tips widened into forked spatulae (Fig. 1).

One hour after the application of 20 mg/L CA, both gametangia still presumed to be male and female were differentiated, but not separated by septa (Fig. 2). Similar continuous double club-like gametangia were formed in CB-treated cultures (20 mg/L) as previously shown with CE-treated cultures (Turian et al. 1992).

In the presence of 60 mg/L CA, CB or CE, only superposed bulb-like gametangia, deprived of intergametangial septa, and filled with yellow, γ-carotene-containing lipid granules were formed
(Fig. 3) indicative of the masculinization of the normally apical, colorless, female gametangia. In cultures left for more than 4 h in the presence of one of the cytochalasins, the masculinized, normally apical, females were dedifferentiated into short, dichotomous and bulbous vegetative hyphae (Fig. 4).

In contrast to the drastic suppression by CD of the apical female gametangia independent their positioning – apical vs. subapical – on the tips of fertile hyphae (Nguyen Thi and Turian 1996), all the three cytochalasins A, B and E actively masculinized Allomyces cultures. To further assess this maleness, we examined (figures not shown) the effects of CA on cultures grown on sodium acetate (2 %) as single carbon source instead of soluble starch in the YpsS medium. This C2 compound, a known carotenogenic precursor, enforced the yellow pigmentation of the superposed couples of partially male–female gametangia still endowed with their normal shape – smaller, roundish male ellipsoidal female – but with cytoplasmic continuity because of the cytochalasin-induced preventive intergametangial septation. Such “bulb-like” gametangia exhibit not only such a generalized dispersion...
of yellow, γ-carotene-containing, lipid granules but also, when tested with toluidine blue, narrow ribosomal crescents capping numerous nuclei, as additional criteria of maleness (Turian 1975).

Fig. 3. Gametangial series of only yellow gametangia (males) following the lack of intergametangial septation (arrows) as a result of CE treatment (10 mg/L) for 4 h at 25 °C. Bar = 20 μm.

Fig. 4. Bulbous structures dichotomously dedifferentiated at the level of the apical masculinized (yellow) gametangia (arrow) of A. arbuscula treated with CA (20 mg/L) for 5 h at 25 °C. Bar = 20 μm.

The fact that cytochalasins A, B and E are also known to be, like CD, anti-actin compounds (Betina 1989) suggests that they also act negatively but on another actin target. This inhibitory targeting may be some kind of contractile ring of actin microfilaments positioned in anticipation of the further deposit of the chitinous intergametangial septum. We propose that, under these conditions, there occurs a generalized spreading of a male-dominating, female suppressor – an acridine-like compound – as already suggested by previous work (Turian et al. 1969; Turian and Ojha 1987).
Actin contractile rings are well known as being involved in the process of animal cell cytokinesis (Inoué 1990) which occurs by an asymmetric or unequal division (Schroeder 1990). A similar contractile ring of actin microfilaments is known to contribute to the formation of the hyphal septum as discovered by Girbardt (1979) in Trametes versicolor and further studied in spore germlings of Uromyces phaseoli (Tucker et al. 1986). Our results suggest therefore that the process of disjunction of superposed male and female, plurinucleated gametangia in Allomyces can be compared to a bipolar asymmetric division.

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REFERENCES


