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Invasive hyphal growth: An F-actin depleted zone is associated with invasive hyphae of the oomycetes *Achlya bisexualis* and *Phytophthora cinnamomi*

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Abstract

We have compared F-actin patterns in invasive and non-invasive oomycete hyphae. In *Achlya bisexualis* an F-actin depleted zone is present in 70% of invasive but only 9% of non-invasive hyphae. In *Phytophthora cinnamomi* these figures are 74 and 20%, respectively. Thus, the F-actin depleted zone appears to be associated with invasive growth. TEM images indicate that it is unlikely to represent areas of vesicle accumulation. Measurements of turgor indicate no significant increase under invasive conditions (0.65 MPa (invasive) and 0.63 MPa (non-invasive)). Similarly we found no difference in burst pressures (1.04 MPa (invasive) and 1.06 MPa (non-invasive)), although surrounding agarose may lead to overestimates of invasive tip strength. An F-actin depleted zone has the potential, along with wall softening, to increase protrusive force in the absence of turgor increases. Staining of F-actin in hyphae under hyperosmotic conditions suggests that decreases in F-actin at growing tips may also enable non-invasive growth at very low turgor.

Keywords: F-actin; Tip growth; Invasive growth; Oomycete; *Achlya bisexualis*; *Phytophthora cinnamomi*; Turgor

1. Introduction

Tip growth, the mechanism by which oomycetes and fungi extend is a complex and dynamic process characterised by localised extension at the apex of the hypha (Geitmann et al., 2001). Models of tip growth have typically stated that it is driven by turgor pressure, the force of which, when exerted on the cell wall physically breaks bonds between wall polymers (Money, 1997). This would also, given substrate attachment, provide a protrusive force which along with sufficient enzymatic substrate breakdown would enable hyphae to force their way through substrata (i.e., grow invasively) (Bastmeyer et al., 2002; MacDonald et al., 2002). These models are attractive, particularly as, in fungi invasive structures generate by far the highest reported pressures in biology (Howard et al., 1991). They become problematic, however, for the oomycetes as species such as *Achlya bisexualis* and *Saprolegnia ferax* appear to be unable to regulate or control, and thereby increase turgor (Lew et al., 2004) and indeed can even grow at levels of very low turgor (Money and Harold, 1993).

An organism in the absence of an ability to increase its turgor could grow invasively because of an increase in tip yielding. There have been indications that the cell wall softens in response to decreasing turgor, this possibly through the increased activity of endoglucanases (Money and Hill, 1997) or alternatively through changes in the rate and/or extent of wall hardening. While cell wall softening appears to be insufficient to allow turgor-less hyphae to exploit solid media the process could certainly increase the protrusive force that a turgid invasive hypha is able to exert. It is important to recognise, however, that such models involving changes to the cell wall only address the problem of

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invasive growth in part; as they ignore any role in tip yielding that might be played by the cytoskeleton. Certainly there is evidence suggesting that microfilaments actually reinforce the hyphal tip of oomycetes, as the disruption of the F-actin cap using a UV micro-beam gives hyphae that are prone to bursting (Jackson and Heath, 1990). There is also evidence suggesting the attachment of F-actin to possible wall-membrane linking molecules, which would be a requirement for the F-actin to resist turgor (Chitcholtan and Garrill, 2005; Kaminskyj and Heath, 1995). Thus, a reduction of F-actin at the tips of oomycete hyphae could, along with cell wall softening, increase tip yielding and thus play a role in invasive hyphal growth.

To the best of our knowledge there have been no reports directly comparing the cytoskeleton of non-invasive (i.e., growing on top of solid media or through liquid media) and invasive hyphae. There have, however, been investigations into the response of the F-actin distribution in *S. ferax* to low turgor conditions (Harold et al., 1996). In media supplemented with 250 mM sucrose there was a normal distribution of F-actin with an apical F-actin cap and subapical plaques and fibrils although there was some fading of the apical F-actin cap. With the addition of 400 mM sucrose 95% of hyphae had no cap and with 600 mM sucrose there was no cap in any of the hyphae examined (Harold et al., 1996). These results are of interest in view of the findings of Jackson and Heath (1990) suggesting that an absence of F-actin at the tip is likely to increase yielding. Furthermore we have recently reported, using an improved methodology for chemical fixation of hyphae, that an F-actin depleted zone at the hyphal apex may be a normal structural component of the F-actin cytoskeleton in *A. bisexualis* (Yu et al., 2004). These studies also raise the question of whether changes in the cytoskeleton may also allow growth under conditions of low turgor.

In view of the above we have compared the F-actin cytoskeleton in invasive and non-invasive hyphae of two species of oomycete, *A. bisexualis* and *Phytophthora cinnamomi*. For *A. bisexualis* we have also made comparisons of vesicle distribution, levels of turgor pressure and hyphal burst pressure. In addition to this we have also imaged the F-actin cytoskeleton in low turgor hyphae.

2. Methods

2.1. Cultures

Hyphae of the oomycetes *A. bisexualis* Coker (a female strain isolated in New Zealand from *Xenopus laevis* dung and available from the University of Canterbury culture collection) and *P. cinnamomi* Rands (obtained from the ICMP collection of Landcare Research, Auckland, New Zealand) were cultured on peptone–yeast–glucose (PYG) plates (containing [in % w/v] peptone [0.125], yeast extract [0.125], glucose [0.3], and agar [2]) that had been overlaid with sterile cellophane.

2.2. F-actin staining in invasive and non-invasive hyphae

To invoke invasive growth conditions rectangular hyphal mats from stock cultures were cut 1 cm behind the growing margins and placed in welled slides. These were then overlaid with 2% low melting point agarose made up with PYG broth (containing [in % w/v] peptone [0.125], yeast extract [0.125], and glucose [0.3]). This had been cooled until it was about to set but was still sufficiently liquid to allow it to be poured over the hyphae. Hyphae were left in the dark at 20 °C until growth resumed and after the resumption of growth for 1 h they were chemically fixed using a combination fixative of (v/v) 4% formaldehyde (ProSciTech., Thiringowa Central, Queensland, Australia) and 0.5% methylglyoxal (Sigma) made up in 50 mM Pipes (Sigma) buffer that had been adjusted to pH 6.8 with KOH (BDH) for 45 min. They were then rinsed twice in buffer solution. The F-actin cytoskeleton was stained using Alexa Phalloidin 488 as described previously (Yu et al., 2004). To prevent photo bleaching the sample was covered with tin foil for 30 min and then washed twice (for 15 min) with buffer solution. The sample was covered with tin foil between washes. Once the second wash had been removed 0.1% (w/v) *p*-phenylenediamine (Sigma) was added as an antifading agent. Hyphae were examined with an MRC1024 confocal microscope (Bio-Rad, Mississauga, Ontario, Canada) and an HQ530/560 filter. Confocal sections were taken every 0.15–0.2 μm for *P. cinnamomi* and every 0.3 μm for *A. bisexualis*.

Non-invasive conditions were invoked as above except that a much thinner layer of 2% low melting point agarose made up with PYG broth was poured over the subapical portion of the hyphal mat to secure the hyphae on the slide. Once this had set the hyphae were submerged in PYG broth. Fixing and staining was carried out as described for the invasive hyphae.

2.3. F-actin staining in non-invasive low turgor hyphae

A rectangular mycelial mat was cut from a stock plate and transferred to a small Petri dish which was then flooded with PYG broth that had been supplemented with the relevant concentration of sorbitol. Mycelia were then allowed to grow at 20 °C for 24 h before they were fixed and stained as described above.

2.4. Transmission electron microscopy

For ultrastructural analysis invasive and non-invasive hyphae were prepared as above and then fixed in 2.5% glutaraldehyde in 0.75 M phosphate buffer pH 7.2 for 1 h. After washing in phosphate buffer three times at 10 min intervals hyphae were embedded in 3% low melting point agar. Prior to dehydration small sections were cut from hyphal mats embedded in the agar very close to the growing edge. These sections were exposed to 1% OsO_4 for 1 h, and then rinsed in phosphate buffer three times, each for 10 min. After