Hyphal Tip Bursting in *Mucor rouxii*: Antagonistic Effects of Calcium Ions and Acid

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INTRODUCTION

The problem of the apical extension growth of fungal hyphae has been approached by investigating the bursting of hyphal tips in response to various experimental treatments. Tip bursting was considered to be a consequence of disturbing a delicate balance between loosening or plasticization of the wall in the tip region and simultaneous deposition of new wall material in the same zone (Bartnicki-Garcia & Lippman, 1972*a*). Circumstantial evidence from such experiments has been used to support the view that the proposed plasticization may be due to localized enzymic lysis of existing wall polymers (Bartnicki-Garcia & Lippman, 1972*b*). Similar models have been suggested for the extension of higher plant cells but in this case, there is evidence against the participation of enzymes which hydrolyse either the cellulose fibrils (Ruesink, 1969) or the matrix polysaccharides of the cell wall (Evans, 1974).

We investigated the effects of both osmotic protection and Ca^{2+} ions on the bursting of *Mucor rouxii* hyphal tips at low pH. It is difficult to reconcile our data with a role for polysaccharide hydrolases as one component of the balance which allows hyphal extension to occur whilst maintaining the structural integrity of the apex.

METHODS

All chemicals were analytical grade where possible; the polyethylene glycol (PEG) had a molecular weight of 400. *Mucor rouxii* strain 68072 was obtained from the Commonwealth Mycological Institute, Kew, and maintained on potato-dextrose-agar medium. Fungal cultures were grown in 60 mm diameter Petri dishes on 10 ml of a dilute, sterile medium containing Difco yeast extract (0.19 g/l), Difco Bactopeptone (0.63 g/l) and D-glucose (1.25 g/l), made up with glass-distilled water and solidified with 2 % Difco-Agar (Noble). The nutrient components represent a 16-fold dilution of the medium used by Bartnicki-Garcia & Nickerson (1962*a*). The plates were inoculated in the centre with a suspension of sporangiospores in distilled water and grown for 13 to 17 h at 28 °C in the dark. The effects of test solutions (5 ml) on the bursting of hyphal tips were observed at room temperature with the aid of a Nikon stereoscopic binocular microscope. In some experiments, colonies were pretreated with 0.2 ml of 0.25 M- CaCl_2 before flooding with 5 ml test solution. Burst tips were easily recognized by the extrusion of a globule of cytoplasm. Note that even with a maximal response (designated as 'extensive tip bursting'), only a proportion of the tips burst since the mycelial hyphae are branched and non-septate;

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presumably a single burst within connected tips would release the turgor pressure. The bursting pattern is similar to that observed by Bartnicki-Garcia & Lippman (1972*a*) where the leading hypha or a side branch (but rarely both) burst.

RESULTS AND DISCUSSION

We have found, in agreement with Bartnicki-Garcia & Lippman (1972*a*), that exposure to acid (pH 3.4 to 5.5; 0.01 M-acetic acid adjusted with 1.0 M-NaOH) and to chelating agents (0.01 M-EDTA pH 7.0 and 0.01 M-sodium hexametaphosphate pH 5.7 to 6.8) both caused extensive bursting of hyphal tips within a few seconds of flooding. Exposure to high pH (NaOH, 0.01 to 100 mM; pH 9 to 13) or to distilled water did not lead to bursting. We extended these observations to investigate the effects of both osmotic protection and Ca^{2+} ions on the response of hyphal tips to low pH.

The effects of acid and polyethylene glycol

In the absence of PEG, the bursting of tips in response to acid occurred over the range pH 3.4 (maximum bursting) to pH 5.0 (no bursting). The bursting was rapid, occurring within 3 s of flooding. However, inclusion of 0.15 M-PEG in the flooding medium prevented bursting at pH 4.5. Presumably, the osmotic protection afforded by the non-penetrating PEG prevented rupture of the acid-weakened wall. At pH 4.0 and pH 3.6, bursting was less extensive and less rapid than without PEG, but at pH 3.4, extensive bursting again occurred within 3 s.

In a separate experiment, colonies were exposed for 10 s to an acidic hypertonic medium (0.01 M-sodium acetate pH 3.8, containing 0.2 M-PEG), drained, and then flooded with either an acidic hypotonic medium (0.01 M-sodium acetate pH 3.8) when the tips burst within 10 s, or with distilled water when the tips remained intact. Transfer from distilled water to 0.01 M-sodium acetate pH 3.8 resulted in extensive bursting within 10 s. These observations indicate that no irreversible weakening of the wall had occurred during initial exposure to the acidic hypertonic medium. The data are difficult to reconcile with the operation of lytic enzymes having acidic pH optima that are located in the cell wall at the hyphal tip unless such an enzymic lysis were reversibly inhibited by the osmotic protectant.

The effect of Ca^{2+} pretreatment on the acid-bursting response

Exposure to CaCl_2 solutions leads to swelling of the apical region and occasionally to bursting. This effect has been interpreted in terms of a Ca^{2+} -mediated increase in wall lytic activity relative to wall deposition (Bartnicki-Garcia & Lippman, 1972*a*). We studied the effect of pretreating colonies with 0.2 ml of 0.25 M- CaCl_2 for varying times followed by flooding with 0.01 M-sodium acetate pH 4.0. The time of initiation of bursting was scored (Fig. 1). The Ca^{2+} pretreatment delayed but did not prevent the initiation of bursting by acid, which was nevertheless always extensive. Flooding with distilled water after pretreatment with CaCl_2 caused no bursting. These observations are again difficult to reconcile with a Ca^{2+} -stimulated cell wall lytic process that has enhanced activity at low pH. Rather, the H^+ ions and Ca^{2+} ions appear to be working in opposition.

Our knowledge of the detailed molecular composition and architecture of fungal walls is limited, although the polymers of *M. rouxii* walls are quite well characterized (Bartnicki-Garcia, 1968; Bartnicki-Garcia & Reyes, 1968*a, b*). *Mucor rouxii* hyphal walls contain polymers capable of conferring both form and tensile strength, chitin and chitosan

Fig. 1. Effect of pretreatment with Ca^{2+} ions on the time of initiation of bursting on flooding with acid (pH 4.0).

(Bartnicki-Garcia & Nickerson, 1962*b*), and polymers which may form a matrix in which the fibrillar components of the wall are embedded. The integrity of the wall at the hyphal tip may depend not only on the distribution and orientation of the fibrils, but also on the properties of the matrix material which contains the acidic polymers mucoran and mucoric acid (Bartnicki-Garcia & Reyes, 1968*a, b*; Bartnicki-Garcia & Lindberg, 1972).

Although it is possible to account for our results on an *ad hoc* basis by a lytic theory of wall plasticization, a simpler explanation may be that the reversible and antagonistic effects of H^+ and Ca^{2+} ions on the integrity of hyphal tips are due to a modification of the physical properties of the matrix wall polymers rather than to the cleavage of covalent bonds. In this connexion, there is some heuristic value in a comparison with the primary cell walls of higher plants where the acidic pectic polysaccharides exist mainly as the Ca^{2+} salt and constitute a major proportion of the matrix. Cell extension is inhibited by exposure to Ca^{2+} ions which leads to a hardening of the cell wall (Ray & Baker, 1965) and is promoted by H^+ ions (Davies, 1973). *In vitro* Ca^{2+} enhances the viscosity of pectin solutions, whereas acidification of the calcium pectate makes the solutions more fluid. Ultimately, Ca^{2+} causes pectin solutions to form gels which can be dissolved by acids and chelating agents (P. H. Rubery and A. R. Sheldrake, unpublished observations). In a similar way, mucoran isolated from cell walls by the method of Bartnicki-Garcia & Reyes (1968*b*) forms a precipitate on addition of Ca^{2+} which can be dissolved in acetic acid. High concentrations of HCl alone cause the polymer to precipitate.

If the integrity of the hyphal apex depends on some property of the matrix related to the viscous behaviour of mucoran solutions, then factors leading to an increase in fluidity, such as the action of acids and chelating agents on the Ca^{2+} salt, might be expected to allow tip disintegration by osmotic bursting, whereas treatments which decrease the fluidity of the matrix, such as exposure to Ca^{2+} , might produce tips more refractory to bursting.

It is known that deposition of both matrix and fibrils is essential to maintain tip integrity, since inhibition of chitin synthesis leads to bursting (Bartnicki-Garcia & Lippman, 1972*b*), but our data focus attention on the possibility that interaction of Ca^{2+} and H^+ ions, in modifying the viscous properties of the matrix acidic polymers, may be one element in the maintenance of a plastic region at the apex to allow extension in response to turgor pressure. Such effects could be of general importance in those fungi with acidic polysaccharides in their hyphal walls.

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