SHORT COMMUNICATION

The Effects of Pectin and Plant Root Surface Carbohydrates on Encystment and Development of *Phytophthora cinnamomi* Zoospores

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(Received 30 December 1983)

Citrus pectin induces highly synchronous differentiation in populations of *Phytophthora cinnamomi* zoospores in the absence of a host plant. This action is independent of the presence of Ca^{2+} in the zoospore suspension medium. Water-soluble, ethanol-insoluble material present on the surface of the plant roots also induces differentiation of *P. cinnamomi* zoospore populations with the same time course as pectin. It is proposed that this material provides the natural recognition signal which induces *P. cinnamomi* zoospores to differentiate, and that pectin is sufficiently similar in structure for it to mimic its action.

INTRODUCTION

Contact of zoospores of the fungus *Phytophthora cinnamomi* with the surface of plant roots results in the transition from motile, wall-less cells, through the stage of sessile walled cysts, to germlings which penetrate the host. At temperatures in the range of 20–24 °C the transition to the cyst stage is completed within 20 min of the zoospore arriving in the neighbourhood of the host root. Germination and penetration are completed within an hour (Ho & Zentmyer, 1977). This synchrony of differentiation observed in zoospore populations on or in the neighbourhood of plant roots is in contrast to the situation where they are suspended in water or dilute salt solutions. Here, after 1 h at 22 °C the population contains motile zoospores, cysts and germlings. This indicates the existence of a mechanism by which the spores recognize the presence of a host plant surface, and which allows them to respond by differentiation into cysts and germlings. Previous studies have shown that compounds such as volatile aldehydes and alcohols, which induce positive chemotaxis in *Phytophthora* zoospores (Allan & Newhook, 1974; Cameron & Carlile, 1978), fail to induce differentiation in *P. cinnamomi* (Byrt *et al.*, 1982*b*). However, among the organic compounds tested, pectin, and to a lesser extent polygalacturonic acid, were unique in their ability to induce synchronous encystment and germination. In this communication we show that pectin acts independently of Ca^{2+} , an ion which also induced synchronous encystment and germination of *P. cinnamomi* *in vitro* (Byrt *et al.*, 1982*a*), and that high molecular weight compounds secreted by plant roots act in a manner identical to pectin when added to zoospore populations *in vitro*.

METHODS

The origin of the isolate of *P. cinnamomi* (IMI 252489), the methods of zoospore production, and the assay system have been described previously (Byrt & Grant, 1979; Byrt *et al.*, 1982*a*; H. R. Irving & B. R. Grant, unpublished). The data are expressed as percentages of the population possessing a particular morphology and, unless otherwise stated, are the means of four replicates $\pm$ SD.

Compounds secreted from the root surface were collected from roots of axenically grown seedlings of maize (*Zea mays*) and lupin (*Lupinus angustifolius*). Seeds which had been surface sterilized in 6% (w/v) calcium hypochlorite were allowed to germinate on an aluminium screen over distilled water in a deep Petri dish. Roots extended

through the screen into the water. After 5 d the water was removed, filtered through cellulose acetate membranes (0.45 μm) and ethanol added to make an 80% (w/v) solution. After standing overnight at 5 °C, the precipitate was removed by centrifugation (11 000 g for 20 min), washed with absolute ethanol and dissolved in distilled water. The solution was then taken to dryness in a rotary evaporator at 25 °C and stored over silica gel. The pectin was commercially prepared citrus peel pectin (from Sigma), galacturonic acid content 76%, methoxy content 7.4%, purified by dialysis against EGTA (1 mM, pH 6.5) and then lyophilized. Ca^{2+} activity was measured using a Ca^{2+} ion-specific electrode (Radiometer).

RESULTS AND DISCUSSION

Pectin induced the encystment and germination of *P. cinnamomi* zoospores at concentrations in excess of 250 $\mu\text{g ml}^{-1}$ (Fig. 1), although maximum rates of encystment (90% of the population in 20 min) required 500 $\mu\text{g ml}^{-1}$. Maximum rates of germination were observed at 250 $\mu\text{g ml}^{-1}$; the lower proportion of the population shown as germinating was the result of the choice of a sampling time of 90 min, and not lack of capacity of cells to respond. Exposure to pectin at 250 $\mu\text{g ml}^{-1}$ or higher concentrations for 120 min resulted in more than 90% germination, but the length of the germ tubes on many cells made the samples difficult to count after this degree of development.

Although it had been shown previously (Byrt *et al.*, 1982*b*) that the effect of pectin was not a result of Ca^{2+} contamination, the possibility that pectin was acting through Ca^{2+} remained, since zoospores were released into water containing micromolar concentrations of Ca^{2+} leached from the mycelium. However, zoospores incubated in the presence of 50 μM -EGTA (pH 6.5), which reduced Ca^{2+} activity to below 10^{-7} M, underwent synchronous encystment and rapid germination (Fig. 2*a*) following the addition of pectin. This was indistinguishable from the time course of encystment and germination of populations of zoospores exposed to pectin in the presence of 10^{-5} M- Ca^{2+} (filled symbols in Fig. 2*a*). The time course of encystment and

Fig. 1

Fig. 2

Fig. 1. Effect of pectin concentration on zoospore encystment (○) after 20 min, and germination (●) after 90 min. Four replicates, bars represent two SD.

Fig. 2. Time course of pectin-induced zoospore differentiation in the presence and absence of Ca^{2+} . (a) 500 μg pectin ml^{-1} + 10^{-5} M- Ca^{2+} (filled symbols) or + 50 μM -EGTA (< 10^{-7} M- Ca^{2+}) (open symbols). (b) No added pectin, 10^{-5} M- Ca^{2+} (the curves for encystment and germination in the presence of 10^{-5} M- Ca^{2+} were superimposable and are not shown). Each datum point is the mean of four replicates; bars represent two SD. ○, ●, Zoospores; △, ▲, cysts; □, ■, germlings.

Fig. 3. Time course of zoospore differentiation in the presence of lupin root exudate ($500 \mu\text{g ml}^{-1}$) in the presence of $10^{-5} \text{ M-Ca}^{2+}$. Points represent means of triplicates. ○, Zoospores; △, cysts; □, germlings. Bars represent two SD; where no bars are shown the SD was smaller than the symbol.

germination of *P. cinnamomi* zoospore populations in the absence of pectin and at $10^{-7} \text{ M-Ca}^{2+}$ is shown in Fig. 2*b*. The behaviour of populations incubated in $10^{-5} \text{ M-Ca}^{2+}$ in the absence of pectin was identical (data not shown).

Although pectins are universal in cell walls of flowering plants, they differ in composition (Northcote, 1972) and there are no reports which suggest that they are released from or present at the surface of cells in healthy plant roots. However, root slime, a mucilaginous material rich in carbohydrate, which originates from the root cap, is present on or near the root tip, which is the zone to which *P. cinnamomi* zoospores are attracted and in which they encyst (Zentmyer, 1980). These root slimes are characteristically rich in uronic acids, although the other sugars present vary from one species to another (Harris & Northcote, 1970; Wright & Northcote, 1974; Howlett *et al.*, 1982; Ralton *et al.*, 1982). High molecular weight materials secreted by lupin and maize roots and isolated by the procedure used to prepare crude root slime were as effective as pectin in the induction of encystment and germination of *P. cinnamomi* zoospores (Fig. 3). This result suggests that in nature these root slimes are the compounds recognized by *P. cinnamomi* zoospores and serve as the stimulus for encystment and germination.

Citrus pectin contains chains of galacturonic acid residues, with L-rhamnose molecules inserted at intervals, and with galactose, xylose and arabinose linked to the sugars in the main chain (Aspinall *et al.*, 1968). The similarity between the structure of pectin and root slimes apparently allows the pectin to mimic the effects of the more complex molecules, and this may serve as a useful model to determine which part of the root slime molecule is essential in the zoospore recognition process. At present, there is insufficient information on the detailed chemistry of root slimes to allow generalizations to be made about their structure. However, as *P. cinnamomi* is a root pathogen with a very broad host range, whatever molecular structures are recognized must be common to a high proportion of unrelated plant species.

This work was supported by grants from the Die-back Research Foundation, the Potter Foundation, the Buckland Foundation and the Rowden White Bequest. H. R. I. was supported by a University of Melbourne Post Graduate Fellowship.

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