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Enzimatic mechanisms of penetration and development of arbuscular mycorrhizal fungi in plant

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ABSTRACT

The spores and external mycelium of arbuscular mycorrhizal (AM) fungi possess a complex of pectinase, cellulase and xyloglucanase enzymes. Studies of the production of pectinases, cellulases and xyloglucanases during root colonization by the AM fungus showed increased activity during the logarithmic stage of AM development. Some of the cellulase activities detected in colonized plant roots can be attributed to the fungus, since endoglucanase activity found in the external mycelium of the fungus and in mycorrhizal root extracts showed the same electrophoretic mobility. However, some of the endoglucanase activities from extracts of mycorrhizal plants had electrophoretic mobilities different from those observed in the external

mycelium and in nonmycorrhizal plants. These results indicate that endoglucanase produced by either the plant or the fungus may be involved in the process of host wall degradation and cell wall material mobilization during colonization.

INTRODUCTION

The enhancement of growth in plants colonized by arbuscular mycorrhizal (AM) fungi is well known (1). The process of mycorrhizal colonization of plant roots takes place through a series of phases, one of which involves the penetration of hyphae along or through cell walls. The colonization of plant roots by AM fungi involves the formation of intercellular hyphae, highly branched intracellular arbuscules and vesicles scattered throughout the root (2). These observations suggest that the establishment of intracellular symbiosis between fungus and plant roots requires penetration of the host cell by the fungus. Cell wall-hydrolyzing enzymes such as cellulases, hemicellulases and pectinases may be involved in this process (3).

Most phytopathogenic fungi and bacteria are known to produce enzymes that degrade pectic, cellulolytic and hemicellulolytic substances (4,5). Pectinases degrade the α-1,4 linkages between galacturonyl moieties in polymers of galacturonic acid and cellulase and xyloglucanases, the mayor hemicellulose in the primary cell wall of dicotyledons and non-graminaceous monocotyledons, degrade the β (1,4) linkages between glucose polymers. These hydrolytic enzymes play a fundamental role in pathogenesis (6, 7, 8). Many of these enzymes has been described in plants, fruits, epicotyls, cotyledons and other growing tissues (9, 10, 11, 12). However, research is scarce on these enzymes in plant roots, and on their mode of action in the process of penetration and development of symbiotic microorganisms (13). Infection of roots by mutualistic microorganisms such as *Rhizobium* and *Azospirillum* appears to be mediated by cell wall–hydrolysing enzymes (14, 15). In spite of the low production of hydrolytic enzymes by these mutualistic microorganisms (16), these enzymes seem to be involved in the dissolution of the cell wall which permits *Rhizobium* to enter the host cell (17).

The observation that AM fungi penetrate the plant cell wall at the site of contact during the establishment of intracellular symbiosis (2) indicates that hydrolytic enzymes may be involved in the AM colonization process. However, since AM fungi have not yet been cultured axenically in the absence of plant roots, it is difficult to confirm the production of hydrolytic enzymes by AM fungi or their possible participation in the colonization of the root, owing to the very low levels of enzyme produced, as occurs with the other mutualistic microorganisms (16, 18).

INDIRECT EVIDENCES OF PARTICIPATION OF ENZYMES IN ROOT COLONIZATION

Attempts to demonstrate pectinase, cellulase and hemicellulase production in extracts from AM tissues were not successful (19). However, catabolic repression experiments by Garcia-Romera *et al.* (3) showed that pectolytic enzymes may be involved in the process of root colonization by AM fungi. These experiments were done in glass tubes containing a sand:vermiculite mixture, which was inoculated under sterile conditions with AM spores and alfalfa as the host plant. Pectin, Na–pectate, carboxy-methyl-cellulose (CMC) and Locus bean were added to the rooting medium at final concentrations of 0, 0.05, 0.2 or 0.6%. Tubes treated with 1% pectin and Na–pectate were also used. The delay in the onset of mycorrhizal infection and the decrease in the plateau of AM infection curves paralleled the rise in pectin and Na–pectate concentration. Pectin and Na–pectate did not affect plant growth, ruling out the negative effects of insufficient oxygen diffusion as a possible explanation (3). This is reinforced by the fact that treatments of higher viscosity, e.g. Locus bean solutions or extracellular polysaccharides from *Rhizobium meliloti* used under similar conditions (20) failed to inhibit AM infection. A certain amount of enzyme production therefore seems to be necessary for the initial and subsequent development of mycorrhizal infection. Once AM infection is established in a root, further infection becomes easier (21), but the amount of pectolytic enzyme produced by the inoculated spores or by the mycelium from another part of the infected root seems to be insufficient to produce the same degree of infection in the pectin and Na–pectate added treatments as in the controls. The addition of pectin or Na–pectate to the rooting medium "protected" root walls from pectinases produced by the fungus, delaying the expression of enzymatic activity in the root cell walls for a period which paralleled the amount of substrate added. The presence of substrate in an easily accessible form inhibited enzyme activity due to the accumulation of the product, or to catabolic repression of enzyme synthesis (14). Thus, pectinases may well be involved in the penetration of AM fungi in host plant root cells. However, the effect of cellulases and hemicellulases on the process of infection was not clear from the results of the same experiments. No relationship was noted between the amounts of cellulose and Locus bean added to the rooting medium and the percentage of infection.

PRESENCE OF ENZYMES IN FUNGAL STRUCTURES

Extracts of AM spores produced zones of hydrolysis in agar plates with pectin, CMC and nasturtium seed xyloglucan stained with ruthenium red for the pectinases and congo red for the cellulases and hemicellulases respectively (17). Research has shown that the degradation of pectin is due to the action of a complex of enzymes, including polygalacturonase, pectin esterase and pectin lyase (14). The spores and external mycelium of the fungus possess a complex of pectinolytic [pectinesterase (PE), endopolymethylgalacturonase (endo-PMG), polymethylgalacturonase (PMG) and pectinlyase (PL)], pectolytic [endopolygalacturonase (endo-PG), polygalacturonase (PG), pectate lyase (PAL)] (22). Polygalacturonases have been observed on the fungal cell wall of some extracellular hyphae at the root surface as well as at the tips of the arbuscular branches (23). Cellulolytic [endo- and exocellulases] (24) and xyloglucanolytic (endo- and exoxyloglucanase) enzymes have been also observed (25). Polygalacturonase activity in pathogenic fungi has been found to be higher than in ericoid mycorrhizas, and this activity was higher than those in the external mycelium of AM fungi (2). The presence of these hydrolytic enzymes in spores and external mycelium may be an indication of the types of enzymes that this mycorrhizal fungus is able to produce in the process of root colonization. However, the ability of microorganisms to produce hydrolytic enzymes in vitro constitutes no proof of their pathogenicity (18). Some microorganisms able to produce pectic enzymes on synthetic nutrient media do not always possess the ability to produce them in vivo. Minimal breakdown of the plant cell wall occurs during penetration and colonization by the AM fungus: this does not affect the viability of plant cell (26). Thus, mycorrhizal fungi would not be expected to produce abundant quantities of enzyme which would macerate the cortical root tissue. Confirmation of the production of hydrolytic enzymes may be impeded by the very low levels of enzyme production, as found for other mutualistic microorganisms. Other causes may lie in the chemical composition of the cell walls of plants which repress or control the production of hydrolytic enzymes (27). Besides this protective mechanism, other inhibitors, including phenolic compounds (28, 29), may be produced during the colonization process or during the extraction of enzymes from colonized tissues. The type of extraction solution is known to influence enzyme recovery (16, 24, 30, 31).

PRODUCTION OF ENZYMES DURING ROOT COLONIZATION

The production of hydrolytic enzymes was studied during the process of penetration and development of AM fungus in plant roots, which showed a sygmoidal development of AM colonization (32).

The PE activity was consistently higher in plants inoculated with AM fungi than in controls throughout the process of root colonization. This enzyme is thought to facilitate the action of the other pectinase enzymes (33).

Endo–PMG, PMG and PNL (pectinolytic) activities were higher in plants inoculated with the fungus than in controls during the logarithmic stage of AM development. The increase in fungal structures which penetrate the cell wall during the logarithmic stage of root colonization (34) may explain the increase in endo-PMG, PMG and PNL activities at this time. However, endo–PG, PG and PL (pectolytic) activities in AM plants were similar to those in controls throughout the experiment (32). The lack of differences in these degradative enzymes is not, however, conclusive evidence that they do not participate in the colonization process, in view of the presence of these enzymes in the extracts of spores and external mycelium of AM fungi (22). Specific plant inhibitors of the fungal enzymes might be produced in mycorrhizal symbiosis in response to the fungal colonization (35). These results suggest that the plant controls the production and/or the activity of these enzymes (27), thus avoiding the indiscriminate breakdown of the cell wall. PE, endo-PMG, PMG and PNL seem to be the main pectinases involved in the colonization of plants by the fungus. Thus, a system of pectin–degrading enzymes, rather than sodium pectate–degrading enzymes, may play a role in the process of AM colonization. Endo- and exoglucanase activities increased in AM colonized plants as compared to non-AM colonized plants when the fungus was in its logarithmic stage of growth. No relationship between number of vesicles and endo- and exoglucanase activities was found, although the maximum hydrolytic activities coincided with the beginning of entry points formation and arbuscules development (36). Endoxyloglucanase activity was also significantly higher in AM colonized plants in comparison to control when the fungus was in its logarithmic stage of growth. The level of this activity in non mycorrhizal and mycorrhizal roots were generally increased with time (31).

ROLE OF ENZYMES IN ROOT COLONIZATION

The formation of entry points and arbuscules requires that the fungal hyphae pass through the cell wall, after which an interfacial matrix is formed around the internal hyphae (2). This matrix is composed of different polymers such as cellulose, xyloglucans and pectin materials of host origin (37). These cytochemical observations suggest the production by the fungus of hydrolytic enzymes which allow penetration of the cell wall without affecting host viability (2, 38). However, difficulties exist in detecting the activity of cell wall degrading enzymes, since breakdown of the walls must be localized during fungal development and, consequently, difficult to detect. In fact, hydrolytic activities were extremely low, as might be expected from a mutualistic interaction. Thus, it is difficult to demonstrate a close relationship between the evolution of the different hydrolytic activities and the development of the fungal structures (39).

Plants suppressed the pectolytic activity of AM fungi throughout development of the fungus in the root, and decreased pectinolytic and cellulolytic activities (except for PE enzyme) of the AM endophyte to a level similar to or lower than that in nonmycorrhizal roots. These results suggest that growth of the AM fungus inside the root may be controlled by the plant (19); this control may decrease the production of these hydrolytic enzymes by the fungus, as is the case with other enzymes, avoiding the indiscriminate breakdown of the cell wall (40).

Endoglucanases are present in non-colonized roots during growth and development (9). Several electrophoretic bands of endoglucanase activity observed in colonized plants had the same mobility as in non-colonized plants; however, some of these bands were present at earlier stages of plant growth in mycorrhizal plants than in nonmycorrhizal plants (39). The presence of bands different from those observed in nonmycorrhizal roots or external mycelia suggests that some of this activity may be induced by the fungus in the plant. These findings indicate that endoglucanases produced by either the plant or the AM fungus may be involved in the process of host wall degradation and cell wall material mobilization during colonization, as has been suggested for other symbiotic associations. Some of the endoglucanase activity can be attributed to the extramatrical phase of the AM fungi, since at least one of the endoglucanase activities found in the external mycelium and in the mycorrhizal root extracts showed the same electrophoretic mobility (39).

The sequence of hydrolytic activities observed in the AM association, and the fact that the AM fungal structures showed hydrolytic activities, suggest that these enzymes play an important role in the process of colonization of the roots by AM fungi.

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