J. Agric. Sci. Mansoura Univ., 28(12): 8419 - 8436, 2003

ANTAGONISTIC INTERACTIONS BETWEEN THE BACTERIAL ANTAGONIST *Pseudomonas fluorescens* Q2-87 AND PATHOGENIC *Pythium* SPP. IN RELATION TO HYPHAL ULTRASTRUCTURAL ANALYSIS

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ABSTRACT

Effects of the biocontrol agent *Pseudomonas fluorescens* Q2-87 that is known to produce the antibiotic 2,4-diacetylphloroglucinol (2, 4-DAPG) on phytopathogenic *Pythium* spp. in relation to electron microscopic analysis of the hyphal cell walls were investigated. The outcome of the antagonistic interactions between this strain and *Pythium* spp., by means of light and scanning electron microscope, when co-cultivated on potato dextrose agar was described. Light micrographs are presented that displayed hyphal distortion, swollen shapes and unusual apical branching. In addition, scanning electron micrographs are also presented that displayed degradation of the cell wall layers and revealed the cellulosic wall microfibrillar skeleton. A similar cell wall degradation was observed when PDA medium was amended with the synthetic antibiotic 2,4-DAPG. Thus, it might be concluded that the natural antibiotic 2,4-DAPG is primarily responsible for antagonistic process of the Oomycete *P. ultimum* induced by *P. fluorescens* Q2-87. Our results based on ultrastructural observations provide evidence that the modes of this strain action in inhibition, preventing colonization by *Pythium* are mainly associated with major cell wall degradation, and eventually death of Pythium cells, as a result of extensive cell wall breakdown.

Keywords: 2, 4-diacetylphloroglucinol, *Pseudomonas fluorescens* Q2-87, *Pythium ultimum*, ulturastructural analysis

INTRODUCTION

A number of *Pseudomonas* strains have been intensively studied as possible biocontrol agents for soil-borne fungal pathogens (Dunne *et al.* 1998; Weller and Thomashow *1999*; Thomashow *et al.* 2000; Landa *et al.* 2002). Among them are *P. fluorescens* strains F113 (Fenton *et al.* 1992), CHAO (Schnider *et* al. 1995; Duffy and Defago 1997) and Q2-87 (Harrison *et al.* 1993; Mazzola *et al.* 1995). The biocontrol effect of all three strains is related, in part, to their ability to produce the antibiotic 2, 4-DAPG (Vincent *et al.* 1991; Shanahan *et al.* 1992). However, strain CHA0 also produces a second antibiotic, pyoluteorin (Natsch *et al.* 1998). Pseudomonads that produce the phenolic antibiotic 2,4-DAPG inhibit a broad spectrum of phytopathogenic fungi (Nowak-Thompson *et al.* 1994; Sharifi-Tehrani *et al.*1998) and control a number of root and seedling diseases (Sharifi-tehrani *et al.* 1998). There are three lines of evidence support the importance of 2, 4-DAPG production in biological control. First, mutations in the biosynthetic pathway resulted in reduced biocontrol activity (Vincent *et al.* 1991; Nowak-

Thompson *et al.* 1994). Second, the population magnitude of 2, 4 DAPG producers correlated with disease suppressiveness of the soil and *in situ* antibiotic production (Raaijmakers *et al.* 1999). Third, diverse 2, 4-DAPG-producing *Pseudomonas* spp. have been isolated from the rhizosphere of many different crop plants (McSpadden-Gardener *et al.* 2000; Picard *et al.* 2000).

Inhibition of a plant pathogen by producing antimicrobial compounds via antibiosis, degradation of pathogenicity factors of the pathogen and biosynthesis of cell wall-degrading enzymes have been considered as a major mode of actions in terms of fluorescent pseudomonads (Keel and Défago 1997; Whipps 2000). 2,4-DAPG has been shown to be the only antibiotic compound produced by *Pseudomonas fluorescens* Q2-87 (Harrison *et al.* 1993; Bonsall *et al.* 1997). In addition, it has been confirmed that mutant-negative strains of this bacteria lost the ability to inhibit plant pathogens (Vincent *et al.* 1991). The mechanism by which this antibiotic exerts its mode (s) of action to antagonize a phytopathogen in relation to ultrastructural analysis by means of scanning electron microscopy is not yet studied. Interaction between organisms may involve cellular communication mediated by diffusible substances.

To improve our understanding of the interaction between a genus of Oomycete, *Pythium,* as a model system of a phytopathogen, and *P. fluorescens* Q2-87 producing 2, 4-DAPG, as a biocontrol agent, we investigated the outcome of antagonistic process between these two organisms in relation to ultrastructural changes of hyphal cell walls. To clarify the specific role of the phenolic antibiotic 2, 4-DAPG produced by this strain in this process against *Pythium*, a synthetic 2, 4-DAPG was also applied.

MATERIALS AND METHODS

Pythium isolates and culture conditions

Pythium species used in this study were *P. ultimum*, *P. aphanidermatum* and *P. intermedium*. *P. ultimum* was isolated from cowpea in Georgia, USA and kindly supplied by Dr. Jeffret Hoy (Agricultural Experiment Station, LSU). However, *P. aphanidermatum* and *P. intermedium* were isolated from turfgrass in Michigan State, USA. All cultures were maintained on a solid V8 medium which consisted of 200 ml V8 juice, 3.0 g $CaCO_3$, 15.0 g agar and made up to one liter with distilled water.

Bacterial culture and growth media

Lyophilized *P. fluorescens* strain Q2-87 (NRRL, B-23374) was obtained from the Agricultural Research Service Patent Culture Collection (National Center for Agricultural Research, USDA, Peoria, Illinois). Cultures were grown on 100 ml tryptone soy broth medium (TSB, 30 g/L. Difco, Detroit, MI). They were maintained at 24°C on tryptone glucose yeast medium (TGY) which consisted of 5.0 g tryptone, 5.0 g yeast extract, 1.0 g glucose, 1.0 g K_2HPO_4, 15.0 g agar and made up to one liter with distilled water, pH 7.0, prior to autoclaving.

In vitro antagonistic interactions

P. fluorescens cultures were grown on potato dextrose agar (PDA, Difco, Detroite, Michigan), pH 6.6, at 24°C for 24 h while *Pythium* species cultures were grown for 3-4 days on PDA medium Hyphal tips from the actively growing mycelia were then used in dual cultures. Interactions between these two living organisms were studied on PDA media as described by Schmidli-sacherer *et al.* (1997). A PDA medium was chosen in this study, because the ability of the biocontrol *P. fluorescens* to antagonize the growth of *Pythium* spp. depends on characteristics of the growth medium (Maurhofer *et al.* 1995). Sterilized glass slides (50 x 75 mm) were placed in Petri-dishes and 2 mm depth of PDA was added. After solidification, bacteria were streaked directly onto the glass slides on one side and 5-mm-diameter of *Pythium* plugs were simultaneously co-cultivated at 1-cm along the bacteria on the opposite side. Control slides were cultivated with *Pythium* only. Treated and untreated slides were incubated at 24°C. The interaction, i.e. inhibition, areas began to form after 24 h and were observed *in situ* by light microscope as evidence that the bacterium prevented colonization of the slide by *Pythium* by producing the antibiotic 2, 4-DAPG under these cultural conditions. Light observations were made without use of cover glasses as this in itself could be traumatic *Pythium* cells and affect their dynamic responses, e.g. cytoplasmic streaming, or cause cell lysis. Strips of agar from the intermediate area having *P. ultimum, P. aphanidermatum* or *P. intermedium* were taken, at specified times unless otherwise stated, after bacterial application and were processed for scanning electron microscopy.

Antagonistic activity of synthetic 2,4 DAPG

Preliminary experiments with synthetic 2,4-DAPG (purchased from Toronto Research Chemicals Inc., Toronto, Canada) concentrations ranging from 0 to100 µg/ ml indicated that the growth of *Pythium* spp. was completely inhibited by 50-80 µg/ ml. 2, 4-DAPG was dissolved in 95% ethanol (EtOH) and added to the media. The highest concentration of EtOH in the 2, 4-DAPG media was 0.1% and thus 0.1% of EtOH was used as a control medium. Antagonistic interactions between 2, 4-DAPG and *P. ultimum* representing *Pythium* spp. was performed by the following assay.

Preparation of the glass slides containing media was done as described above. 5-mm-diameter plugs of *Pythium* were excised from the youngest growing margin of a 4-day-old culture and cultivated on one side of the glass slides. 24 h later, aliquots of 80µg/ ml synthetic 2,4-DAPG were applied at 0.5 cm along the bacteria on the opposite side of the plugs. Control slides were cultivated with *Pythium* only and applied with aliquots contained 0.1% EtOH. Treated and untreated cultures were incubated at 24°C. Inhibition zones were observed *in situ* by light microscope after 24 h of 2,4-DAPG application. Strips of agar, from the intermediate area having *P. ultimum* that interacted with diffusible of the synthetic antibiotic, were taken at specified times after application and were processed for scanning electron microscopy.

Scanning Electron Microscopy (SEM)

Mycelial samples from the interaction regions were cut into 5 mm^2 pieces (two pieces from each slide) and fixed by immersion in 4%

glutraldehyde (Sigma Co.) in 0.1 M phosphate buffer (pH 7.0) for 2 h at 25°C. Dehydration was conducted through a graded ethanol series in deionised water (Glauert 1974). When in 100% ethanol, specimens were critical-point dried in an Balzers critical point dryer with CO_2 as the transitional fluid, then mounted on aluminum stubs using adhesive tabs and sputter coated with approximately 24.5 nm of gold for 3.5 min in an Emscope SC 500 unit and purged with argon gas. Specimens were examined at various magnifications *in situ* with a JEOL 6300 scanning electron microscope, manufactures by Japan Electron Optics Limited.

There were 5 replicates for each antagonist-pathogen combination and control. The experiment was repeated two times with similar results. *P. ultimum* induced by synthetic 2, 4-DAPG showed identical results to that induced by natural 2, 4-DAPG produced by *P. fluorescens*. Subsequently, 2, 4-DAPG micrograph at 96 h was just presented.

RESULTS

In vitro antagonistic experiment

In the glass slide single cultures, *P. ultimum* grew actively and colonized the entire agar surface within 4 days following cultivation. However, in dual cultures, contact between *P. fluorescens* producing 2,4-DAPG and *P. ultimum* did not occur by 4 days after co-cultivation. Antagonistic interactions between these two living organisms were clearly discerned by very limited growth and the complete absence of mycelial hyphae in the intermediat area along bacterial colonies (Fig. 1, A and B). Similar result was also noted in terms of the synthetic compound 2, 4-DAPG (Fig. 1, C and D). In addition, when agar samples from the interaction region between *P. fluorescens* and *P. ultimum* were deposited at the margin of an actively growing *Pythium* colony, mycelial growth was halted. By contrast, the *Pythium* easily overgrew agar disks taken from un-inoculated PDA medium (not shown).

Morphological changes under light and scanning electron microscope

At the microscopic level, the control hyphae of *Pythium* have a regular diameter , normal growth and relatively linear shape during the time-course of investigation. *Pythium* hyphae grown in dual culture exhibited morphological changes mainly characterized by hyphal distortions (Fig. 1, E and F). Swollen shapes were clearly observed in *P. intermedium* compared to *P. ultimum* (Fig. 1, F and H). In addition, the surface of the cell wall of treated *Pythium* became smooth instead of rough in all *Pythium* spp. under study compared to their controls (Figs. 1, E-H, 2, and 3).

Time-course investigation of the cell wall events of *P. ultimum* and *P. aphanidermatum*

Fig. 4 shows that sequential degradation of the cell walls of *P. ultimum* after inducing by *P. fluorescens* Q2-87 was observed in the intermediate area where diffusible bacterial metabolite interacting with *Pythium* cells (Fig. 2, B-H).

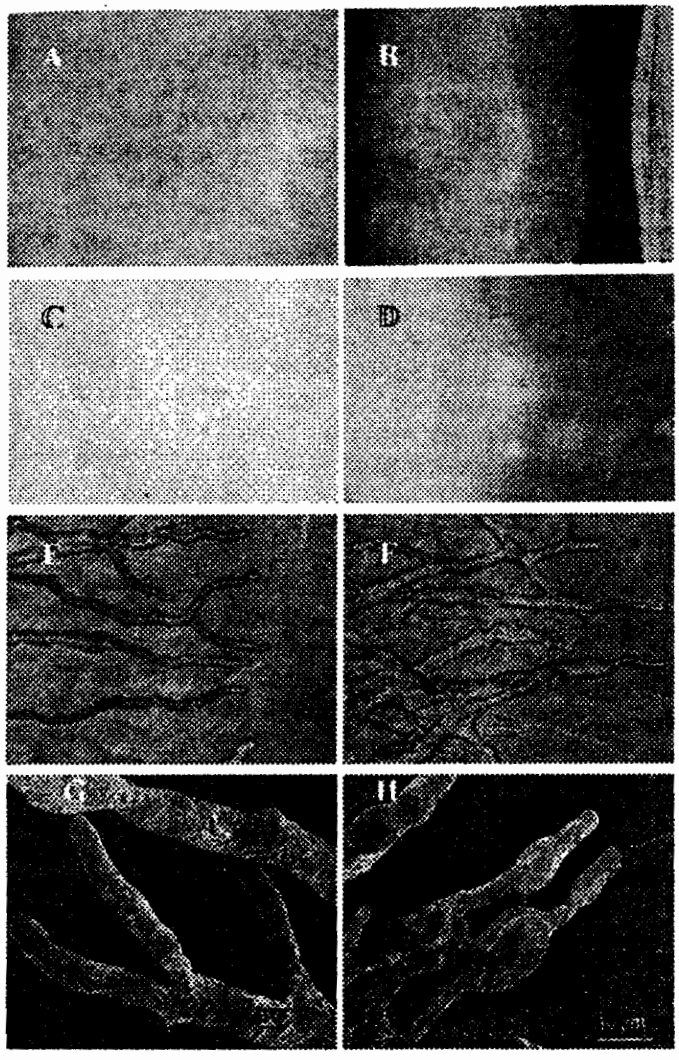

Fig. 1. Light micrographs of *P. ultimum* inhibited by either *P. fluorescens* Q2-87 or synthetic 2,4-DAPG for 96 h . *P. ultimum* grown in single culture (A and C). *P. ultimum* grown in dual culture and PDA media applied with 80 µg/ ml 2,4-DAPG (B and D, respectively). Note: the white area depicts growth of *P. ultimum* and the black area depicts the zone of inhibition. Light micrograph of *P. ultimum* inhibited by *P. fluorescens* Q2-87 for 96 h, in the intermediate area where diffusible bacterial antibiotic and *Pythium* are interacting, mycelial hyphae of *Pythium* grown in a single culture showing normal growth (E), mycelial hyphae of *Pythium* grown in dual culture showing irregular in shape and expanded in size (F). Scanning electron micrographs of *P. intermedium* inhibited by *P. fluorescens* Q2-87 for 96 h, in the intermediate area where diffusible bacterial antibiotic and *Pythium* are interacting, *Pythium* grown in single culture showing normal hyphae having rough outer surface, *Pythium* grown in dual culture showing swollen shapes and smooth outer surface (H).

However, control mycelial hyphae grown in single culture showed no degradation and a rougher outer surface of the cell walls over the time course of study (Fig. 2, A-G). However, *P. ultimum* induced by natural diffusible antibiotic 2, 4-DAPG lost its uniform folding patterns during the progress of degradation process (Fig. 2, B and D). After 72 h, looseness and disconnection of the outer layer of the cell wall was observed (Fig. 2F) followed by complete degradation of this layer and appearance of wall microfibril skeleton by 96 h (Fig. 2H). Identical results were also seen after synthetic 2, 4-DAPG application (Fig. 3D) in comparison with the induction by living biocontrol agent *P. fluorescens* (Fig. 2H). Similar ultrastructural analyses were also noted during the time course study of *P. aphanidermatum* induced by the same strain (Fig. 4) with considering that the folding patterns vary between these two species (Figs. 2A, and 4A). However, microfibrillar skeleton were not markedly seen in *P. aphanidermatum* by 96 h (Fig. 4H).

DISCUSSION

Phenolic antibiotic-produced by fluorescent pseudomonads have been used effectively in biological control of many soilborne phytopathogens (Pierson 1997). Among the modes of biocontrol action mediated by fluorescent pseudomonads are production of the secondary metabolites (Howell and Stipanovic 1980; Howie and Suslow 1991; Thomashow and Weller 1995) and/ or hydrolytic enzymes (Jones *et al.* 1986; Défago *et al.* 1996). According to Haas *et al.* (1991), Vincent *et al.* (1991), Shanahan *et al.* (1993), Harrison *et al.* (1993), Keel *et al.* (1996), and Bonsall *et al.* (1997), *P. fluorescens* Q2-87 has the ability to antagonize a wide range of pant pathogens via producing the secondary metabolite 2,4-DAPG. Indeed, mutant strains of *P. fluorescens* lacked the ability to produce 2,4-DAPG, also lost the ability to inhibit plant pathogens (Fenton *et al.* 1992; Carrol *et al.* 1995). In agreement with the former authors, we isolated and identified 2,4-DAPG from a chemically-defined broth culture of the strain Q2-87 by chromatographic means(data will be reported elsewhere). In the present study, *in vitro* antagonistic process between *Pythium* and the Q2-87 or synthetic 2,4-DAPG was observed (Fig. 1, A-D). These data are in agreement with previously reported inhibitory activities of *P. fluorescens* on various plant pathogens, including *Pythium* spp. (Fenton *et al.* 1992; Levy *et al.* 1992; Shanahan *et al.* 1992; Harrison *et al.* 1993; Hultberg *et al.* 2000). At microscopic level, *Pythium* spp. cultivated in dual culture relative to that cultivated in single culture revealed that mycelial hyphae were mainly characterized by irregular in shapes and expanded in size (Fig. 1, E and F). Our results are in line with Paulitz *et al.* (2000) who reported that alterations of *Pythium* cells were occurred after application with *P. aureofacien* producing the antibiotic 2,4-DAPG. They described the nature of these morphological changes as swollen and abnormal appearance of the hyphae. In the present study, scanning electron micrographs of *Pythium* in dual culture relative to that in control documented the sequential cell wall degradation, over the time course study, induced by the Q2-87 strain (Figs. 2,

and 4). The outer cell wall layer, i.e. amorphous layer, at least, was completely degraded and revealed the underlying microfibrillar ß-1, 4-glucan skeleton (Fig. 2H). However, the cell wall microfibrils were not seen in *P. aphanidermatum* that induced by the same strain (Fig. 6) indicating that degradation process did not reach to the level that cell wall skeleton might be seen. Extending the time-course of analysis may reveal this ultrastructure. Similar results here were also observed after synthetic 2,4-DAPG application (Fig. 3), confirming that Q2-87 strain may exert its biocontrol action via producing the antibiotic 2,4-DAPG. In terms of revealing cell wall microfibrils presented here, similar results were obtained by Bartnicki-Garcia and Wang (1983) via scanning electron microscopic examination after exo-ß-1,3-glucanase application. These authors reported that amorphous glucan layer that completely covers the mycrofibrils of *Phytophthora palmivora* could be selectively removed by hydrolyzing with exo-ß-1,3-glucanase activity to reveal the underlying mycrofibrillar ß-1, 4 glucan skeleton. In addition, Chérif *et al.* (1992) found that application of exoglucanase, a ß-1,4 glucan cellobiohydrolase with specific affinity for ß-1,4-linked glucans, to ultrathin sections of *Pythium ultimum* showed that gold particles were preferentially associated with the internal wall portion closely appressed against the plasma membrane whereas the outer portion was devoid of labeling. Consequently, it may be concluded that constituents of microfibrils are considered to be cellulosic (ß-1,4 glucan) and they disappeared from the mycelial walls upon hydrolyzing with cellulase, following a previous hydrolyze with ß-1,3-glucanase as also reported by Bartnicki-Garcia and Wang 1983. However, constituents of amorphous material are considered to be noncellulosic glucans (ß-1,3- and ß-1,6 linked glucan) as suggested by Bartnicki-Garcia and Wang (1983).

Based on the foregoing results, the scanning electron micrographs (Figs. 2, and 3) indicate that noncellulosic ß-glucans existing in the outer layer of *Pythium* cells was completely degraded by ß-1,3 glucanase to reveal the internal wall layers, cellulosic ß-1,4 glucan microfibrils (Figs. 2, and 3D). Further, comparing figures 2H and 3Dwith the correspondence control (Figs. 2G, and 3A), the present results suggest that there are two different structural elements in *Pythium* cells: long microfibril and nonfibrillar or amorphous substance. This architectural design is similar to that of other fungal walls as reported by Bartnicki-Garcia and Wang (1983), and Wessels (1990). Additionally, microscopic investigations reveal that growth inhibition of both *P. ultimum* and *P. intermedium* induced by either *P. fluorescence* Q2-87 or the synthetic compound 2,4-DAPG correlated with both hyphal degradation observed via SEM (Figs 4 and 5) and marked structural changes including vaculation, condensation and aggregation of the cytoplasm, as well as cytoplasmic organelles breakdown (Figs. 5, A-D). Because such cell reactions were, in fact, induced in the absence of intimate contact between *Pythium* cells and *P. fluorescens*, the possibility that diffusible 2,4-DAPG metabolite (s) may be responsible for the observed disturbances appears realistic.

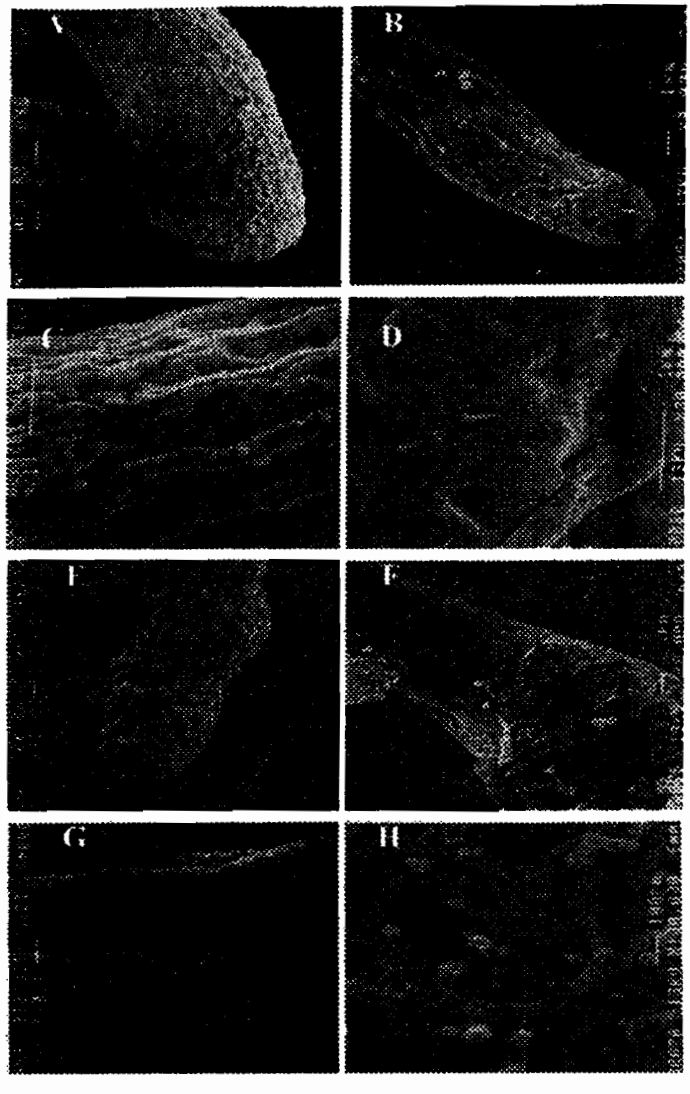

Fig. 2. Scanning electron micrographs of *P. ultimum* induced by *P. fluorescens* Q2-87 during the time-course indicated of cell wall hyphal degradation, in the intermediate area where diffusible bacterial antibiotic and *Pythium* are interacting. Mycelial hypha was grown in single culture for 24 h having normal growth and much rougher outer surface (A) compared to that grown in dual culture showing less rougher outer surface (B). Mycelial hypha was grown in single culture (C) for 48 h compared to that grown in dual culture (D) showing relatively smooth cell wall relative to the control. Mycelial hypha was grown in single culture (E) for 72 h compared to that grown in dual culture (F) showing looseness and disconnection of the outer layer of the cell wall. Mycelial hypha was grown in single culture (G) for 96 h compared to that grown in dual culture (H) showing complete degradation of the outer layer, at least, of the cell wall and revealing wall microfibril skeleton. Magnifications were indicated within Figs.

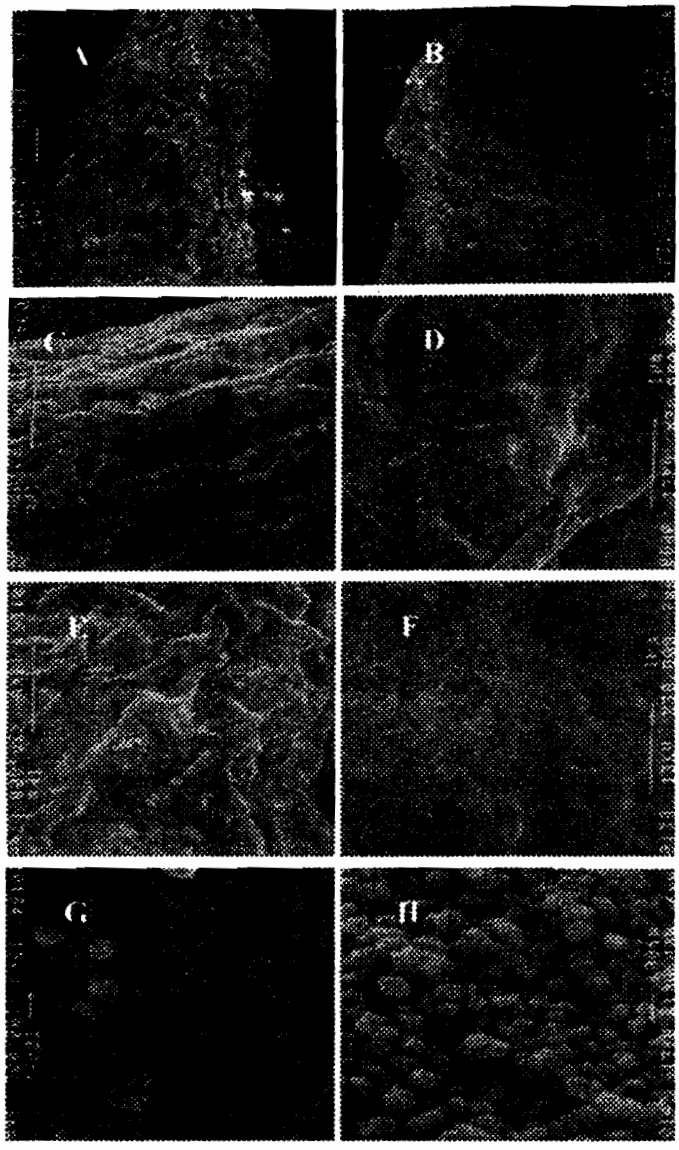

Fig. 3. Scanning electron micrographs of *P. ultimum* induced by the synthetic 2,4-DAPG at 80 µg/ ml for 96 h. *Pythium* grown in single culture (A) compared to that grown in dual culture showing complete degradation of the outer layer, at least, of the cell wall and revealing wall microfibril skeleton (B). The same as in B at higher magnifications indicated (C and D).

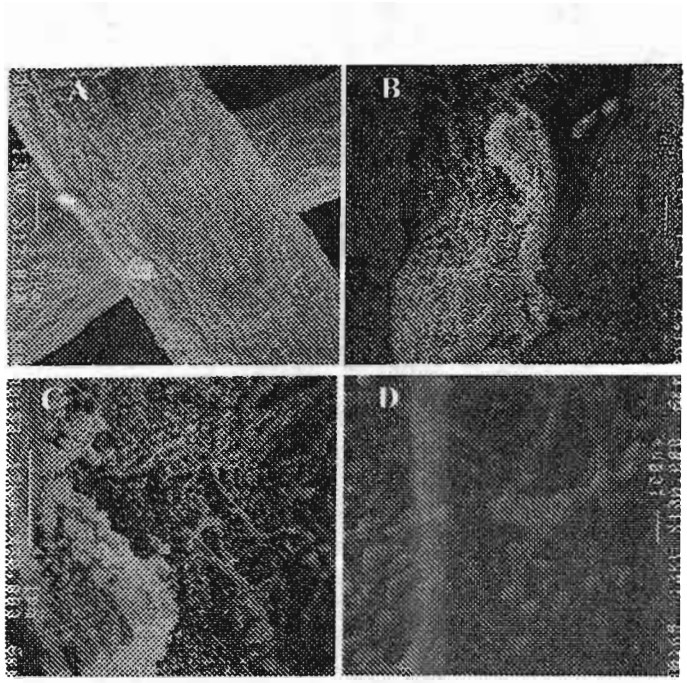

Fig. 4. Scanning electron micrographs of *P. aphanidermatum* induced by *P. fluorescens* Q2-87 producing 2,4-DAPG, during the time-course of hyphal degradation, in the intermediate area where diffusible bacterial antibiotic and Pythium are interacting. Mycelial hypha was grown in single culture for 24 h showing normal hypha with a much rougher outer surface (A) compared to that grown in a dual culture (B). Mycelial hypha was grown in single culture (C) for 48 h compared to that grown dual culture (D) showing a relatively smooth cell wall relative to that in control. Mycelial hypha was grown single culture (E) for 72 h compared to that grown in dual culture (F) which show a smooth outer layer of the cell wall. Mycelial hypha was grown in single culture (G) for 96 h compared to that grown in dual culture (H) which show looseness and disconnection of the outer layer of the cell wall. Magnifications were indicated within the Figs.

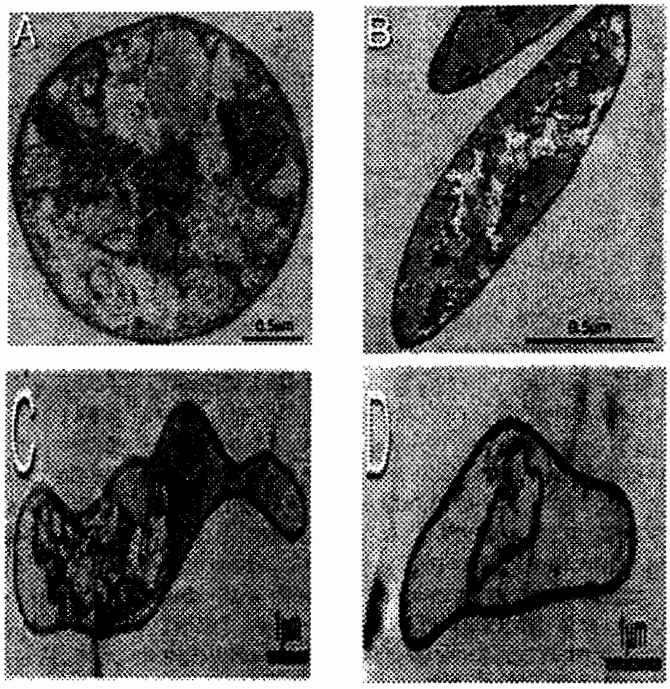

Fig. 5. Transmission electron micrographs of *P. ultimum* induced by P. fluorescens for 48 h, in the intermediate area where diffusible bacterial antibiotic interacting with hyphae of *P. ultimum*. Cross section of *P. ultimum* grown in single culture having normal structural components (A). Angle of section through a hypha of *P. ultimum* grown in dual culture showing structural changes mainly characterized by an increase in the number of vacuoles (B). Induced *P. ultimum* for 96 h showing disorganization of cytoplasmic organelles and a cell was devoid of all living components (C and D, respectively). Bars indicated in within figures.

Similar disturbances in the overall fungal organization have been reported in the case of fungal cells exposed to either antibiotics (Klecan *et al.* 1990; Hajlaoui and Bélanger 1993, Benhamou *et al.* 1996), or Sterol-binding fungicides (Fuller *et al.* 1990). Because of plasma membranes have been reported to be a potential target for antibiotics (Lewis and Papavizas 1987), changes in the plasma membrane permeability may have promoted internal osmotic imbalances, leading to alterations such as cytoplasm disorganization and aggregation. Since *P. fluorescence* Q2-87 is well known to produce the secondary metabolite 2,4-DAPG during its stationary phase, strong support for the concept of antibiosis as the main mechanism by which Q2-87 operates against *Pythium* cells is provided by the finding that agar disks collected from the bacterial-free intermediate region between Pythium cells and Q2-87 could trigger the same sequence of events seen by SEM and transmission electron microscopy (not shown). These biocontrol effects led ultimately to cell wall degradation and cell death (Figs. 2, B-H, 3, B-D, and 4, B-H). So far, little is known about pathogen cell (wall) degradation mediated by *Pseudomonas*-produced antibiotics. In fact, most studies have been concentrated on the role of hydrolytic enzymes in the antagonistic process against some diseases (Saksirirat and Hoppe 1990) and not on the possible involvement of antibiotics. Askary *et al.* (1997) were the first to suggest that antibiosis, causing cytoplasm disorganization, preceded parasitism and subsequent internal colonization of the plant pathogenic *S. fuliginea.* Antifungal synergisms between enzymes and either toxic or antibiotic metabolites have just been reported over the past few decades (Köller 1992; Yang *et al.* 1993; Shirmböck *et al.* 1994). One of the examples being the fungal antagonist, *Gliocladium virens*, while gliotoxin, a fungitoxic metabolite, has long been thought to be the main mechanism of biocontrol by this fungus, recent studies have shown that cell wall degrading enzymes were also involved in this process (Di Pietro *et al.* 1993). These authors suggest that synergistic effect of the enzymes was likely due to partial cell wall hydrolysis which facilitated rapid diffusion of the toxin. Similar results by us were also found during the interaction between *Pythium* cells and *Pseudomonas* strain Q2-87, since higher levels of ß-1, 3 glucanase and cellulase (ß-1, 4, glucanase), and protease were found in *Pythium* cells after 2,4-DAPG application (data will be reported elsewhere). Thus, antibiotics and hydrolytic enzymes may be involved in the antagonistic process mediated by *Pseudomonas* strain Q2-87.

Overall, marked ultrastructural changes induced in *Pythium* cells by Q2-87 (Figs. 2H, and 4H) were found to be associated with hyphal distortion, swollen shapes, abnormality, and inhibited growth that seen by light and scanning electron microscopy (Figs. 1, A-H). These significant changes may be explained by the fact that two cell wall textures described above including the outer layer might be required for *Pythium* growth and development and may be related to the need for controlled plasticity of the apical zone where expansion takes place, as also suggested by Bartnicki-Garci and Wang (1983). Thus, one might conclude that cell wall degradation would affect hyphal growth and development because of the importance of amorphous layer in hyphal wall morphogenesis. In line with our results, Meyer *et al.*

(1976) found in a related study that germination of the Oomycete *Phytophthora palmivora* cysts after application with exo-ß-1,3-glucanase, the outer wall layer is eroded and the germ tubes were distorted, swollen in shapes and unusual in apical branching. It might be concluded that degradation of *Pythium* cell walls, which contain, ß-1,3 glucan and ß-1,4 glucan, require enzymes that can hydrolyze polymers of glucose with various glycosidic linkages (Bartnicki-Garcia and Wang 1983). ß-1,3-glucanase and cellulase (ß-1,3-glucanase) are recognized as important hydrolytic enzymes because they attack the most common cell wall-forming polymers in Oomycete hyphae. As a result, partial and/ or complete degradation of these structural polymers would have adverse effects upon the growth and differentiation of fungi (Bartnicki-Garcia and Wang 1983; Poulose 1992; Fridlender *et al.* 1993; Schirmböck *et al.* 1994).

CONCLUSION

The results of the present investigation demonstrate that antibiosis of *Pythium* spp. triggered by *P. fluorescens* Q2-87 induces cell wall degradation that adversely affects Pythium growth and development and shed more light on the mechanism by which antibiosis is expressed at ultrastructural level. Our results suggest that there are, of course, hydrolytic enzymes participated in the cell wall degradation of *Pythium*. However, the source and nature of these enzymes are required further investigation. Could these have been produced by the bacteria? Could the mechanism of biocontrol involve enzyme production as well as antibiotic production. These questions led us to further study of the mechanism of action of biocontrol *P. fluorescense* Q2-87 of a major plant pathogen, *Pythium*.

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التفاعلات المضادة بين المضاد البكتيرى سيدوموناس فلوريسنس Q٢-٨٧ (*Pseudomonas fluorescens* Q2-87) والمسبب المرضى أنواع *Pythium* فيما يتعلق بالتركيب الدقيق للهيفات

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تم دراسة تأثيرات عامل المقاومة الحيوية سلالة Q2-87 المعروف بإنتاج المضاد الحيوي 2,4-diacetylphloroglucinol على المسبب المرضى بيثيم فيما يتعلق بالتحليل الدقيق لهيفاته. تمت تلك الدراسة بواسطة كل من الميكروسكوب الضوئي و الإلكتروني عند تنمية كل منهما معا على بيئة البطاطس– دكستروز– أجار. لقد أوضحت الصور الضوئية المأخوذة بالميكروسكوب الضوئي أن الهيفات أصبحت مشوهة وبها مناطق منتفخة و تفريعها غير عادي بالمقارنة بالكنترول. بالإضافة الي ذلك فان الصور الإلكترونية المأخوذة بالميكروسكوب الإلكتروني المجسم أظهرت تحللا لطبقات الجدر الخلوية الخارجية مما أدى آلي ظهور الهيكل الداخلي للهيفات الدقيقة السليولوزية. لوحظ أيضا تحلل مماثل لتلك الجدر عند إحلال المادة المصنعة 2,4-diacetylphloroglucinol لعامل المقاومة الحيوية.

بناءا علي ماسبق يمكن إستنتاج أن المضاد الحيوي الطبيعي الناتج من بكتيريا سيدوموناس فلوريسنس سلالة Q2-87 هو المسؤل بصفة أساسية عن عملية التضاد السابقة وأن طبيعة عمل تلك السلالة البكتيرية فى تثبيط النمو لخلايا البيثيم يكون مصاحبا بتحلل جدرها الخلوية ويلي ذلك موت الخلايا عقب التحلل الكامل لجدرها.