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EFFECT OF 2, 4-DIACETYLPHLOROGLUCINOL PRODUCED BY *Pseudomonas* FLUORESCENS Q2-87 ON ULTRASTRUCTURE, PROTEINASE AND RIBONUCLEASE OF *Pythium ultimum*

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ABSTRACT

Effects of exogenous application of 2, 4-diacetylphloroglucinol (2, 4-DAPG) produced by the biocontrol agent *Pseudomonas fluorescens* Q2-87 on ultrastructure, activities of protease and ribonuclease (RNase) of *Pythium ultimum* Trow were investigated during the time-course studied after application. Electron microscopic examination of treated *Pythium* cells relative to the untreated ones revealed that hyphal cell walls, plasma - membranes, mitochondria and nuclei membranes showed disruption and degradation. Cytoplasm was aggregated and cytoplasmic organelles were disorganized. Increased vacuolation was also a characteristic of the treated cells. In addition, many hyphal cells were devoid of all living components. Total soluble proteins, protease and RNase activities were assayed spectrophotometrically while native polyacrylamide gel of protease and RNase activities were analyzed by electrophoretically. Higher levels of protease, RNase and their specific activities and total soluble proteins were found in treated cells of *Pythium* compared to controls. The present results suggest that 2,4-DAPG causes disorganization, degradation and death later of *P. ultimum* by promoting elevated levels of proteinase and RNase activities. It is concluded that 2, 4-DAPG exerted its mode of biocontrol agent action by increasing the concentration and /or activities of such hydrolytic enzymes that may affect its normal ultrastructure.

Keywords: 2, 4-diacetylphloroglucinol, Protease, *Pseudomonas fluorescens* Q2-87, *Pythium ultimum*, ribonuclease

INTRODUCTION

Numerous studies have demonstrated that the secondary phenolic metabolite 2,4-DAPG produced by some strains of *P. fluorescens,* including Q2-87 strain has antifungal, antibacterial, and antiviral activities against a wide range of phytopathogens including *Pythium*-mediated damping-off of many plants (Tada *et al.* 1990; Vincent *et al.* 1991; Fenton *et al* 1992; Harrison *et al.*1993; Nowak-Thompson *et al.* 1994; Bonsall *et al.* 1997; Duffy and Défago, 1997; Rodriguez and Pfender 1997; Sharifi-Tehrani *et al.* 1998; Thomashow *et al.* 2000; Landa *et al.* 2002). Mutant strains lacking the ability to produce 2,4-DAPG, also lost the ability to inhibit plant pathogens (Carroll *et al.* 1995), indicating that this phenolic antibiotic is responsible for antagonistic interactions between a phytopathogen and *P. fluorescens.* As a result, phenolic antibiotic-producing fluorescent Pseudomonads have been used effectively in biological control of many phytopathogens *in vitro* and *in vivo* (Hultberg *et al.* 2000).

Cell disorganization is a cited mechanism of antagonism used by fungi and bacteria against phytopathgens. Microbial antibiotics cause cell disruption in *Erysiphe graminis* var. *tritici* (Haijlaoui and Bla n ger 1993) and *Erysiphe graminis* f.sp. *Hordei* (Klecan *et al.* 1990) after application with the living biocontrol *Sporothrix flocculosa* and *Tilletiopsis pallescens,* respectively. Similar results were reported in *Penicillium digitatum* after the treatment with the biocontrol *Verticillium lecanii* (Benhamou and Brodeur 2000). Protease is a hydrolytic enzyme that has been participated in the biocontrol of plant pathogens (Chr i f and Benhamou 1990; Haab *et al.*, 1990; Sacherer *et al.* 1994; Dunne *et al.* 1997; Elad and Kapat 1999 Helist. *et al.* 2001). In addition, RNase activity is ubiquitous in living organisms (Green 1994) and may play a role in responding to 2,4-DAPG. Exogenous application of the isolated secondary metabolite 2,4-DAPG produced by *P. fluorescens* Q2-87 to a plant pathogen in relation to transmission electron microscopic (TEM) ultrastructure, protease and ribonuclease activities that might cause degradation and self-destructive processes of the *P. ultimum* cells has not been previously studied.

In the present investigation, *P. ultimum* was used as a model of a phytopathogen to study the effectiveness of 2, 4-DAPG on the ultrastructure, activities of protease and RNase to follow the physiological and biochemical changes that may elucidate the mode of 2, 4-DAPG action against a major plant pathogen, *Pythium.*

MATERIALS AND METHODS

Organisms and culture conditions

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 maintained at 28°C in Tryptone Glucose Yeast medium (TGY) consisting of tryptone 5.0 g, yeast extract 5.0 g, glucose 1.0 g, K_2HPO_4 1.0 g, agar 15.0 g in 1 liter distilled water, pH 7.0. *P. ultimum* was isolated from cowpea in Georgia and provided by Dr. Jeff Hoy (Louisiana State University). *Pythium* cultures were grown in V8 medium which consisted of V8 juice 200 ml, $CaCO_3$ 3 g, agar 15 g, distilled water 820 ml. The following experiments were conducted in potato dextrose broth (PDB, 24 g/L. Difco, Detroit) and potato dextrose agar (PDA, 39 g/L. Difco).

Antagonism *in vitro* and the process for transmission electron microscopy (TEM)

P. fluorescens Q2-87 cultures were grown on PDA at 24°C for 24 h prior to use as seed culture. *Pythium* cultures were grown for 3-4 days on PDA medium, and hyphal tips from the actively growing mycelia were used in the antagonistic experiment as described by Youssef *et al.* (2003). Four days after treatment applications were made, mycelial samples (5 mm^2) were collected from the interaction region of dual cultures, as well as samples from pure culture of *Pythium.* Samples were dissected into 1-mm^2 pieces; immediately fixed for 2 h at room temperature in 2.5% (v/v) glutaraldehyde + 1% sucrose in 50 mM sodium cacodylate buffer, pH 7.1, followed by washing

5 times in 50 mM in the same buffer, each for 20 min. Samples were post-fixed with 1% (w/v) osmium tetroxide (OsO_4) in the same buffer for 1 h at 4°C, followed by washing 4 times in distilled H_2O for 20 min each, then fixed with 1% uranyl acetate for 1 h, and subsequently washed 2 times in distilled H_2O, each for 15 min. The fixed specimens were dehydrated in a graded series of acetone and subsequently infiltrated and embedded in Spurr resin ERL 4206 (Spurr 1969). They were infiltrated with a mixture of 2: 2 acetone resins for 2 h and 3:1 mixture of acetone resin overnight. Several changes in 100% resin were made and then embedded in quetol. Blocks were made using silicone molds and polymerized for 48 h at 60°C. Ultrathin sections were collected on 200-mesh nickel grids coated with Formvar and stained with uranyl acetate for 30 min followed by lead citrate for 3 min (Reynolds 1963). Examinations were made using a JOEL 1200 EX transmission electron microscope (JOEL Ltd., Tokyo, Japan) operating at 120 kV. Mycelial samples were collected from five Petri dishes and within each dish from different points of contact, to obtain a representative sampling of the interaction. For each sample, 10 ultrathin sections were examined.

Isolation of the metabolite 2,4-DAPG produced by *P. fluorescens* strain Q2-87, treatment and sampling

The natural product 2, 4-DAPG was isolated according to a method described by Shanahan *et al.* (1992) and modified by Youssef *et al.* (2004). *Pythium* mycelia were grown in freshly prepared PDB media for 4 days at room temperature in a rotary shaker, then the medium was replaced by another one supplemented with isolated 2,4-DAPG (2.5 g /L, w/v) under sterilized conditions and 2,4-DAPG free medium was used as a control. Treated and untreated *Pythium* mycelia were collected at specified times during the time-course of study. Samples were weighed, frozen immediately in liquid nitrogen, lyophilized and stored at 20°C until analysis.

Protease extraction and its spectrophotometer assay

Lyophilized mycelia were homogenized in 50 mM Tris-HCl buffer at pH 7.0 containing 1mM EDTA, 3 mM $MgCl_2$, 0.02% (v/v) β-mercaptoethanol and 1% polyvinylpyrolidone (1: 3, w/v). The homogenates were centrifuged at 10,000 g at 4°C for 30 min. The supernatants were decanted and the pellets were re-suspended in the same amount of buffer, and centrifuged under the same conditions as above. The two supernatants were combined and used immediately for protease assay and its detection by native polyacrylamide gel electrophoresis (PAGE). The assay was carried out according to a modified method of Sankhla *et al.* (1985). The reaction mixtures consisted of 1 ml of 0.5% casein, 3 ml of 0.2 M phosphate buffer at pH 7.0 and 0.2 ml enzyme extract. After 25 min incubation at 37°C, the reactions were terminated by adding 1 ml of 10% trichloroacetic acid (TCA). After centrifugation, the absorbances were measured at 280 nm. One unit of protease activity was equal to an increase in absorbance of one in 1 h under the above specified conditions. Protease activities were expressed as units h^{-1} g^{-1} FW and units mg^{-1} protein.

RNase extraction and its spectrophotometer assay

Lyophilized mycelia were homogenized in 25 mM Tris-HCl buffer at pH 7.5 (1: 3, w/v). The homogenates were centrifuged at 10,000 g at 4°C for 30 min. The supernatants were decanted and pellets were re-suspended in the same amount the buffer and centrifuged under the same conditions as above. The two supernatants were combined and used to assay RNase and native electrophoretic analysis. RNase activity was assayed by a modified method of Altiman *et al.* (1977). The reaction mixture contained 0.4 ml of RNA substrate (15 mg highly polymerized RNA, Sigma, in 16 ml of 6.2 mM acetate buffer pH 5.5 which was added to 0.1 ml of enzyme extract and incubated at 28؛C for 30 min. The reaction was terminated by adding 0.5ml of 2.5% w/v TCA containing 0.3% uranyl acetate. The samples were then placed in ice for 15 min and centrifuged at 10,000 rpm for 15 min. The supernatant was diluted appropriately and absorbance was read at 260 nm. RNase activity was expressed in the same manner as protease. The separation of each sample using PAGE was repeated three times with identical results.

Detection of proteinase and ribonuclease and by native PAGE

Non-denaturing proteinase PAGE [10% (w/v) acrylamide] amended with 0.15% casein before polymerization was carried out without SDS using the buffer system according to Laemmli (1970). Detection of proteinase activity, during maximum activity measured spectrophotometrically after 2,4-DAPG application, was performed according to a modified method of Taylor *et al.* (1997). After electrophoresis, the gel was incubated overnight at 37؛C in protease activity buffer, 50 mM sodium phosphate, pH 6.8. Following overnight incubation in activation buffer, the gel was rinsed with water, stained with Coomassie Brilliant blue, and destained for 1 h. Protease activity was visualized as clear zones in a blue background. The separation of each sample using PAGE was repeated three times with identical results.

Non-denaturing ribonuclease PAGE [12% (w/v) acrylamide] was carried out without SDS using the buffer system according to the method of Laemmli (1970). Detection of RNase activity, during maximum activity measured spectrophotometrically after application, was performed by washing the gels in 150 mM Na-acetate buffer pH 5.6 for 2ص10 min, followed by incubating gel in substrate solution [150 mM Na-acetate pH 5.6, 2.5 mM EDTA and 0.4% (w/v) yeast RNA] at 37°C for 30 min. After rinsing off adhering substrate solution with buffer, gel was stained in 0.2% w/v toluidine blue, 0.5% (v/v) acetic acid for 5 min and was destained in 0.5% acetic acid (Abel and K.ck 2001). RNase activity was visualized as clear zones in a blue background. The separation of each sample using PAGE was repeated three times with identical results. Protein concentration was determined by the method of Bradford (1976) using Bio-Rad protein assay.

RESULTS

Effect of 2,4-DAPG produced by *P. fluorescens* Q2-87 on *Pythium* ultrastructure

Electron microscopic examinations reveal that untreated *P. ultimum* showed typical ultrastructure with regular cylindrical hyphae. Cells also had a normal hyphal wall and enriched cytoplasm containing normal cytoplasmic organelles. They also had a large vacuole and storage vesicles as shown in Fig. 1A. However, treated *P. ultimum* grown in dual cultures with *P. fluorescens* shows that hyphal cell wall was disrupted and vacuolation was increased after three days of application as shown in Fig. 1B. Close examination of a nucleus in untreated *Pythium* showed double membrane and nucleolus were present (Fig. 1C). In contrast, the nucleus in the dual culture was disrupted in its shape (Fig. 1D). Close examination of a mitochondrion shows that the double membrane was also disrupted and possibly degraded in treated *Pythium* cell (Fig. 1F) but intact double membrane was present in the untreated cells (Fig. 1E). The hyphal cell wall and cell membrane in the untreated *Pythium* was evident and regular, but they were disrupted and in some areas was totally degraded in case of treated hyphae (Fig. 1G and H). In terms of that, Scanning electron microscopic observation revealed that the cell wall of *P. ultimum* was effectively degraded revealing the underlying wall microfibrillar skeleton (Fig. 1I). Four days after inoculation, the main feature of treated *Pythium* showed irregular cell shape and disorganized cell organelles. In addition, the cytoplasm and its organelles was aggregated (Fig. 1 J). Further, some *P. ultimum* cells were significantly devoid of their components (Fig. 1 K).

Effect of 2,4-DAPG produced by *P. fluorescens* Q2-87 on protease activity

As a response of *Pythium* treated with 2, 4-DAPG produced by *P. fluorescens*, protease activity and its specific activity levels were increased during the time-course of study and reaching the maximum levels after 48 and 72 h, respectively, of treatment as compared to control (Fig. 2). Total soluble proteins also increased during most of the time-course of study and reached the maximum level after 2 h of application in comparing to the control. Thereafter, total soluble protein was decreased during 48 and 72 h after application (Fig. 2). In untreated samples, protease activity, protease specific activity and total soluble proteins were determined during all the time-course indicated in the Fig. 2. There were no significant differences (5% variation) could be found between the time intervals. For that reason, the initial zero time only before treatment was just plotted in the Figure to represent untreated *Pythium*. Detection of protease activity by native PAGE gel (Fig. 4A) during the maximum activity over-times are also correlated with RNase activity that previously discussed (Fig. 2). In general, less activity of protease was found in control cells whereas more activity was found in 2,4-DAPG treated cells.

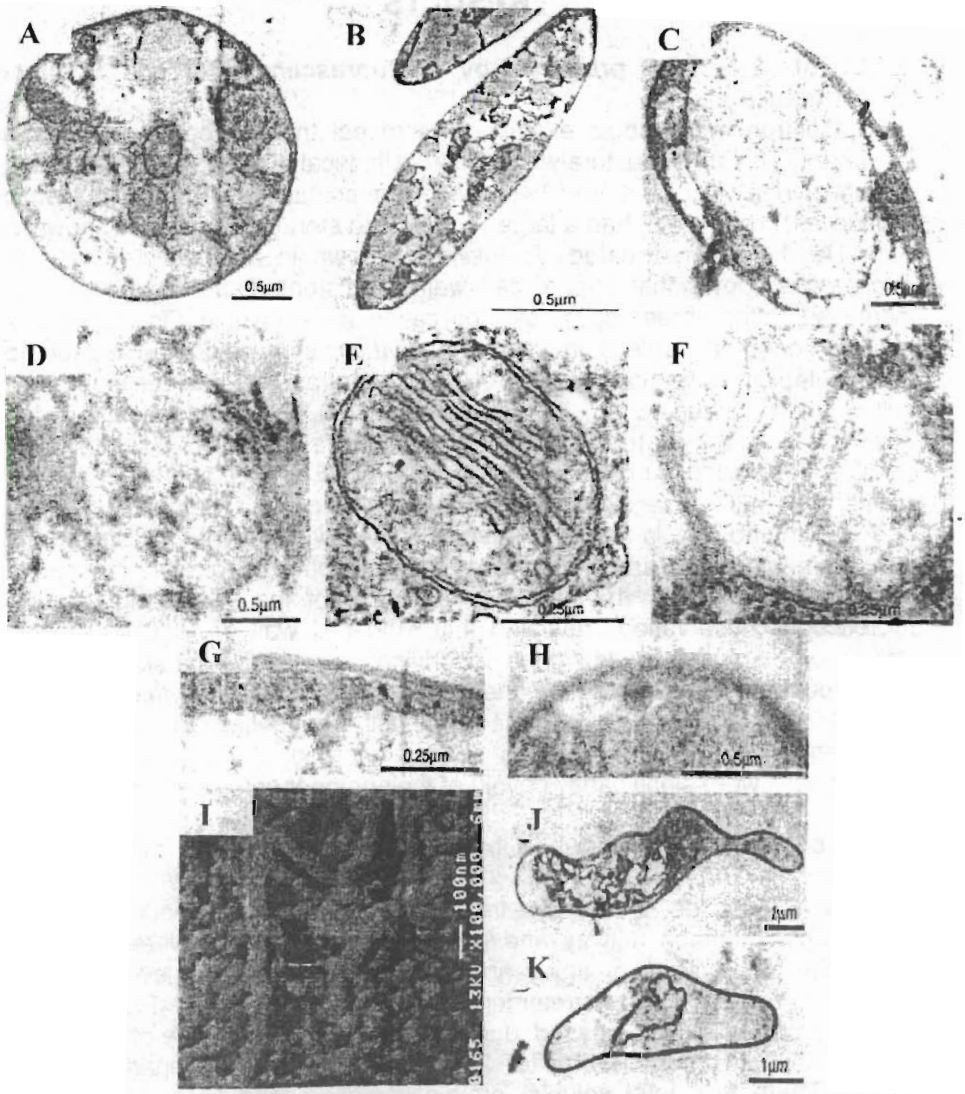

Fig. 1: Transmission electron micrographs of *P. ultimum* treated or not with *P. fluorescens* Q2-87 producing 2, 4-DAPG, showing *P. ultimum* grown in a single culture (A), note the hyphal cell wall is cylinder and containing normal organelles and enriched cytoplasm. An oblique section of *P. ultimum* grown for 72 h in dual culture (B), note increase of vacuoles. Untreated hypha has a normal nucleus surrounded with a double membrane, (C). Treated *P. ultimum* hypha for 72 h showing the double membrane is degraded (D). Untreated hypha has normal mitochondrion (E), note the inner membrane is invaginated. The mitochondrion double membrane is degraded in treated hypha (F). Untreated hypha having normal cell wall and plasma membrane (G). Treated hypha for 72 h showing degradation of cell wall and plasma membrane (H). Scanning electron micrographs of *P. ultimum* induced by natural 2,4-DAPG for 96 h showing complete degradation of the outer layer of the cell wall and revealing the microfibril skeleton (I), Treated hypha for 96 h showing cytoplasm aggregation and organelles disorganization (J) or a cell was devoid of all living components (K).

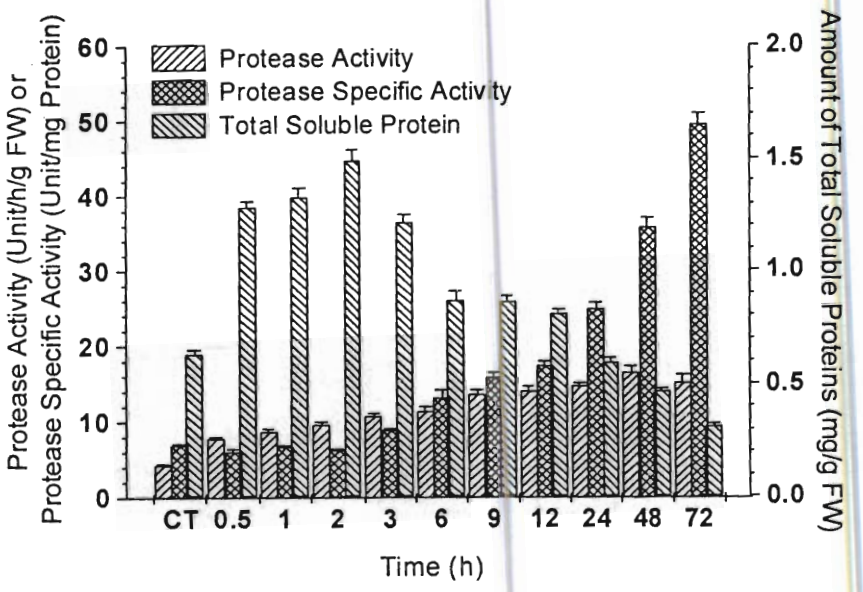

Fig. 2. Effect of 2,4-DAPG produced by *P. fluorescens* Q2-87 on *P. ultimum* protease activity, specific protease activity, and total soluble protein levels during the time-course indicated after application. Vertical bars represent SD of three replicates.

Effect of 2,4-DAPG produced by *P. fluorescens* Q2-87 on RNase activity

A higher level of RNase activity was found in treated *Pythium* comparing to the control during the time-course of study reaching the maximum level during the first hour after application. However, RNase specific activity during the first hour after treatment was lower comparing to the control, then gradual increase was found to reach the maximum level during 48 h after application followed by a decrease (Fig. 3). Total soluble protein level was found to be higher during the time-course of study reaching the maximum level during the first hour after application as shown in Fig. 3. In untreated samples, RNase activity, specific activity and total soluble proteins were assayed during all the time-course indicated in Fig. 3. There were no significant differences (5% variation) could be found between the specific time intervals. For that reason, the initial zero time only before treatment was just plotted in the Fig. 3. Electrophoretic RNase activity as shown in Fig. 4*B* is also correlated with its RNase activity that previously discussed (Fig. 3). In general, less activity of RNase was found in control cells whereas more activity was found in 2,4-DAPG treated *Pythium* cells. It seems that the three RNase isoforms are relatively close in their molecular weights as shown in Fig. 4B.

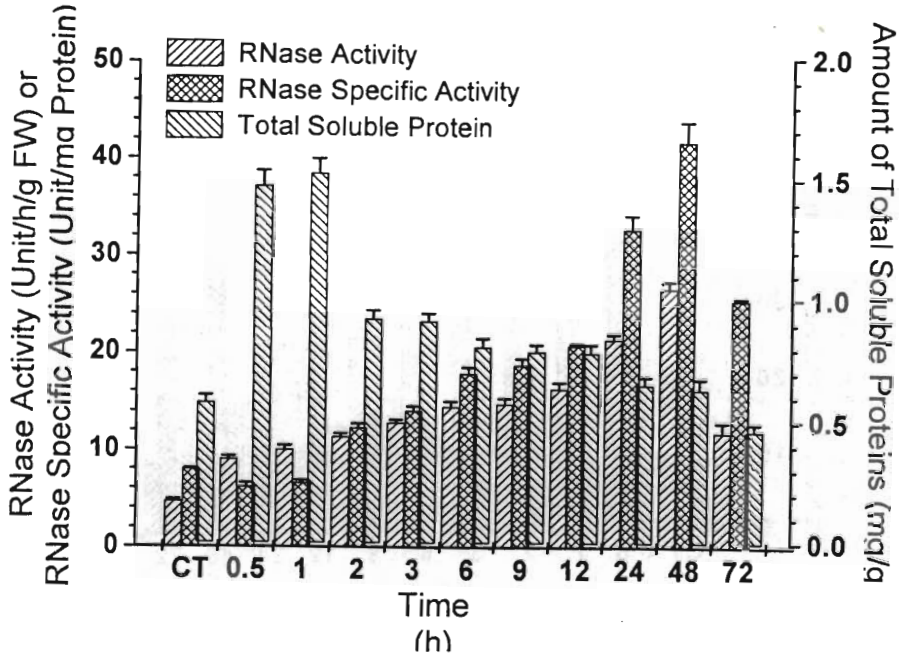

Fig. 3: Effect of 2,4-DAPG produced by *P. fluorescens* Q2-87 on *P. ultimum* RNase activity, specific RNase activity, and total soluble protein levels during the time-course indicated after application. Vertical bars represent SD of three replicates.

DISCUSSION

Ability of fluorescent *Pseudomonas* strains to control plant pathogens via production of antimicrobial metabolites that involved in cell disorganization was documented (Ahl *et al.* 1986; Lambert *et al.* 1987; Homma *et al.* 1989). The present results indicate that the secondary metabolite 2, 4-DAPG significantly caused cell degradation, increased vacuolation and death of *Pythium* later. In agreement with the ultrastructure findings in this study, Kilecan *et al.* (1990), Haijlaoui and Bl anger (1993), Askary *et al.* (1997) and Benhamou and Garand (2001) found that other biocontrol agents caused cell destruction and cytoplasic disorganization and increased vacuolation and that increase was related to sign of cell death. They attributed such ultrstructural changes of phytopathogens cells to secretion of metabolizing toxic compounds and/ or antibiotics. Weete (1980) also demonstrated that disruption of lipids and phospholipids constituents of cell membranes affected the selective permeability of these membranes leading to cell plasmolysis. The same conclusion of the affected plasma membrane was put forward by Lewis and Papavizas (1987). The latter authors suggested that 2,4-DAPG is

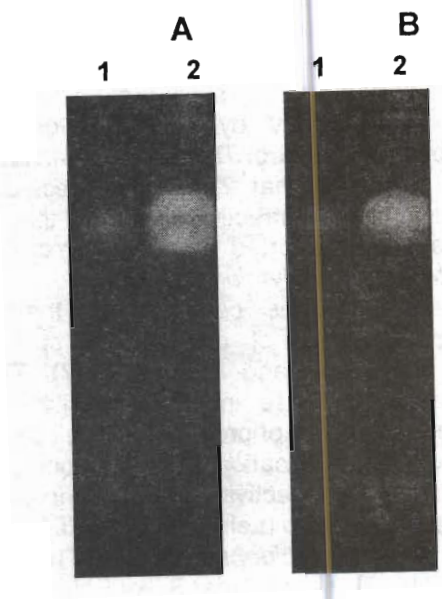

Fig. 4: Detection of proteinase (A) and ribonuclease (B) activities during 48 h after application by native polyacrylamide gel electrophoresis of untreated (1) and treated (2) *Pythium* cells. 30 g protein was loaded on the gel.

Table to diffuse into cells or act from outside the cell without diffusion and caused cell wall disruption and cytoplasm disorganization and aggregation.

A correlation between pathogenicity and protease activity has been reported in terms biocontrol of phytopathogens. Proteases may play a significant role in biological control by inducing cell lysis (Ries and Albersheim 1973; Flores *et al.* 1997). The present results indicate that *Pythium* protease activity may increase as a result of the exogenous application by 2,4-DAPG (Fig. 2). Low level of protease in untreated healthy *Pythium* (Fig. 2) may serve to break down the proteins present in old cells of *Pythium* to facilitate its transport to actively growing hyphal tip. However, whether protease is present and active following 2,4-DAPG application remains to be elucidated. In line with our present results, Dunne *et al.* (1997) found that extracellular proteolytic enzymes are able to inhibit *P. ultimum*. These authors indicated that incubation of *Pythium* cells with free supernatants of antagonistic *Stenotrophomonas maltophilia* W81 producing proteases caused hyphal lysis of *Pythium* that lead to loss its ability to grow. They also demonstrated that protease negative mutant strain had no effect on growth compared with the wild type producing protease. In addition, Dunne *et al.* (1998) showed that

combining two biocontrol agents, namely *P. fluorescens* producing 2, 4-DAPG and *S. maltophilia* W81 producing extracellular proteases, against *P. ultimum*, resulted in synergistic interaction in effectively controlling *Pythium* that was similar to what they had obtained with a fungicide in the field experiment. In addition, Sivan and Chit (1989) found that pre-treating *Fusarium oxysporum* hyphae with such proteolytic enzymes increased markedly their lysis susceptibility by another hydrolytic enzyme, β-1,3 glucanase, secreted by the biocontrol *Trichoderma harzianum*. Their findings supports the present results in that 2,4-DAPG affected *Pythium* cells by elevating the activities of self-destructive enzymes that determined in this investigation and others that reported elsewhere (Youssef *et al.* 2004) to effective synergy in biocontrolling *Pythium* cells.

Ribonucleases are ubiquitous components in the living cells and their role is to terminate the life span of different RNA species by hydrolytic or phosphorolytic action (D Alessio and Riordan 1997). The present results suggest that the observed increase in RNase activity and its isoforms (Fig. 4B) were probably due to induction of programmed cell death in *Pythium* cells as a result of the exogenous application of the metabolite 2,4-DAPG. In fact, increase of ribonuclease LX activity was observed associated with programmed cell death in tomato (Lehmann *et al.* 2001), senescent tomato leaves (Lers *et al.* 1998) and *Arabidopsis thaliana* (Taylor *et al.*1993). In this respect, Galiana *et al.* (1997) reported that S-like RNase in tobacco was able to inhibit *Phytophthora parasitica* from leaf colonization. They concluded that this extracellular ribonuclease was participated in the defense mechanism of tobacco. In addition, Hugot *et al.* (2002) proposed that pathogen inhibition was mediated by interacellular degradation of the pathogen RNase after translocation of the protein through Golgi bodies. Thus, the present results may suggest that increased vaculation and RNase activity are signs of programmed cell death in *Pythium*. However, low level of RNase activity in untreated *Pythium* (Fig. 3) may serve to turnover of cellular RNAs. Whether RNase are present and active following 2,4-DAPG application remains to be elucidated. However, the demonstration that RNase activity increase in *Pythium* cells after treatment provides an easily controlled system in which to study their activation, e.g. whether the increase in activity is a consequence of gene induction or of post-translational modification or processing of existing inactive or precursor proteins. Additionally, presented data indicate that the total soluble proteins were increased during the early cellular events of *Pythium* following 2,4-DAPG application. Higher levels of protein content in treated *Pythium* are in agreement with a similar study conducted by Pozo *et al.* (1998). Accumulation of total soluble proteins in treated *Pythium* during the first few hours after application appeared to be a result of the exogenous application of the phenolic antibiotic 2, 4-DAPG. This quantitative alteration in protein expression may be explained by transient activation of *Pythium* defense mechanisms occurred as a consequence of 2, 4-DAPG as also suggested by Pozo *et al.* (1998), Dumas-Gaudot *et al.* (1994), and Benabdellah *et al.* (1998) in similar studies.

CONCLUSIONS

Based on the foregoing results, elevated levels of protease and RNase activities in *P. ultimum* might contribute to disruption and degradation of *P. ultimum* cells that were markedly found by electron microscopic observation. The results of the present investigation suggest that the secondary metabolite 2,4-DAPG produced by *P. fluorescens* Q2-87 might participate in causing cell degradation, disorganization and death later by actively increasing higher activities in protease and ribonuclease. Increased levels of these hydrolytic enzymes might elucidate the mode of action by which antagonistic microorganism *P. fluorescens* Q2-87 might attack a large variety of phytopathogens including *P. ultimum* responsible for certain major crop diseases. The significant increase in RNase in *Pythium* following the application with 2,4-DAPG is considered as a sign of the programmed cell death of an organism. Hence, determinations of nuclear DNA as well as other biological substances of *Pythium* are required to fully elucidate the mode of *P. fluorescens* Q2-87 action against plant pathogenic *Pythium*.

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تأثير المضاد الحيوي داي اسيتيل فلوروجلوسينول الناتج من بكتيريا سيدوموناس فلوريسنس سلالة Q2-87 علي التركيب الدقيق و إنزيمات تحلل البروتينات و الأحماض النووية الريبوزية لبيثيم ألتيمم.

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أجري هذا البحث بهدف دراسة تأثيرات معاملة بيثيوم بالمضاد الحيوي ٢،٤ داى أسيتيل فلوروجلوسينول، الناتج الثانوي لبكتيريا سيدوموناس فلوريسنس سلالة Q2-87 علي التركيب الدقيق وأنشطة إنزيمات تحلل البروتينات و الأحماض النووية الريبوزية لبيثيم ألتيمم. أظهرت الصور الإلكترونية المأخوذة بالميكروسكوب الإلكتروني العادي لخلايا بيثيوم المعاملة بمقارنتها بغير المعاملة أن هناك تشوية وتحلل للتركيب الدقيق لخلايا بيثيوم المعاملة وزيادة تكوين الفجوات بها. و لقد أوضحت النتائج أيضا أن هناك مستويات عالية من البروتينات الذائبة الكلية وإنزيمات تحلل البروتينات والأحماض النووية الريبوزية والأنشطة النوعية لكل إنزيم في هيفات بيثيوم المعامل بمقارنته بغير المعامل.

من النتائج التي تم الحصول عليها تم إقتراح أن المضاد الحيوي البكتيرى هو المسئول عن مقاومة بيثيوم حيويا بإحداث تكوين مستويات عالية لإنزيمات التحلل المائى، البروتيز والريبونيوكليز، المسئولة عن تحلل خلايا بيثيوم المعاملة و فقدان تكوينها.