pH Regulates Endoglucanase Expression and Virulence of *Alternaria alternata* in Persimmon Fruit

Dani Eshel,^{1,2} Itay Miyara,¹ Tong Ailing,¹ Amos Dinoor,² and Dov Prusky¹

¹Department of Postharvest Science of Fresh Produce, Institute for Technology and Storage of Agricultural Products, The Volcani Center, Agricultural Research Organization, Bet Dagan 50250, Israel; and ²Department of Plant Pathology and Microbiology, Faculty of Agricultural, Food and Environmental Quality Sciences, The Hebrew University of Jerusalem, Rehovot 76100, Israel

Submitted 31 December 2001. Accepted 13 April 2002.

The phytopathogenic fungus *Alternaria alternata* produces one endo-1,4- β -glucanase, *AaK1*, which is an important factor in disease development in persimmon fruit. During growth of *A. alternata* in media containing acidified yeast extract or cell walls from persimmon fruit, the fungus secreted ammonia and raised the medium pH. A rise in media pH from 3.8 to 6.0 in the presence of cell walls induced the expression of *AaK1*, whereas a glucose-induced decline in pH to 2.5 repressed transcription and enzymatic production. Treatments with buffered solutions at pH 6.0 during growth of *A. alternata* in the presence of glucose derepressed *AaK1* expression and endo-1,4- β -glucanase production and enhanced decay development on the fruit. The results suggest that conditions affecting environmental pH modulate gene expression of *AaK1* and virulence of *A. alternata* in persimmon fruit.

Additional keyword: postharvest diseases.

Alternaria alternata (Fries) Keisel. is the causal agent of black spot disease in persimmon (*Diospyros kaki* L.) (Prusky et al. 1981). The pathogen mainly produces one endo-1,4- β -glucanase (EG) (EC 3.2.1.4) when grown on persimmon cell walls (CW) (Eshel et al. 2000). Symptom development of *A. alternata* is directly enhanced by purified EG, and host susceptibility is closely related to EG activity on persimmon CW (Eshel et al. 2000).

Filamentous fungi produce a battery of extracellular enzymes capable of degrading the polymers of plant cell walls. Targeted mutational studies have found that individual enzyme genes play only a partial role, if any, in pathogenesis (Annis and Goodwin 1997; Kim et al. 2001; Scott-Craig et al. 1998; Walton 1994). This raises the question if mutational analysis of *A. alternata* genes is the only approach to determine their involvement in pathogenicity (Issshiki et al. 2001). Recent findings by Yakoby and associates (2000) and Prusky and associates (2001) indicate that specific genes in the battery of genes encoding for extracellular enzymes are expressed under specific environmental conditions, which might determine their role in pathogenesis.

Corresponding author: Dov Prusky, Department of Postharvest Science of Fresh Produce; Telephone: +972-3-9683880; Fax: +972-3-9683622; E-mail: prusky33@netvision.net.il.

D. Eshel and I. Miyara are joint first authors.

The nucleotide and amino acid sequence data reported are available in the EMBL database under accession number AF176572.

Environmental conditions are known to affect protein production and secretion in various organisms (Annis and Goodwin 1997; Condemine and Robert-Baudouy 1995; MacKenzie et al. 1993). Dean and Timberlake (1989) found that *Aspergillus nidulans* secretes polygalacturonase at low pH values and pectate lyase when the media pH increases and becomes conducive to pectate lyase activity. Recent reports suggest that pathogens are able to modulate host pH as a mechanism to increase virulence (Prusky et al. 2001; St. Leger et al. 1998). *Colletotrichum gloeosporioides* alkalized the tissue as a mechanism to enhance the expression of *peIB* during host colonization (Prusky et al. 2001). St. Leger and associates (1998) hypothesized that ambient pH affects pathogenicity by altering the expression of cuticle-degrading enzymes and hydrophobin in the insect pathogen *Metarhizium anisopliae*.

In the present report, we evaluated the environmental effect of pH on *AaK1* expression. We describe environmental pH as a critical factor in *AaK1* expression and EG production by *A. alternata*. Acidified conditions, created during glucose repression, were used as a tool to demonstrate that environmental pH represents a significant factor for gene expression and virulence regulation in *A. alternata*-persimmon interactions.

RESULTS

Ammonia accumulation and pH increase associated with growth of *A. alternata* on yeast extract medium and in persimmon fruit.

When *A. alternata* was grown in acidified yeast extract, it increased ammonia content and the pH of the media. Ammonia concentration increased from 2,000 μmol per g of dry weight 4 h after inoculation, to 32,000 μmol per g of dry weight 16 h later (Fig. 1). The increase in ammonia concentration corresponded to an increase in pH from 4.5 to 7.0 (Fig. 1). Evaluation of the pH changes in fruit showed that pH of the pericarp and mesocarp of unripe persimmon fruits was 5.1 ± 0.1 , and it increased to 5.6 ± 0.1 in mature fruit at commercial harvesting conditions. Fruit is resistant to fungal attack during the first two to three months of storage. Following storage for three months, fruit becomes susceptible to fungal attack, and pH in the pericarp rose to 5.8 ± 0.1 . An increase in pH was also observed in decaying mature persimmon fruit within 10 days of fruit inoculation with *A. alternata* (Fig. 2). These changes occurred parallel to an increase in ammonia content from 33.3 in healthy fruit to 162.7 μmol per l in decayed tissue (Fig. 2), suggesting that pH changes account for this ammonia increase.

A. alternata was found to modify pH not only in persimmon fruit but in several other fruits (Table 1). In tomato fruit, *A. al-*

ternata inoculation increased pH by almost 2 pH units, while in inoculated melon fruit, the increase in pH was only 1 unit. Accumulation of ammonia was detected in all the infected fruits, but while the accumulation increase in tomato was 3.5-fold, the increase in melon was 10-fold.

Effect of pH and glucose on *AaK1* expression and endoglucanase production.

A. alternata growing in the presence of persimmon CW raised the pH of the media from 3.8 to 6.0 after 144 h (Fig. 3A) and the amount of accumulated ammonia from 12,563 to 18,937 μmol per g of dry weight. Expression of *AaK1* from *A. alternata* and EG production increased dramatically as the pH of the media was raised (Fig. 3). In the presence of glucose, the pH of the media decreased from 3.8 to 2.5 (Fig. 3A) and ammonia concentration did not increase during that period (staying in the low range of 3,500 to 4,250 μmol per g). *AaK1* expression and EG production were completely repressed in the presence of glucose.

Effect of controlled pH on *AaK1* expression, endoglucanase production, and decay development by *A. alternata*.

Growth of *A. alternata* for 96 h in the presence of persimmon CW increased initial pH from 4.0 to 5.5 and induced *AaK1* expression and EG production (Fig. 4). Growth of *A. alternata* for 96 h in the presence of CW in citrate phosphate buffer at pH 6.0 increased the pH to 7.3 and induced higher *AaK1* expression than in the unbuffered treatment (Fig. 4A). In the presence of CW and phthalate sodium hydroxide buffer at pH 6.0, the pH increased to 6.4 after 96 h (Fig. 4A). *AaK1* expression was already observed 24 h after induction initiation and had further increased 72 h later. In the treatments buffered to pH 6.0, EG production was 130 to 190% higher than in the unbuffered treatment (Fig. 4B). When persimmon fruit was prick-inoculated with *A. alternata* in the presence of buffers at pH 6.0, decay development was enhanced throughout the incubation period at 20°C (Fig. 5).

Effect of glucose treatment under controlled pH on *AaK1* expression, endoglucanase production, and decay development by *A. alternata*.

Growth of *A. alternata* for 96 h in the presence of CW and glucose decreased pH from the initial 4.0 to 2.5, and *AaK1* expression and EG activity were repressed (Fig. 6). Growth of *A. alternata* for 96 h in the presence of CW and glucose media in citrate phosphate buffer or phthalate hydroxide buffer at pH 6.0 increased the pH to 6.8 and 6.2, respectively, and induced higher *AaK1* expression than the unbuffered treatment (Fig. 6A). During the first 24 h after induction initiation in the pres-

Fig. 1. Accumulation of ammonia in acidified yeast extract liquid medium during the growth of *Alternaria alternata*. Error bars indicate standard error.

ence of CW and glucose in phthalate hydroxide buffer, *A. alternata* showed *AaK1* expression, which was not found in the nonbuffered control. In media containing only CW, EG production was higher than in media with CW and glucose; however, under buffered conditions with a pH of 6.0, activity increased more than twofold (Fig. 6B).

Inoculation of *A. alternata* on persimmon fruit in the presence of glucose inhibited decay development by 40%, but decay development was restored and was further enhanced in the presence of glucose buffered to pH 6.0 (Fig. 7).

DISCUSSION

Host pH modulation by pathogens was recently suggested as an important factor for enhancing *Colletotrichum* sp. virulence during postharvest attack. Alkalinization of the ambient pH by secretion of ammonia enhanced the expression of *pelB* in *C. gloeosporioides* (Prusky et al. 2001). *A. alternata*, a broad-host-range phytopathogenic fungus, was also found to enhance the accumulation of ammonia and to increase pH in acidified yeast extract, CW-containing media, and several decayed fruits. This is of particular interest since, in unripe persimmon fruit, the pH of the cortical tissue is 5.1 and increases to 5.6 when mature fruits are harvested and, when decayed by *A. alternata*, pH may increase even further, up to 5.8. In *C. gloeosporioides*, local accumulation of ammonia enhanced the alkaline environment by increasing one pH unit, which enhanced the expression of *pelB* and its secretion (Yakoby et al. 2000). The increase in pH in decaying persimmon fruit was, however, smaller (only 0.2 pH units), suggesting that changes in pH levels are dependent on the initial pH of the host and the specific genes that the pathogen will activate to colonize that particular host. In apples, the pH during *Colletotrichum accutatum* attack increased from 3.9 to 6.5, while in avocado fruit with a higher

Fig. 2. pH changes and ammonia accumulation induced by *Alternaria alternata* on persimmon fruit. pH values represent direct pH measurements, and ammonia concentration was determined in the juice of the fruit. Measurements were taken 10 days after inoculation. Error bars indicate standard error.

Table 1. pH changes and ammonia concentrations induced by *Alternaria alternata* on fruit^a

Fruit	pH values ^b		Ammonia (μmoles)	
	Healthy	Decayed	Healthy	Decayed
Tomato	5.6	7.4 ^c	96.7	352.9 ^c
Pepper	5.4	7.6 ^c	56.1	482.7 ^c
Melon	6.3	7.5 ^c	150.0	1500.0 ^c

^a Fruit was wound inoculated with *A. alternata* spores (100 μl with 10^4 spores). Tomato fruit was from cv. 189, pepper from cv. Maor, and melon from cv. Galia.

^b pH values represent direct pH measurements taken 7 to 10 days after inoculation.

^c Significant difference compared to healthy tissue in the same fruit.

initial pH, the increase was only 0.5 pH units. In this case, the increase in pH in the tissue enhanced *pelB* expression (Prusky et al. 2001). Analysis of the EG *AaK1* from *A. alternata* indicated that pH can regulate enzyme levels through gene transcription of *AaK1*. Maximal expression is observed at pH levels higher than 6.0, values that are similar to those present in decayed tissues in which maximal virulence is observed. *A. alternata* was also able to increase ammonia accumulation and the pH level of infected tissue in tomato, pepper, and melon fruits with different initial pH levels. Interestingly, the extent of ammonia accumulation was not related to the pH increase, which suggests that it may depend on the buffer capacity of the tissue.

How can the pathogenicity of *A. alternata* in hosts with different environmental pH levels be explained? We envision that in polyphage pathogens, different genes encoding for pectolytic enzymes might be activated at different environmental pH levels. This could require the pathogen to use different enzymes out of the battery of enzymes available as virulent factors in each interaction (Eshel et al., in press). Endopolygalacturonase was reported to be essential for citrus black rot caused by *Alternaria citri* (Isshiki et al. 2001), while in our case, endoglucanase of *A. alternata* may affect virulence in persimmon fruit. This may explain the cases in which inactivation of a single pectolytic gene did not result in any discernible effect on the pathogenicity of the fungus on its host plant (Gao et al. 1996; Scott-Craig et al. 1998) and suggests that a clear charac-

terization of the genes expressed in vivo should be performed before proceeding to knock out their activity.

To date, many extracellular depolymerases in various combinations have been disrupted. Disruption of both the pectate lyase genes *pelA* and *pelD* in *Fusarium solani* f. sp. *pisi* resulted in mutants with a reduced pathogenicity on pea (Rogers et al. 2000). In other cases, however, even various combinations of disrupted genes did not affect reduced pathogenicity of *Cochliobolus carbonum* (Kim et al. 2001). However, disruption of regulatory protein kinase *ccSNF1* in *C. carbonum*, which is essential for expression of glucose-repressed genes, resulted in a strain with reduced depolymerase gene expression and strongly reduced virulence (Tonukari et al. 2000). Although not directly supporting a role for extracellular pectolytic enzymes in virulence, the results of Tonukari and associates (2000) suggested that down regulation by glucose affects pathogenicity. In the present work, *A. alternata* *AaK1* expression, EG production, and virulence were also repressed by glucose (Rohne 1995; Ruijter and Visser 1997). However, this repression was ac-

Fig. 3. Northern analysis of **A**, *AaK1* and **B**, endo-1,4- β -glucanase (EG) production by *Alternaria alternata* when grown on persimmon cell walls (CW) or glucose medium. After 5 days of growth on minimal medium (MM) containing 1% glucose, hyphae were washed and transferred to MM containing 1% persimmon CW or glucose. Experiments were repeated three times, and the presented results are those of one experiment. Similar results were obtained in the other experiments. Error bars indicate standard error.

Fig. 4. Effect of pH control on **A**, *AaK1* expression and **B**, endo-1,4- β -glucanase (EG) production by *Alternaria alternata* in the presence of persimmon cell walls (CW). After 5 days of growth on minimal medium (MM) containing 1% glucose, hyphae were washed and transferred to MM containing 1% persimmon CW. The pH was adjusted in the media by adding 4 ml of sterile 1 M citrate phosphate buffer (CPB) or 0.5 M phthalate sodium hydroxide buffer (PHB) at pH 6.0 to the 36 ml of media. NB = nonbuffered medium. Hyphae were subjected to RNA extraction at the indicated time points (24 and 96 h). EG production was assayed after 96 h as described in text. Experiments were repeated three times, and the presented results are those of one experiment. Similar results were obtained in the other experiments. Error bars indicate standard error.

accompanied by a significant decrease in pH from 3.8 to 2.5. Repression of pathogen virulence by glucose is probably due to the acidification of the fruit tissue, since buffer restored the virulence. This is not the first report of a pathogen decreasing the environmental pH in the presence of glucose. Acidification of glucose-amended media has been described in *Fusarium oxysporum* f. sp. *lini* (Brandao et al. 1992), *Penicillium cyclopium* (Ponitz and Werner 1994), and in yeast cells (Kotyk et al. 1999; Lapathitis and Kotyk 1998). Internal alkalization and external acidification, apparently resulting from H⁺-ATPase activity, are associated with the growth induction of yeast cells in glucose-containing media. Glucose causes an increase in the intercellular level of cAMP as well as H⁺-ATPase in *F. oxysporum* var. *lini* cells (Brandao et al. 1992). However, acidification of the media by *A. alternata* in the presence of glucose raises the question of whether or not the gene repression caused by glucose is a result of decreased environmental pH created during glucose metabolism. When *A. alternata* was grown in the presence CW and glucose at pH 6.0, significantly higher transcript expression, enzyme production, and virulence were observed. The present work suggests that ambient pH derepressed glucose repression in the modulation of *AaK1* expression. This is supported by early findings on *Botrytis cinerea* where *Bcpg* gene expression in the presence of glucose was also affected by the pH level (Wubben et al. 2000). This suggests that pH regulation of gene expression is a key factor in the expression of specific genes affecting virulence. In specific cases, the pathogen activates the mechanism of alkalization, and in others, the mechanism is acidification. In *Sclerotinia sclerotiorum*, the acidic environment is necessary for the activity of polygalacturonase (Rollins and Dickman 2001). In avocado fruit, modulation of pH occurs by the natural increase in host pH and the capability of *C. gloeosporioides* to secrete ammonia (Prusky et al. 2001). In the persimmon-*A. alternata* system the optimal pH level for EG activity, 5.5 to 6.0 (Eshel et al. 2000), is significantly higher than that of the acidified conditions caused by glucose. This pH regulation system ensures that extracellular enzymes and other exported metabolites are produced under the pH conditions at which they function best (Denison 2000). The cytoplasm and the vacuole of plant cells have buffering capacities, as well as the

Fig. 5. Effect of buffer treatment on symptom development by *Alternaria alternata* in persimmon fruit cv. Triumph. *A. alternata* spores were suspended in sterile ddH₂O (H₂O), sterile 0.1 M citrate phosphate buffer (CPB), pH 6.0, or sterile 0.05 M phthalate sodium hydroxide buffer (PHB), pH 6.0. Fruit was inoculated according to Eshel and associates (2000). In all treatments, 10 µl of the respective solution was added every 48 h to each pricked site. Error bars indicate standard error. Average values marked with different letters are significantly different according to one-way analysis of variance at $P \leq 0.05$.

ability to activate other regulatory systems to restore the optimal pH and buffering capacity of the cell (Guern et al. 1991). Such metabolic production or consumption of H⁺ outside the cytoplasm can be sensed as a pH signal by invading pathogens and can trigger gene expression and pathogenicity.

MATERIALS AND METHODS

Fungal cultures and plant material.

Persimmon fruit (*Diospyros kaki* L.) were used for the isolation and inoculation of *A. alternata*, the causal agent of black spot disease, as previously described (Eshel et al. 2000). Each treatment included 120 inoculations (30 fruits × 4 replicates). In all treatments, 10 µl of the respective solution was added every 48 h to each inoculation site. The inoculated fruits were incubated in a moist chamber at 20°C.

Persimmon fruit CW were prepared as previously described (Eshel et al. 2000). In short, flesh (300 g mesocarp) was ground in 350 ml of acid-methanol (4% HCl, vol/vol) and filtered through filter paper (No. 1; Whatman, Maidstone, England). The

Fig. 6. Effect of pH control on **A**, *AaK1* expression and **B**, endo-1,4-β-glucanase (EG) production by *Alternaria alternata* in the presence of persimmon cell walls (CW) and glucose (Glu). After 5 days of growth on minimal medium (MM) containing 1% glucose, hyphae were washed in sterile water and transferred to MM containing 1% persimmon CW with or without 1% glucose. The pH was adjusted in the media by adding 4 ml of sterile 1 M citrate phosphate buffer (CPB) or 0.5 M phthalate sodium hydroxide buffer (PHB) at pH 6.0 to the 36 ml of media. NB = non-buffered medium. Hyphae were subjected to RNA extraction at the indicated time points (24 and 96 h). EG production was assayed after 96 h. Experiments were repeated three times, and the presented results were obtained from one experiment. Similar results were obtained in the other experiments. Error bars indicate standard error.

residue was collected from the filter paper and blended twice more in a similar volume of methanol until no phenols could be detected in the filtrate. Phenols were detected as previously described (Eshel et al. 2000). The residue was blended with 200 ml of cold acetone three times and air-dried overnight before weighing. Air-dried residue was kept at -20°C until used as a carbon source in liquid media.

pH measurement of fruit.

pH was measured with a flat electrode (Sensorex; Stanton, CA, U.S.A.) according to the method described previously for avocado fruit (Yakoby et al. 2000). Pericarp pH levels were determined in an approx. 0.2-mm-deep cut that was made with a scalpel. Mesocarp pH levels were determined after peeling the pericarp to a depth of 2 mm. All measurements were repeated on 10 fruits at three different places (30 measurements) around the calyx of each fruit.

Detection of ammonia in the liquid media and in the tissue.

Ammonia concentration was determined in filtered culture medium or tissue homogenate. Spores from 10- to 20-day-old

cultures were harvested from M_3S medium (Tu 1985). Erlenmeyer flasks (125 ml) containing 40 ml liquid medium of 1% yeast extract (Sigma, St. Louis) at pH 4.0 were inoculated with *A. alternata* (1×10^6 spores per flask) and grown at 25°C on an orbital shaker (150 rpm).

Ammonia was measured with ammonia electrode model 95-12 (Orion, Beverly, MA, U.S.A.) in 0.1- to 1-ml aliquots (depending upon the concentration found) at pH 10.0, sampled at different times after inoculation. Different concentrations of NH_4Cl in a range of 7 to 700 μM were used as standards. All measurements were repeated on 10 fruits at three different places (30 measurements) around the calyx of each fruit.

RNA extraction.

For RNA extraction, the fungus was grown in 125-ml flasks for 5 days on liquid minimal medium (MM) (Mandels and Weber 1969) containing 1% glucose. Hyphae were washed twice in sterile water and then transferred to MM containing 1% persimmon CW or glucose.

Hyphae were subjected to RNA extraction at the indicated time points (24, 48, 96, and 144 h). The mycelia (0.2 g) were lyophilized, ground with a mortar and pestle under liquid nitrogen, transferred into 5 ml of extraction buffer [0.3 M Tris-HCl, pH 8.0; 90 mM LiCl; 4.5 mM EDTA; 1% sodium dodecyl sulfate (SDS); diethyl pyrocarbonate (DEPC)-treated water], and mixed well. Following homogenization, 3 ml of phenol was added, and the tube was vortexed for 1 min before centrifugation for 20 min at $1700 \times g$. The supernatant was collected, mixed with 5 ml of chloroform, vortexed for 1 min, and then centrifuged for 15 min at $1700 \times g$. The upper phase was collected again and mixed with 5 ml isopropanol, incubated at -20°C for at least 2 h, and then centrifuged for 15 min at $1700 \times g$. The pellet was resuspended in 5 ml of DEPC-treated water, mixed with $1/3$ volume of 8 M LiCl, and incubated at -20°C overnight. The pellet was resuspended in DEPC-treated water and precipitated with ethanol. RNA was quantified using GeneQuant (Pharmacia Biotech, Cambridge, U.K.).

Northern blot analyses.

Northern blot analysis was conducted by running 10 μg of total RNA on a 1.1% formaldehyde denaturing agarose gel (Sambrook et al. 1989). The RNA was blotted onto a Hybond+ nylon membrane (Amersham, Buckinghamshire, U.K.) by the capillary method (Sambrook et al. 1989), using $20\times$ SSC ($1\times$ SSC is 0.015 M NaCl plus 0.15 M sodium citrate). The RNA was fixed by baking for 2 h at 80°C and subjected to hybridization with full-length *AaKI* cDNA. All hybridizations and washes were carried out at 65°C , and the products were washed with $0.1\times$ SSPE ($1\times$ SSPE is 0.18 M NaCl, 10 mM NaPO_4 , and 1 mM EDTA, pH 7.7) plus 0.1% SDS. As a control for RNA loading, ethidium bromide stains are presented.

Enzyme production assay.

After freeze-drying, filtrates were dissolved in 2 ml of water, dialyzed overnight at 4°C against water, and freeze-dried. Prior to the assay, samples were resuspended in 1 ml of water. Reaction mixtures contained 1 to 8 μg total protein. Blanks always contained the same components, but incubation was at 4°C . Specific activities were expressed as units per milligram protein as measured by a dye-binding assay (Protein Assay Kit, Bio-Rad, Hercules, CA, U.S.A.) using crystalline bovine serum albumin as the reference standard.

Endoglucanase activity was assayed in 50-mM sodium acetate buffer, pH 5.5, containing 0.5% sodium carboxymethylcellulose (CMC) (Sigma). Activity was determined after 1 h of incubation at 47°C , described previously as optimum conditions (Eshel et al. 2000). The method was based on that of Somogyi

Fig. 7. Effect of glucose treatment under buffered conditions on symptom development caused by *Alternaria alternata* in persimmon fruit. *A. alternata* spores were suspended in either ddH₂O (H₂O), sterile 0.1 M citrate phosphate buffer (CPB), pH 6.0, or sterile 0.05 M phthalate sodium hydroxide buffer (PHB), pH 6.0, the last two with 1% glucose (Glu). **A**, Decay development after inoculation, and **B**, inoculated fruit. Error bars indicate standard error. Average values with different letters are significantly different according to one-way analysis of variance at $P \leq 0.05$.

(1952) and Nelson (1944). The product was analyzed by measuring the optical density at 660 nm. One unit of enzyme activity was defined as the amount of enzyme hydrolyzing 0.5% CMC to produce reducing sugar equivalent to 1 μ mol of D-glucose per minute under standard assay conditions.

ACKNOWLEDGMENTS

This research was supported by research grants to D. P. from the United States-Binational Agricultural Research and Development Fund (BARD) and the U.S.-Israel Cooperative Development Research (CDR) Program. D. E. and I. M. were granted personal scholarships from the Israel Fruit Marketing Board.

LITERATURE CITED

- Annis, L. S., and Goodwin H. P. 1997. Recent advances in the molecular genetics of plant cell wall-degrading enzymes produced by plant pathogenic fungi. *Eur. J. Plant Pathology* 103:1-14.
- Brandao, R. L., Castro, I. M., Passos, J. B., Nicoli, J. R., and Thevelein, J. M. 1992. Glucose-induced activation of the plasma membrane H⁺-ATPase in *Fusarium oxysporum*. *J. Gen. Microbiol.* 138:1579-1586.
- Condemine, G., and Robert-Baudouy, J. 1995. Synthesis and secretion of *Erwinia chrysanthemi* virulence factors are coregulated. *Mol. Plant-Microbe Interact.* 8:632-636.
- Dean, A. R., and Timberlake, E. W. 1989. Regulation of *Aspergillus nidulans* pectate lyase gene (*pelA*). *Plant Cell* 1:275-284.
- Denison, S. H. 2000. pH regulation of gene expression in fungi. *Fungal Genet. Biol.* 29:61-71.
- Eshel, D., Ben-Arie, R., Dinoor, A., and Prusky, D. 2000. Resistance of gibberellin-treated persimmon fruit to *Alternaria alternata* arises from the reduced ability of the fungus to produce endo-1,4- β -glucanase. *Phytopathology* 90:1256-1262.
- Eshel, D., Lichter, A., Dinoor, A. and Prusky, D. Characterization of *Alternaria alternata* glucanase genes expressed during infection of resistant and susceptible persimmon fruits. *Molecular Plant Pathology*, In press.
- Gao, S., Choi, G. H., Shain, L., and Nuss, D. L. 1996. Cloning and targeted disruption of *enpg-1*, encoding the major in vitro extracellular endopolygalacturonase of the chestnut blight fungus, *Cryphonectria parasitica*. *Appl. Environ. Microbiol.* 62: 1984-1990.
- Guern, J., Felle, H., Matthieu, Y., and Kurkdjian, A. 1991. Regulation of intracellular pH in plant cells. *Int. Rev. Cytol.* 127:111-173.
- Isshiki, A., Akimitsu, K., Yamamoto, M., and Yamamoto, H. 2001. Endopolygalacturonase is essential for citrus black rot caused by *Alternaria citri* but not brown spot caused by *Alternaria alternata*. *Mol. Plant-Microbe Interact.* 14:749-757.
- Kim H., Ahn, J.-H., Görlach, J. M., Caprari, C., Scott-Craig, J. S., and Walton, J. D. 2001. Mutational analysis of β -glucanase genes from the plant-pathogenic fungus *Cochliobolus carbonum*. *Mol. Plant Microbe Interact.* 14:1436-1443.
- Kotyk, A., Lapathitis, G., and Krenkova, S. 1999. Glucose- and K(+)-induced acidification in different yeast species. *Folia Microbiol.* 44:295-298.
- Lapathitis, G., and Kotyk, A. 1998. Different sources of acidity in glucose-elicited extracellular acidification in the yeast *Saccharomyces cerevisiae*. *Biochem. Mol. Biol. Int.* 46:973-978.
- MacKenzie, A. D., Jeenes, D. J., Belshaw, N. J., and Archer B. D. 1993. Regulation of secreted protein production by filamentous fungi: Recent developments and perspectives. *J. Gen. Microbiol.* 139:2295-2307.
- Mandels, M., and Weber, J. 1969. The production of cellulases. Pages 391-413 in: *Cellulases and Their Applications*, Advances in Chemistry, Series 95. E. J. Hajny and E. T. Reese, eds. American Chemical Society, Washington, D.C.
- Nelson, N. 1944. A photometric adaptation of the Somogyi method for the determination of glucose. *J. Biol. Chem.* 153:375-380.
- Ponitz, J., and Werner, R. 1994. A glucose-activated electron transfer system in the plasma membrane stimulates the H⁺-ATPase in *Penicillium cyclopium*. *J. Bacteriol.* 176:5429-5438.
- Prusky, D., Ben-Arie, R., and Guelfat-Reich, S. 1981. Etiology of black spot disease caused by *Alternaria alternata* in persimmon fruits. *Phytopathology* 71:1124-1128.
- Prusky, D., McEvoy, J. L., Leverentz, B., and Conway, L. S. 2001. Local modulation of host pH by *Colletotrichum* species as a mechanism to increase virulence. *Mol. Plant Microbe Interact.* 14: 1105-1113.
- Rogers, L. M., Kim, Y.-K., Guo, W., Gonzales-Candelas, L., Li, D., and Kolattukudy, P.E. 2000. Requirement for either a host- or pectin-induced pectate lyase for infection of *Pisum sativum* by *Nectria hematococca*. *Proc. Nat. Acad. Sci. U.S.A.* 97:9813-9818.
- Rollins, J. A., and Dickman, M. B. 2001. pH signaling in *Sclerotinia sclerotiorum*: Identification of *pacC*/RIM1 homolog. *Appl. Environ. Microbiol.* 67:75-81.
- Ronne, H. 1995. Glucose repression in fungi. *Trends Genet.* 11:12-17.
- Ruijter, G. J. G., and Visser, J. 1997. Carbon repression in *Aspergilli*. *FEMS (Fed. Eur. Microbiol. Soc.) Lett.* 151:103-114.
- Sambrook, J., Fritsch, E. F., and Maniatis, T. 1989. *Molecular Cloning: A Laboratory Manual*. 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, U.S.A.
- Scott-Craig, J. S., Cheng, Y.-Q., Cervone, F., DeLorenzo, G., Pitkin, J. W., and Walton, J. D. 1998. Targeted mutants of *Cochliobolus carbonum* lacking the two major extracellular polygalacturonases. *Appl. Environ. Microbiol.* 64:1497-1503.
- Somogyi, M. 1952. Notes on sugar determination. *J. Biol. Chem.* 195:19-23.
- St. Leger, R. J., Joshi, L., and Roberts, D. 1998. Ambient pH is a major determinant in the expression of cuticle-degrading enzymes and hydrophobin by *Metarhizium anisopliae*. *Appl. Environ. Microbiol.* 64:709-713.
- Tonukari, N. J., Scott-Craig, J. S., and Walton, J. D. 2000. The *Cochliobolus carbonum* SNF1 gene is required for cell wall-degrading enzyme expression and virulence on maize. *Plant Cell* 12:237-248.
- Tu, J. C. 1985. An improved Mathur's medium for growth, sporulation and germination of spores of *Colletotrichum lindemuthianum*. *Microbios* 44:87-93.
- Walton, J. D. 1994. Deconstructing the cell wall. *Plant Physiol.* 104:1113-1118.
- Wubben, J. P., ten Haven, A., van Kan J. A. L. and Visser, J. 2000. Regulation of endopolygalacturonase gene expression in *Botrytis cinerea* by galacturonic acid, ambient pH and carbon catabolite repression. *Curr. Genet* 37:152-157
- Yakoby, N., Kobiler, I., Dinoor, A., and Prusky, D. 2000. pH regulation of pectate lyase secretion modulates the attack of *Colletotrichum gloeosporioides* on avocado fruit. *Appl. Environ. Microbiol.* 66:1026-1030.