Green Fluorescent Detection of Fungal Colonization and Endopolygalacturonase Gene Expression in the Interaction of *Alternaria citri* with Citrus

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ABSTRACT

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Alternaria citri, a postharvest pathogen, produces endopolygalacturonase (endoPG) and causes black rot on citrus fruit. We previously described that an endoPG-disrupted mutant of *Alternaria citri* was significantly reduced in its ability to macerate plant tissue and cause black rot symptoms on citrus. In order to investigate colonization of citrus fruit tissues by *Alternaria citri*, pTEFEGFP carrying a green fluorescent protein (GFP) gene was introduced into wild-type *Alternaria*

citri and its endoPG-disrupted mutant (M60). Green fluorescence was observed in spores, germ tubes, appressoria, and infection hyphae of transformants G1 (derived from wild type) and GM4 (derived from M60). Hyphae of G1 but not GM4 vertically penetrated the peel, but the hyphae of both G1 and GM4 spread equally in the juice sac area of citrus fruit. Green fluorescence of *Alternaria citri* transformant EPG7 carrying a GFP gene under control of the endoPG gene promoter of *Alternaria citri* was induced by pectin in the peel during the infection stage, but repressed completely in the juice sac area, likely by carbon catabolite repression by sugars in the juice.

Additional keywords: cell wall-degrading enzyme, pectinase.

Alternaria citri Ellis & N. Pierce, causes Alternaria black rot, a postharvest fruit disease, on a broad range of citrus cultivars (4,5). *Alternaria citri* infects at the stem end of the fruit and causes internal black discoloration and maceration of the fruit core during storage (4,5).

Fungal pathogens including *Alternaria citri* make extracellular enzymes that can degrade cell wall polymers during the infection stage. Cell wall-degrading enzymes and their genes have been studied for their possible role in pathogenicity, including penetration, maceration, nutrient acquisition, plant defense induction, and symptom expression (7,8,33). In previous work, we purified endopolygalacturonases (endoPG, EC 3.2.1.15) that can degrade pectic polysaccharides in plant cell walls (16) and cloned their genes from two different species of *Alternaria*: one (*AcpG1*) from the Alternaria black rot pathogen *Alternaria citri* and the other (*AapG1*) from the citrus brown spot pathogen *Alternaria alternata* rough lemon pathotype (17). The sequences of these genes and biochemical characteristics of the enzymes they encode are highly similar. However, when we disrupted these genes in the respective pathogens by gene targeting, the phenotypes of the mutants were completely different (16,17). An endoPG mutant of *Alternaria citri* was significantly reduced in its ability to cause black rot symptoms on citrus as well as in the maceration of potato tissue and could not colonize citrus peel segments (17). In contrast, an endoPG mutant of *Alternaria alternata* was unchanged in pathogenicity (17). These results indicate that an endoPG could play different roles in the pathogenicity of different plant-pathogenic fungi. The role of endoPG depends on the type of disease but not the taxonomy of the fungus. The importance of *Alternaria citri*

endoPG in development of disease symptoms (black discoloration and maceration in citrus fruit) has been established. However, the role of endoPG in fungal colonization of citrus tissues as well as the spatial and temporal regulation of endoPG gene expression in different tissues during infection needs to be elucidated.

In this study, we monitored the colonization of an *Alternaria citri* mutant expressing a gene encoding green fluorescent protein (GFP) from the jellyfish *Aequorea victoria* (6) in citrus tissues. The gene encoding GFP has been successfully expressed and used as a vital marker for growth of several phytopathogenic fungi in their host plants, including *Ustilago maydis* (29), *Cochliobolus heterostrophus* (21), *Colletotrichum lindemuthianum* (13), *Mycosphaerella graminicola* (28), *Fusarium oxysporum* (24), and *Magnaporthe grisea* (2). We also analyzed the pattern of endoPG gene expression in different tissues in citrus fruit by using the GFP gene under the control of the *AcpG1* promoter.

MATERIALS AND METHODS

Fungal cultures and enzyme assay. *Alternaria citri* (strain ATCC 58171) (9) and its endoPG-disrupted mutant M60 (17) were grown on potato dextrose agar (PDA) or V8-juice agar. PG activity was measured by a relative viscosity assay as described previously (17).

Nucleic acid manipulations. *Alternaria citri* wild type and its mutant strains were grown in 50 ml of potato dextrose broth (PDB) in 200-ml Erlenmeyer flasks at 28°C for 2 to 3 days with shaking (120 rpm). Genomic DNA was isolated from mycelia grown in PDB as described previously (16,17,22). Total RNA was isolated, according to the methods of Masunaka et al. (22), from mycelia grown at 24°C for 25 days in Richard's medium (10 g of KNO₃, 5 g of KH₂PO₄, 2.5 g of MgSO₄, 0.02 g of FeCl₃, and 0.005 g of ZnSO₄ each per liter) containing combinations of 1% (wt/vol) pectin and different concentrations of glucose (0.001, 0.01, 0.1, 1, and 2% [wt/vol]).

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For blot analysis, an internal 608-bp region of cEGFP (originally from jellyfish *Aequorea victoria*) from pTEFEGFP (32) was amplified by polymerase chain reaction (PCR), and the amplified partial cEGFP was labeled for Southern blots by the digoxigenin (DIG) system (Boehringer Mannheim, Mannheim, Germany). The same fragment was also used for DIG RNA probe preparation for northern blots as described previously (17). PCR was performed in a DNA thermal cycler (Perkin-Elmer, Wellesley, MA) as described previously (17). The primers used were gfp1 (5'-AGCTGGACGCGACGTAA-3') and gfp2 (5'-GACCATGTGATCGCGCTT-3'). Restriction enzyme digestion, hybridization, and detection conditions were performed as described previously (16,17,22).

Plasmid construct preparation. GFP vector pTEFEGFP containing cEGFP under the control of *Aureobasidium pullulans* TEF promoter (32) was provided by D. Cullen, A. Van den Wymelenberg, and J. Andrews (University of Wisconsin). Plasmid pPGGFP11, which contains the cEGFP gene fused to the putative *Acp1* promoter region (Accession no. AB101001) of 813 bp located upstream of the initiation codon of *Acp1* (Accession no. AB047543), was constructed. The 813-bp *Acp1* promoter fragment was fused with the cEGFP gene by introducing *EcoRI* sites at the 3' terminus of the *Acp1* region and 5'-terminal of the cEGFP gene. The 813-bp fragment of *Acp1* was amplified from genomic fragment in pPGNCG1 containing the 813-bp region and entire open reading frame of *Acp1* by a pair of PCR primers, PG20 (5'-CAGATGTGGCGTTTTCGC-3') and Pro2 (5'-GGGAATTCTGTGAAAGATGC-3'). The cEGFP gene with the *gla* terminator of *Aspergillus awamori* was amplified from pTEFEGFP (32) by PCR primers Pro1 (5'-GGGAATTCATGTTGAGCAAG-3') and P6 (5'-CGCGGCCGCGGATCCCTG-3'). These two PCR products were digested with *EcoRI* and then separated in low-melting-point agarose. The purified PCR products were ligated and reamplified by PCR using a set of primers PG20 and P6. The amplified fusion fragment was subcloned into pT7Blue-2 vector (Novagen, Madison, WI), and the resulting plasmid was named pPGGFP11.

Fungal transformation. Protoplast preparation and polyethylene glycol transformation of *Alternaria citri* were performed as described previously (17). Plasmid pTEFEGFP was introduced by cotransformation into *Alternaria citri* wild type with the plasmid pSH75 (19), which contains a cassette conferring hygromycin resistance. pTEFEGFP was also introduced into the endoPG-disrupted mutant of *Alternaria citri* M60 (already expressing hygromycin resistance) with the plasmid pII99 (30), which contains a cassette conferring geneticin resistance. Plasmid pPGGFP11 was introduced into *Alternaria citri* wild type with pSH75. The colonies growing on the selective medium were transferred to PDA containing 50 µg of hygromycin B per ml or 100 µg of geneticin per ml and purified to nuclear homogeneity by single-spore isolation as described previously (17).

Fungal inoculations. A spore suspension (10^6 spores per ml) was placed under the pedicels of surface-sterilized citrus fruits or injected directly into the central axis of sterilized fruits of sweet orange (*Citrus sinensis*) cv. Valencia and Washington navel oranges (*Citrus sinensis* (L.) Osbeck) as described previously (17). The citrus fruits were sterilized by soaking for 1 to 3 h in 1% (vol/vol) sodium hypochlorite, rinsed in sterilized water, and incubated after the inoculation in a sealed sterilized plastic box at 24°C for 3 weeks.

A spore suspension (10^6 spores per ml) was also placed on cellulose membrane on a glass slide in a moist chamber or 1% agar plates (wt/vol) with or without different concentrations of various types of carbon sources and incubated at 24°C for various periods.

Detection of green fluorescence. GFP expressed in transformant cells was detected with an Olympus IMT2-RFL microscope (Olympus, Tokyo) or an Olympus BX60 microscope with a B

excitation filter. GFP expression in infected citrus peels was detected by an Olympus IMT2-RFL microscope or irradiation with a hand-held long-wave (365 nm) UV source. For examination of development of fungal hyphae in citrus fruits, a spore suspension (10^6 spores per ml) of G1 or GM4 was injected directly into the central axis of citrus fruits (Washington navel orange or sweet orange cv. Valencia), which were incubated for 3 weeks after inoculation. A total of 120 cross sections (Fig. 1I and J) at a range of distances (0 to 20 mm) from the edge of macerated area from 60 fruits were observed under microscope. The percentage of area occupied in a given field by fungal hyphae (Fig. 2) was determined by the area of green fluorescence from the hyphae using the MacSCOPE program, version 2.32 (Mitani Corporation, Tokyo). For examination of the regulation of the endoPG gene promoter, green fluorescence from germinating spores of transformant EPG7 was determined on agar plates containing either 1% (wt/vol) pectin or polygalacturonic acid (PGA) with or without addition of different concentrations of glucose, sucrose, or fructose. Activation or repression of the endoPG gene promoter was determined after 24 h incubation at 24°C by detection of the percent area of green fluorescence from fungal hyphae occupied in a given field using the MacSCOPE program. Ten different fields were observed for each treatment, and the experiments were repeated independently at least 10 times.

RESULTS

Characterization of *Alternaria citri* transformants expressing the GFP gene. To investigate the colonization of citrus fruit by *Alternaria citri* and the role of endoPG in this process, plasmid pTEFEGFP carrying the GFP gene (32) was introduced into *Alternaria citri* wild type and its endoPG-disrupted mutant (M60) (17) by transformation. Transformants G1 and GM4 were selected for further study and their GFP genotype were confirmed by genomic Southern blot analysis using the GFP gene as a probe (data not shown) as well as by exhibition of green fluorescence in germinating spores on cellulose membranes (Fig. 1A and B). Ten of thirteen hygromycin-resistant transformants derived from wild type and all of 11 geneticin-resistant transformants derived from M60 showed a GFP signal. The cultural morphologies of the transformants expressing the GFP gene were unchanged compared with the corresponding original strains. These transformants derived from the wild type and M60 sporulated and grew well on minimal medium and no differences were found in spore germination, appressorium formation, or infection hypha formation.

Green fluorescent detection during fungal colonization of citrus by G1 and GM4. Transformants G1 and GM4 exhibited strong green fluorescence in germinating spores of the various morphologies typical of this species on cellulose membranes (Fig. 1A and B) but no fluorescence background was detected in the wild-type strain (data not shown). Green fluorescence was not detected in the cell walls of G1 spores (Fig. 1B). A droplet of spore suspension of G1 or GM4 was placed under the pedicels of surface-sterilized navel orange fruit. After 2 weeks incubation, although the inoculation sites of G1 and GM4 were not distinguishable by optical observation (Fig. 1C and D), hyphae of G1, but not GM4, vertically penetrated the peel (Fig. 1E to H) as detected by green fluorescence. Similar results were obtained when G1 or GM4 was inoculated into fruits of sweet orange cv. Valencia (data not shown).

Spore suspensions were also injected directly into the central axis of navel orange fruit. Although germ tubes of G1 spread faster and in larger amounts than that of GM4 (Fig. 2), germ tubes of both G1 and GM4 spread in the juice sac area (Fig. 1I and J). Despite the growth of G1 and GM4 in the juice sac area, development of rotting in the inoculated area differed as described previously (17). G1 caused severe rotting, whereas GM4 caused rotting only at the injected area.

Characterization of *Acpg1* promoter using the GFP gene as the reporter gene. Plasmid pGGFP11 containing the GFP gene fused with the 813-bp region (Accession no. AB101001) located upstream of the initiation codon of *Acpg1* was introduced into *Alternaria citri* wild type to express the GFP gene in citrus fruit

Fig. 1. Green fluorescence detection of transformants G1 and GM4 in citrus tissue. **A**, Optical observation of germinating spores of G1 on cellulose membrane. **B**, Green fluorescence detection of G1 with B excitation filter. **A and B**, Scale bars represent 30 μ m. **C and D**, Cross sections 2 weeks after inoculation with spore suspensions (10^6 spores per ml) of **C**, G1 and **D**, GM4 under the pedicel of navel orange fruit. **E and F**, Fluorescence of **E**, G1 and **F**, GM4 in peel of the inoculated fruit as detected by hand-held long-wave (365 nm) UV source. White arrows indicate the sites of fluorescence. **G**, Green fluorescence from hyphae of G1 in peel of navel orange was detected with an Olympus IMT2-RFL microscope (Olympus, Tokyo) with B excitation filter. **H**, Green fluorescence of GM4 in peel of navel orange was not detected with an Olympus IMT2-RFL microscope with B excitation filter. **G and H**, Scale bars represent 40 μ m. **I and J**, Fungal hyphae of **I**, G1 and **J**, GM4 developing in juice sac area were observed with an Olympus IMT2-RFL microscope with B excitation filter. **I and J**, Cross section in the area of 0 to 5 mm from the edge of macerated area 3 weeks after inoculation by injection into the central axis of navel orange fruit. **I and J**, Scale bars represent 80 μ m. All experiments were repeated at least 10 times with similar results.

under control of the putative promoter region of *Acpg1*. Transformants containing pGGFP11 were selected for hygromycin resistance and screened by PCR using a primer combination for GFP gene amplification (data not shown).

The green fluorescence of EPG7, which contains the GFP gene fused to the putative *Acpg1* promoter region, was detected in germinating spores on agar medium containing 1% (wt/vol) pectin as a sole carbon source after 24 h incubation at 24°C (Fig. 3A). Similar fluorescence was also detected on agar medium containing 1% (wt/vol) PGA, although this fluorescence was not detected in germinating spores on agar medium without any carbon source added (Fig. 4) or with 1% (wt/vol) cellulose as the carbon source (data not shown). The green fluorescence was detectable after 8 h incubation with pectin and after 6 h incubation with PGA at 24°C. Green fluorescence was not induced on pectin media containing 1% (wt/vol) or higher concentrations of glucose (Figs. 3B and 4).

A droplet of spore suspension of EPG7 was placed under the pedicel or injected directly into the central axis of citrus fruit (sweet orange cv. Valencia). The green fluorescence of EPG7 fungal hyphae was detected in peel (Fig. 3C) but not in the juice sac area (Fig. 3D). Similar results were obtained when EPG7 was inoculated into the fruits of navel orange (data not shown). To investigate the possibility that the repression of *Acpg1* promoter in the juice sac area was due to carbon catabolite repression by sugar sources in the juice, spore suspensions of EPG7 were placed on pectin or PGA plates containing 2% (wt/vol) fructose or sucrose, which are major sugars in citrus juice together with glucose (23,31). Green fluorescence of germinating spores was not detected on the plates with fructose or sucrose (Fig. 5), as well as on the plate with glucose (Fig. 4).

Carbon catabolite repression in *Alternaria citri*. Carbon catabolite repression of gene expression of *Acpg1* and enzyme

Range of distance from the edge of maceration

Fig. 2. Colonization of transformants G1 and GM4 in juice sac area of citrus fruits. A spore suspension (10^6 spores per ml) of G1 or GM4 was injected directly into the central axis of citrus fruits (Washington navel orange), which were incubated for 3 weeks after inoculation. A total of 120 cross sections (Fig. 1I and J) for each range of distance from the edge of macerated area from 60 fruits were observed under microscope. The percentage of area occupied in a given field by fungal hyphae was determined by the area of green fluorescence in each range; 1, 0 to 5 mm; 2, 5 to 10 mm; 3, 11 to 15 mm; and 4, 16 to 20 mm from the edge of macerated area using MacSCOPE program, version 2.32 (Mitani Corporation, Tokyo). Each column indicates an average of independent repeats with a vertical bar representing the standard deviation of at least 10 replicates.

synthesis of endoPG was examined using a wild-type strain of *Alternaria citri*. *Alternaria citri* was cultured in liquid medium containing 1% (wt/vol) pectin with different concentrations of glucose (0.001, 0.01, 0.1, 1, and 2% [wt/vol]) for 25 days, and the culture filtrate and mycelial mat were collected for detection of endoPG activity and *Acpgl* gene expression. EndoPG activity and accumulation of *Acpgl* transcripts were repressed when 1 or 2% (wt/vol) glucose was added to the pectin medium (Fig. 6A and B).

DISCUSSION

Expression of GFP in *Alternaria citri* provided a valuable tool for visualization and measurement of growth and endoPG expression during citrus tissue colonization. Green fluorescence was observed in the various morphologies typical of germinating spores of *Alternaria citri* but not in the cell walls of spores from strains G1 and GM4 transformed with the GFP gene. This result may suggest that the GFP accumulated in cytoplasm of *Alternaria citri* cells. GFP probably does not interfere with any major physiological pathways of this fungus because phenotypes of these transformants were unchanged and GFP appeared stable even after multiple subcultures on different types of media with or without hygromycin.

Fungal hyphae of G1 derived from wild-type *Alternaria citri* penetrated vertically from citrus pedicels to the peel, whereas those of GM4, derived from an endoPG-disrupted mutant of *Alternaria citri*, could not penetrate the tissues. The penetration of G1 into citrus fruit was limited to the pectin-rich part of peel and did not pass through the cuticle and cell wall layers. Interestingly, hyphae of G1 never developed on the surface of the fruit (data not shown). This observation fits well to the characteristics of this pathogen. *Alternaria citri* infects internal tissues of the fruit and causes black discoloration and maceration, but unlike other storage pathogens of citrus, it never causes symptoms over the surface of the fruits (4,5). In contrast, other storage pathogens of citrus including *Penicillium digitatum* or *P. italicum* colonize the surface of citrus fruit, and the infected fruit soon becomes covered with green or blue mold, respectively (14). Garber et al. (15) proposed the importance of pectinase in rotting symptoms caused by these pathogens. However, Barkai-Golan and Karadavid (3) suggested that cellulases play a more important role in the early stages of

pathogenesis. It is unclear why *Alternaria citri* is restricted to internal tissues and cannot colonize or cause symptoms on the surface of citrus fruits. However, we have established that endoPG plays an essential role for penetration from pedicel to the internal part of the fruit through the peel because hyphae of the endoPG-disrupted mutant were never observed in the peel. This observation, made possible by visualization of hyphae expressing GFP, correlates well with our previously published gene disruption results. When *Alternaria citri* or its endoPG-disrupted mutant (M60) was inoculated at the pedicels of sterilized citrus fruits, the wild type caused rotting, mainly in the central axis of the inoculated fruit, and the fungus spread into the juice sac area through the vascular bundles (17). However, growth of M60 was restricted to the inoculation site, and rotting never developed in the central axis or juice sac area (17).

When the fungi were aided in breaching the peel barrier by injecting spore suspensions directly into the central axis of citrus fruits, both wild type (G1) and endoPG-disruptant (GM4) grew and colonized the juice sac area. Colonization of G1 (derived from wild type) was faster than that of GM4 (derived from endoPG-disrupted mutant), and this is likely because the tissue between the juice sac area and the central axis contains pectin. However, upon reaching the juice sac area (which is rich in simple sugars), the

Fig. 3. Green fluorescence detection of transformant EPG7. Green fluorescence of transformant EPG7 detected in germinating spores on agar plates containing 1% (wt/vol) pectin **A**, without 1% (wt/vol) glucose or **B**, with 1% (wt/vol) glucose. **A and B**, Scale bars represent 50 μ m. **C**, Green fluorescence of EPG7 fungal hyphae in peel of sweet orange fruit cv. Valencia was detected by an Olympus BX60 microscope with B excitation filter (Mitani Corporation, Tokyo). **D**, No green fluorescence from EPG7 fungal hyphae was detected in juice sac area of the sweet orange fruit. **C and D**, Scale bars represent 80 μ m. All experiments were independently repeated at least 10 times with similar results.

Fig. 4. Activation and repression of the endopolygalacturonase (endoPG) gene promoter by various carbon sources. Green fluorescence of germinating spores of transformant EPG7 after 24 h incubation at 24°C was detected on agar plates containing either 1% (wt/vol) pectin or polygalacturonic acid (PGA) with or without addition of different concentrations of glucose. Activation and repression of the endoPG gene promoter was determined by the percent area of green fluorescence from fungal hyphae occupied in a given field using MacSCOPE program, version 2.32 (Mitani Corporation, Tokyo). Each column indicates an average of 10 independent repeats of 10 observations for one treatment, with a vertical bar representing the standard deviation of replicates. No Glucose indicates agar plates containing pectin or PGA alone. No carbon sources indicates a simple agar plate without any carbon source. Pectin WT indicates *Alternaria citri* wild type on agar plate with 1% (wt/vol) pectin alone.

endoPG-disrupted mutant grew as well as the wild type. This result indicates that endoPG is essential for rotting (17) and penetration through the central axis from the pedicel during the infection stage of *Alternaria citri*, but endoPG is not important for fungal colonization of the juice sac area. Results from studies of GFP under the control of the *Acpgl* promoter indicate that expression of the endoPG gene is inducible by substrates like pectin and PGA but is repressed in the juice sac area, possibly by carbon catabolite repression by sugars in the juice. The major sugars in citrus juice are glucose, fructose, and sucrose, and the compositions of these sugars in juice are at concentrations of 5 to 10% (23,31). These concentrations of sugars are likely high enough to repress the endoPG gene expression in juice area by carbon catabolite repression. In fact, our results showed that these sugars, at a concentration of 2%, repressed the endoPG gene expression on pectin plates, and the green fluorescence of EPG7 carrying a GFP gene under control of endoPG promoter was probably induced by pectin in the peel during infection stage but repressed completely in the juice sac area. These sugars, instead of pectin, in the juice sac area might also be used as a major nutrient source for fungal growth.

Our results revealed that the 813-bp upstream fragment of the endoPG gene is functional as the promoter with a possible involvement of regulatory elements for induction and repression of the gene expression by various carbon sources. Pectin or PGA induced green fluorescence of EPG7 but neither glucose nor cellulose in agar plates induced green fluorescence of EPG7. No fluorescence was induced by combinations of pectin and glucose,

fructose, or sucrose. The green fluorescence of EPG7 induced by PGA was detected 2 h faster than that induced by pectin. This result may reflect the difference in structures of pectin and PGA. Pectin consists primarily of partially methylated PGA residues containing minor amounts of neutral sugars such as arabinose and galactose. In contrast, PGA does not contain methyl groups and monosaccharides. Thus, the degradation of PGA by endoPG, as well as the uptake of its degraded products, is likely to occur faster than that of pectin.

The process of one compound preventing the utilization of a second compound is called catabolite repression and generally results in the repression of a range of genes when readily utilized carbon sources such as glucose are present in the medium for microorganisms. Carbon catabolite repression of fungal PGs was reported for *F. oxysporum* f. sp. *lycopersici* (25), *Aspergillus niger* (20), *Aspergillus nidulans* (1), *Cochliobolus carbonum* race 1 (34), and *P. expansum* (26). *Alternaria citri* also showed the typical features of carbon catabolite repression. Although the mechanisms of fungal carbon catabolite repression are not well elucidated, *creA*, which encodes a negative regulator of carbon catabolite repression, was cloned from *Aspergillus nidulans* (11,12). The product of this gene, CREA, has a zinc finger domain and its binding site (5'-SYGGRG-3') was identified in the DNA region between the *prnD* and *prnB* genes of the proline degradation cluster of *Aspergillus nidulans* (10). Similar CREA binding sites

Fig. 5. Carbon catabolite repression of transformant EPG7 by sucrose and fructose. Green fluorescence of germinating spores of transformant EPG7 after 24 h incubation at 24°C was detected on agar plate containing either 1% (wt/vol) pectin or polygalacturonic acid (PGA) with or without addition of 2% (wt/vol) sucrose and fructose. None indicates agar plates containing 1% (wt/vol) pectin or PGA alone.

Fig. 6. Carbon catabolite repression of endopolygalacturonase (endoPG) synthesis in the wild-type strain of *Alternaria citri*. **A**, Production of endoPG by *Alternaria citri* wild type in 25-day-old culture in Richard's medium containing 1% (wt/vol) pectin with different concentrations of glucose. PG activity was determined by a relative viscosity assay. **B**, Northern blot analysis of endoPG gene expression in mycelial mats of 25-day-old cultures in Richard's medium containing 1% (wt/vol) pectin with different concentrations of glucose. Equality of RNA loading in each lane was estimated by rRNA stained with methylene blue. The number on the left indicates the position of RNA size markers in kilobases.

were found in the promoter regions of *pgxA* encoding exoPG from *Aspergillus tubingensis* (18) and *pgI* from *Sclerotinia sclerotiorum* (27). In the *AcpgI* promoter region (Accession no. AB101001), several putative CREA binding sites are present at positions -84, -406, -494, -562, and -798 from the initiation codon, suggesting that a regulatory system similar to that involving CREA may regulate *AcpgI* transcription under carbon catabolite repression.

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