Glyoxysomal Nature of Microbodies Complexed with Lipid Globules in *Botryosphaeria dothidea*

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ABSTRACT

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The glyoxysomal nature of microbodies was determined in *Botryosphaeria dothidea* hyphae based on morphology and in situ enzyme characteristics by transmission electron microscopy and cytochemistry. Bound by a single membrane, microbodies had a homogeneous matrix and varied in size ranging from 200 to 400 nm in diameter. Microbodies often had crystalline inclusions that consisted of parallel arrays of fine tubules in their matrices. Microbodies and lipid globules were placed in close association with each other, forming microbody-lipid globule complexes in hyphae. The cytochemical activities of catalase and malate

synthase were localized in microbodies, showing intense electron density of the organelle. In addition, immunogold labeling detected the presence of catalase in a multivesicular body-like organelle and the cell wall as well as in the matrix and crystalline inclusion of microbodies, supporting the enzyme secretion outward. Meanwhile, isocitrate lyase was localized only in matrices of microbodies. These results suggest that the microbodies complexed with lipid globules in *B. dothidea* hyphae are functionally defined as glyoxysomes which may enable the fungus to survive latent periods using lipids via the glyoxylate cycle and catalase secretion.

Additional keywords: active oxygen species, *Fusicoccum aesculi*, latent infection, peroxisomes, white rot.

Microbodies are morphologically defined organelles that are bound by a single membrane and have a homogeneous granular matrix in eukaryotic cells (16,20). They often contain inclusions in the matrix and are topographically associated with other organelles. Based on their functions, microbodies in higher plants are usually classified into two groups: (i) peroxisomes for photorespiration in photosynthetic leaves and (ii) glyoxysomes for lipid utilization in germinating seeds (20). All groups of microbodies contain catalase (EC 1.11.1.6) which detoxifies hydrogen peroxide to oxygen and water. The glyoxysomes are a site for the glyoxylate cycle, which leads to the synthesis of succinate from acetyl-CoA derived from fatty acids, and the key enzymes are (i) isocitrate lyase (EC 4.1.3.1) which splits isocitrate to succinate and glyoxylate, and (ii) malate synthase (EC 4.1.3.2) which converts glyoxylate and acetyl-CoA to malate.

In fungi, microbodies have been intensively studied as to the basis of their occurrence and peroxisomal biochemistry (16); however, little information is available on the glyoxysomal nature of microbodies in plant-pathogenic fungi. However, there is growing evidence that glyoxysomes and the glyoxylate cycle are involved in critical processes including appressorium formation of plant-pathogenic fungi (12,27). The activity and presence of catalase in plant-pathogenic fungi are highly relevant to pathogenicity and infection processes, because plant disease resistance may include an increase in hydrogen peroxide concentration within host cells (14). The role of a fungal extracellular catalase in pathogenicity and suppression of host defense responses in *Claviceps purpurea*-rye interaction has been reported (4).

The ascomycetous fungus *Botryosphaeria dothidea* Ces. & De Not. (anamorph *Fusicoccum aesculi* Corda) causes white rot, one of the most destructive diseases of apple (*Malus domestica* Borkh.) in humid areas (25). The disease produces a soft and light-colored rot on fruits and extensive cankers on limbs, eventually leading to tree death. The fungus forms appressoria on the fruit surface or enters fruits through natural openings such as lenticels and surface cracks (10). Apple fruits can be infected by conidia within 7 weeks after petal fall, and symptoms usually become noticeable 6 to 8 weeks before harvest (21). It is generally accepted that fungitoxic compounds including benzoic acid in immature fruits and fruit substrates unfavorable to the fungal growth are responsible for suppressing pathogen invasion and delaying symptom appearance (24). Our previous study (11) revealed the frequent occurrence of microbody-lipid globule complexes in *B. dothidea* hyphae invading immature apple fruits. Based on the presence of crystalline inclusions in the matrix and topographical association with lipid globules, the microbodies were considered to be glyoxysomes with catalase crystals. However, the biochemical nature of these organelles was not cytochemically characterized. Specific cytochemical localization of enzymes unique to glyoxysomes can be used for in situ identification of glyoxysomes. Either isocitrate lyase or malate synthase might be suitable for this purpose, because they are the key enzymes of the glyoxylate cycle occurring in glyoxysomes. In situ activity and subcellular localization of glyoxysomal enzymes through enzyme cytochemistry and immunocytochemistry will provide a better understanding of the microbody-lipid globule complexes and the fungal survival mechanisms underlying dormant hyphal growth and latent infections in immature apple fruits. The objectives of this study were to (i) investigate the morphology and in situ enzyme characteristics of the microbodies and (ii) provide evidence for the glyoxysomal nature of microbodies in *B. dothidea* hyphae.

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MATERIALS AND METHODS

Fungal isolate and culture conditions. An isolate (BD-3) of *B. dothidea* was obtained from a naturally infected apple fruit showing typical white rot symptoms. The isolate was grown and stored on autoclaved barley (*Hordeum vulgare* L.) grains at 25°C (9). The isolate was transferred to potato dextrose agar (PDA) plates and grown under a 12-h photoperiod of fluorescent light at 25°C. The cultures were subjected to specimen preparation for transmission electron microscopy.

Hyphal ultrastructure. Three types of *B. dothidea* hyphae were collected as follows: (i) actively growing tips from the margin of a 3-day-old culture grown on PDA, (ii) nonmarginal hyphae from a 30-day-old culture grown on PDA, and (iii) nonmarginal hyphae from a 30-day-old culture grown on autoclaved barley grains. The hyphal tips and samples taken from either of the 30-day-old cultures were designated in this study as young and old hyphae, respectively. Squares of young hyphae (each 2 × 2 mm² with approximately 1 mm thickness of underlying agar blocks) were excised with a sterile razor blade from the margin of actively growing 3-day-old PDA cultures. Squares of the same size were obtained from the center of 30-day-old PDA plates and autoclaved barley grains. The specimens were fixed with modified Karnovsky's fixative (8) consisting of 2% (vol/vol) glutaraldehyde and 2% (vol/vol) paraformaldehyde in 50 mM sodium cacodylate buffer (pH 7.2) at 4°C overnight and washed with the same buffer three times each for 10 min. The specimens were postfixed with 1% (wt/vol) osmium tetroxide in the same buffer at 4°C for 2 h and washed briefly with distilled water two times. The postfixed specimens were en bloc stained with 0.5% (wt/vol) uranyl acetate at 4°C overnight and dehydrated in a graded ethanol series: 30, 50, 70, 80, 95, and 100%, and three times in 100% each for 10 min. The specimens were further treated with propylene oxide two times each for 30 min as a transitional fluid and embedded in Spurr's resin. Ultrathin sections (approximately 50 nm thick) were cut with a diamond knife using an ultramicrotome (MT-X; RMC Inc., Tucson, AZ). The ultrathin sections were mounted on copper grids and stained with 2% uranyl acetate and Reynolds' lead citrate (23) each for 7 min. They were examined with a transmission electron microscope (JEM-1010; JEOL Ltd., Tokyo) operated at an accelerating voltage of 80 kV. More than 10 ultrathin sections per specimen, from a total of three specimens per hyphal type, were examined with the microscope.

Enzyme cytochemistry. Cytochemical localization of catalase activity in hyphae was carried out by the 3,3'-diaminobenzidine tetrahydrochloride (DAB) staining method (4,31) with slight modifications as follows. Squares of the 30-day-old hyphae grown on PDA were sampled as described previously. The specimens were fixed with modified Karnovsky's fixative consisting of 0.5% (vol/vol) glutaraldehyde and 4% (vol/vol) paraformaldehyde in 0.05 M sodium cacodylate buffer (pH 7.2) at 4°C for 2 h and washed as described previously. They were then incubated in 0.05 M sodium cacodylate buffer (pH 9.5) containing 0.2% (wt/vol) DAB and 0.06% (vol/vol) hydrogen peroxide at 37°C for 30 min. This incubation step was performed with gentle shaking under continuous darkness conditions and repeated two times. Other specimens were also incubated in the incubation medium lacking hydrogen peroxide as a negative control. The specimens were then washed in 0.05 M sodium cacodylate buffer (pH 7.2) three times each for 10 min. The specimens were postfixed, further processed, embedded in LR white resin, and examined as described previously.

To localize malate synthase activity in hyphae, another cytochemical staining method (30) was employed with slight modifications as follows. Squares of the 30-day-old hyphae grown on PDA were fixed and washed as described in the catalase cytochemistry study. They were then preincubated in 0.01 M potassium ferricyanide and 0.02 M sodium cacodylate buffer (pH 7.2)

at room temperature (20 to 24°C) under continuous darkness conditions. After 30 min, they were immersed in an incubation medium (30) with 0.065 M sodium cacodylate buffer (pH 7.2). The incubation step was performed at 37°C under continuous darkness conditions for 30 min and repeated once. Other specimens were also treated with the same incubation medium, except lacking acetyl-CoA, as a negative control. The specimens were postfixed and further processed as described in the catalase cytochemistry study.

Western blot analysis. The fungus was grown in potato dextrose broth at 25°C for 21 days. Whole protein extracts were prepared by homogenizing mycelia with liquid nitrogen in a buffer containing 50 mM Tris-HCl (pH 7.4), 1 mM EGTA, 0.2% Triton X-100, 1 mM phenylmethyl sulfonyl fluoride, and 10 µg/ml each of leupeptin and pepstatin (7). The homogenates were clarified by centrifugation at 10,000 × g for 10 min at 4°C and then at 25,000 × g for 60 min at 4°C. The proteins in supernatants were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (12% acrylamide gel) and transferred to a polyvinylidene difluoride membrane. The membrane was washed with 0.1 M Tris-HCl (pH 8.0) and treated with a blocking solution containing 5% (wt/vol) nonfat dry milk in Tris-buffered saline (TBST; 100 mM Tris-HCl and 0.05% Tween 20, pH 8.0) at room temperature for 1.5 h. The membrane was then incubated for 1.5 h with rabbit polyclonal antisera of (i) catalase (Polysciences Inc., Warrington, PA) raised against bovine liver catalase and (ii) isocitrate lyase raised against spinach isocitrate lyase diluted 1:1,000 with the 0.2% blocking solution. After briefly washing two times with TBST, the membrane was further treated for 1.5 h with alkaline phosphatase-conjugated goat anti-rabbit immunoglobulin G (IgG) (Pierce Biotechnology Inc., Rockford, IL) diluted 1:1,000 with the 0.2% blocking solution. The membrane was washed with TBST and stained with a combination of nitroblue tetrazolium chloride and 5-bromo-4-chloro-3'-indolylphosphate *p*-toluidine salt to visualize antigen-antibody complexes on the blotted membrane.

Immunocytochemistry. To localize catalase and isocitrate lyase in hyphae, immunogold labeling was performed using polyclonal antisera raised against each enzyme. Squares of old hyphae grown on PDA were fixed and washed as described in the enzyme cytochemistry study. After en bloc staining with 0.5% uranyl acetate at 4°C overnight, they were dehydrated in a graded ethanol series (30, 50, 70, 80, and 95%) each for 10 min and embedded in LR white resin. The reaction conditions and optimum dilution ratios of immunogold labeling mixtures were determined in preliminary studies. Ultrathin sections mounted on Formvar-coated nickel grids were treated with a blocking solution containing 5% (wt/vol) nonfat dry milk in Tris-buffered saline (TBS; 50 mM Tris-HCl and 150 mM NaCl, pH 7.2) at room temperature for 10 min. The grids were incubated in a moist chamber overnight with the rabbit polyclonal antisera of catalase and isocitrate lyase diluted 1:250 with the blocking solution. After briefly washing three times with TBS, they were further treated for 1 h with goat anti-rabbit IgG conjugated with 10 nm colloidal gold particles (Sigma Chemical, St. Louis) diluted 1:50 with TBS. They were then washed thoroughly with TBS followed by sterilized distilled water, stained, and examined as described previously. Negative control experiments were also performed to prove the specificity of the labeling through the replacement of the primary antisera with preimmune serum of rabbit.

RESULTS

Hyphal ultrastructure. Transmission electron microscopy of young hyphae revealed numerous ribosomes in the cytoplasm enclosed by an electron-translucent cell wall (Fig. 1A). The hyphae contained nuclei, mitochondria, and vacuoles in cytoplasm. Microbodies were usually round in cross sections, bound by a single

membrane, and had a homogeneous matrix in young hyphae. Unlike mitochondria with cristae projecting into matrices, microbodies did not have internal membrane systems in such matrices. Old hyphae grown on PDA had thicker cell walls than young hyphae, and the overall appearance of the cell wall from old hyphae differed from that of the cell wall from young hyphae (Fig. 1B). The cytoplasm had large vacuoles, lipid globules, and mitochondria. Microbodies varied in size, ranging from 200 to 400 nm in diameter, and often had crystalline inclusions which consisted of parallel arrays of fine tubules in their matrices (Fig. 1C). In addition, microbodies and lipid globules were placed in close association with each other, forming microbody-lipid globule complexes in old hyphae grown on either PDA plates or autoclaved barley grains (Fig. 1D). The morphology and size of

microbodies in old hyphae grown on autoclaved barley grains were similar to those of the organelle found in young or old hyphae grown on PDA.

Enzyme cytochemistry. Microbody-lipid globule complexes were found in old hyphae grown on PDA (Fig. 2A). However, it was common to observe more electron-dense microbodies after the DAB staining with the complete incubation medium including hydrogen peroxide than when staining in medium lacking hydrogen peroxide (compare Fig. 2B with 2A). The staining of catalase activity was uniform throughout the matrix of microbodies, so even crystalline inclusions were nearly indiscernible within some intensely stained microbodies (Fig. 2C). In addition to microbodies, some mitochondria exhibited cytochemical staining (Fig. 2D).

Fig. 1. Ultrastructure of *Botryosphaeria dothidea* hyphae. **A**, A section near a hyphal tip grown on potato dextrose agar (PDA). The cytoplasm was filled with numerous ribosomes. CW = cell wall, M = mitochondrion, Mb = microbody, N = nucleus, V = vacuole. Bar = 500 nm. **B**, A 30-day-old hypha grown on PDA. The cytoplasm was mainly characterized by large vacuoles (V) and lipid globules (L). Arrow indicates electron-dense outer layer of the cell wall. Bar = 500 nm. **C**, A microbody in the 30-day-old hypha. The organelle contained a crystalline inclusion (arrow) in the matrix. ER = endoplasmic reticulum. Bar = 100 nm. **D**, A 30-day-old hypha grown on autoclaved barley grains. Microbody-lipid globule complexes were often found in cytoplasm. Bar = 300 nm.

Hyphae had microbodies and mitochondria with normal electron density when the incubation medium for malate synthase cytochemistry lacked acetyl-CoA (Fig. 3A). However, incubations with the complete incubation medium including acetyl-CoA for malate synthase cytochemistry resulted in the intense and uniform deposition of electron-dense reaction products throughout the matrix of the microbodies and mitochondria with cristae (Fig. 3B).

Western blot analysis. The SDS-PAGE of whole protein extracts showed that a number of soluble proteins were induced and accumulated in the mycelium. The anti-catalase antiserum raised against bovine liver catalase detected two major protein bands in western blots (Fig. 4A). The molecular masses of these immuno-

reactive proteins were estimated to be approximately 40 and 45 kDa. In addition, two major protein bands at molecular masses of approximately 50 and 60 kDa were detected with the anti-isocitrate lyase antiserum raised against spinach isocitrate lyase (Fig. 4B).

Immunocytochemistry. As usual in the nonosmicated specimens embedded in LR white resin, ultrathin sections showed membrane systems of low contrast. The control experiment gave negative results, with only nonspecifically scattered gold particles on ultrathin sections (Fig. 5A). When ultrathin sections were incubated with the anti-catalase antiserum, gold particles were specifically localized in both crystalline inclusions and matrices

Fig. 2. In situ localization of catalase activity in *Botryosphaeria dothidea* hyphae. **A**, A 30-day-old hypha after the 3,3'-diaminobenzidine tetrahydrochloride (DAB) staining with the incubation medium lacking hydrogen peroxide (negative control). Bar = 500 nm. **B**, A 30-day-old hypha after the DAB staining with the complete incubation medium including hydrogen peroxide. Microbodies were more electron-dense than those in **A**. Bar = 400 nm. **C**, Higher magnification of microbodies in a 30-day-old hypha after the DAB staining with the complete incubation medium. Even crystalline inclusions were indiscernible within intensely stained microbodies. Bar = 200 nm. **D**, A 30-day-old hypha after the DAB staining with the complete incubation medium. Some mitochondria showed cytochemical staining. S = septum. Bar = 500 nm.

of microbodies (Fig. 5B). The gold labeling was also evident in a multivesicular body-like organelle containing small bubble-like vesicles and in the cell wall. The density of labeling indicated about 13% of the gold particles localized to microbodies in the micrograph (Fig. 5B).

When ultrathin sections were incubated with the anti-isocitrate lyase antiserum, gold particles were specifically localized only in the matrices of microbodies (Fig. 5C). Crystalline inclusions were not labeled with gold particles, even though the inclusions were present in the gold particle-labeled matrices of microbodies. In some cases, the matrices of microbodies were not labeled with gold particles when crystalline inclusions occupied a majority of the matrix of the organelle in ultrathin sections. About 62% of gold particles were localized to microbodies in the micrograph (Fig. 5C). Two different quadrangular crystalline inclusions were often found in the matrices of microbodies, where only the matrices were specifically labeled with gold particles (Fig. 5D). Occasional nonspecific gold particles were observed in other cell components in addition to microbodies.

DISCUSSION

Plant-pathogenic fungi are exposed to limited nutrient conditions during the saprophytic or parasitic phase, and thus need to use storage compounds for survival. They commonly use lipids, the dominant form of stored carbon, and coordinate the topographical associations of organelles for the efficient production of energy (11,27). This study demonstrated the glyoxysomal nature of microbodies in *B. dothidea* hyphae by the criteria of the exami-

Fig. 4. Western blot analysis of catalase and isocitrate lyase in *Botryosphaeria dothidea* hyphae. **A**, Detection of catalase from 21-day-old hyphae. Note the immunoreactive protein bands at the molecular masses of approximately 40 and 45 kDa. **B**, Detection of isocitrate lyase from 21-day-old hyphae. Note the immunoreactive protein bands at the molecular masses of approximately 50 and 60 kDa. The approximate sizes of the proteins are shown at the left. Arrowheads indicate the immunoreactive protein bands in each lane.

Fig. 3. In situ localization of malate synthase activity in *Botryosphaeria dothidea* hyphae. **A**, A 30-day-old hypha after the cytochemical staining with the incubation medium lacking acetyl-CoA (negative control). Microbodies (Mb) and mitochondria (M) showed normal electron density. **B**, A 30-day-old hypha after the cytochemical staining with the complete incubation medium including acetyl-CoA. Microbodies and a mitochondrion with cristae (arrowhead) were more electron-dense than those in **A**. Bars = 200 nm.

nation of organellar morphology and cytochemistry of enzymes unique to glyoxysomes. The morphological characteristics such as shape, size, and matrix feature of the microbodies were consistent with those of the glyoxysomes in other fungi (16). In particular, the proximity of microbodies with lipid globules in hyphae was strikingly similar to that found in zoospores of chytrids or germinating seeds, where microbodies (glyoxysomes) were involved in lipid utilization via the glyoxylate cycle (20, 22,28). Furthermore, enzyme cytochemistry and immunocytochemistry provided evidence for in situ activity and subcellular localization of glyoxysomal enzymes in microbodies of hyphae, respectively. These results suggest that the microbodies com-

plexed with lipid globules in *B. dothidea* hyphae are functionally defined as glyoxysomes, where glyoxysomal enzymes are biochemically active for the glyoxylate cycle to be a metabolic pathway in gluconeogenesis. To our knowledge, this is the first report of the in situ activity and subcellular localization of glyoxysomal enzymes in plant-pathogenic fungi.

Microbodies were invariably found in cytoplasm regardless of the hyphal types tested; however, crystalline inclusions in the organellar matrix were often observed in old hyphae. As inferred from the previous observation of the inclusions in *B. dothidea* hyphae invading immature apple fruits (11), it is likely that the inclusion formation would increase while nutrients in culture or

Fig. 5. Subcellular localization of catalase and isocitrate lyase in *Botryosphaeria dothidea* hyphae. **A**, A 30-day-old hypha treated with the preimmune serum of rabbit (negative control). Gold particles were nonspecifically labeled on the ultrathin section. **B**, Immunogold labeling of catalase in a 30-day-old hypha. Note the gold particles labeled in a microbody (Mb), a multivesicular body-like organelle (Mu), and a cell wall (CW). Arrowheads indicate bubble-like vesicles. **C and D**, Immunogold labeling of isocitrate lyase in a 30-day-old hypha. Gold particles were specifically labeled in the matrix of a microbody (Mb). Bars = 200 nm.

plant substrate were being depleted during hyphal growth. Also, such limited nutrient conditions might favor the formation of microbody-lipid globule complexes, considering the frequent occurrence of the complexes in old hyphae. The cytochemical localization of catalase activity indicated that the enzyme was biochemically active in microbodies, as reported from other fungi (4,31). In the presence of exogenously added hydrogen peroxide, catalase has been known to mediate the oxidation of DAB into an osmiophilic polymer, which reduces osmium tetroxide, resulting in electron-dense deposits (6). The activity of malate synthase was also localized in microbodies, suggesting that the enzyme would be biochemically active for the glyoxylate cycle to provide the fungus with energy and 4-carbon intermediates of the tricarboxylic acid cycle (17,20). Considering the components of the incubation medium, the pronounced electron-dense materials in the malate synthase cytochemistry study would likely correspond to copper ferrocyanide (30). Meanwhile, the electron-dense staining and reactivity of mitochondria may be due to the peroxidative reaction of cytochrome c peroxidase or several mitochondrial heme proteins (29).

The immunocytochemistry detected the presence of catalase in matrices and crystalline inclusions of microbodies, where catalase activity was demonstrated by the DAB staining method. These findings support that the crystalline inclusions are composed of biochemically active catalase molecules that undergo crystallization in the matrix (26). Moreover, the immunogold labeling indicated the presence of catalase in a multivesicular body-like organelle and a cell wall, suggesting the enzyme secretion outward. This result was consistent with the observation that *Claviceps purpurea* catalase was secreted via fungal secretory pathway through multivesicular bodies (4). The distribution of gold particles on the nonmicrobody regions could be due to the extracellular secretion of catalase. Meanwhile, the specific location of isocitrate lyase in matrices of microbodies in this study implies that the glyoxylate cycle occurs in the microbodies, as also suggested from the in situ activity of malate synthase. The relatively high density of labeling also supported the specific localization of the enzyme in microbodies.

In addition, the specific cross-reactivity of heterologous antisera produced against catalase and isocitrate lyase with the corresponding enzymes of *B. dothidea* was shown in this study. Considering the specific labeling of gold particles, it is likely that catalase and isocitrate lyase of the fungus share a considerable number of epitopes with those of the heterologous hosts. Moreover, the results from the western blot analysis of catalase and isocitrate lyase supported that the antisera raised against the two enzymes from heterologous hosts had cross-reactivity with the fungal-derived proteins. In particular, the molecular mass of a protein band having the binding specificity with the anti-isocitrate lyase antiserum was estimated to be approximately 60 kDa, which was similar to that of isocitrate lyase from *Aspergillus nidulans* (3) and *Magnaporthe grisea* (32). The putative molecular masses of protein bands that strongly reacted with the anti-catalase antiserum in this study were not in exact agreement with those of catalase from other fungi (2,4,5). The discrepancy between the results can be explained by assuming that fungi contain multiple catalase genes that form a quite diverse group of enzymes with respect to their structures, locations, and functions (5). It is considered that the anti-catalase antiserum was mainly bound to the microbody-localized and extracellularly secreted catalase(s) from the entire catalase populations in *B. dothidea*. Future work remains to be done to characterize the biochemical nature of the glyoxysomal enzymes from *B. dothidea* that may have different isoforms or subunits in structure and conformation.

The putative involvement of glyoxysomes in the survival and pathogenicity of *B. dothidea* could be proposed in this study. One of the earliest and most effective responses of host plants after pathogen recognition is the oxidative burst, cascade generation of

active oxygen species including superoxide radical, hydrogen peroxide, and hydroxyl radical (14). Hydrogen peroxide is closely associated with a variety of host defense responses such as cell wall fortification, hypersensitive response, and direct antimicrobial action (1,14,19). To establish compatible interactions with host plants, *B. dothidea* might counteract the oxidative burst by secreting catalase as an enzymatic defense against hydrogen peroxide. Diffusible catalase could counter hydrogen peroxide acting on host cell walls at the host-parasite interface, suppressing hydrogen peroxide-mediated host defense responses. We suggest, therefore, that the glyoxysomes enable the fungus to endure latent periods and adverse conditions, such as nutrient deficiency and host defense responses, allowing the use of lipids via the glyoxylate cycle and catalase secretion until the fungus meets suitable nutrients. Although suppressed in immature fruits, *B. dothidea* is thought to survive unfavorable conditions and resume its active growth as the sugar content in fruits increases (13). The crucial roles of catalase and the glyoxylate cycle have been well established in the pathogenesis of human and plant diseases caused by fungi and bacteria, indicating the widespread utility of the enzyme and the pathway in microbial pathogenesis (15,18,32,33).

In conclusion, this study increased our knowledge of the cytology and in situ enzyme characteristics of glyoxysomes in *B. dothidea* hyphae. The implication of glyoxysomes with fungal survival suggests that the organellar enzymes and glyoxylate cycle would perhaps present site-specific novel targets for the control or intervention of plant diseases caused by fungi. It remains to be elucidated in further studies whether the glyoxylate cycle is essential to the latent infection or endophytic colonization of host tissues by *B. dothidea*. Also, the knowledge of the developmental transition and regulation of enzyme activities and the occurrence of individual enzyme isoforms will enhance our understanding of the nature of fungal pathogenicity and survival.

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