Increased Activity of a Cationic Peroxidase Associated with an Incompatible Interaction Between Xanthomonas oryzae pv oryzae and Rice (Oryza sativa)\textsuperscript{1}

Peter J. Reimers\textsuperscript{2}, Ailan Guo, and Jan E. Leach* 

Department of Plant Pathology, Throckmorton Hall, Kansas State University, Manhattan, Kansas 66506–5502

ABSTRACT

Rice (Oryza sativa L.) cultivar Cas 209 carries the gene Xa-10 for resistance to race 2 of Xanthomonas oryzae pv oryzae, the bacterial blight pathogen. When seedling leaves of Cas 209 plants were infiltrated with bacterial cell suspensions of strain PX086\textsuperscript{R} (race 2, incompatible), total peroxidase activity in extracts from extracellular spaces increased almost threefold between 16 and 24 hours after inoculation. The increase in total peroxidase activity in extracellular extracts was correlated with the appearance of a 43-kilodalton peroxidase isoenzyme with an isoelectric point of 8.6. Increases in the activities of two anionic peroxidase isoenzymes also were associated with the incompatible interaction. Later during the interactions, total peroxidase activities increased in both compatible (cv Cas 209 infiltrated with race 1, PX061\textsuperscript{R}) and control (Cas 209 infiltrated with water) treatments, but final activity levels were less than that observed in the incompatible combination. Similarly, the cationic peroxidase was detected in all three treatments by 48 hours after infiltration, but at reduced levels in compatible and water-infiltrated control treatments relative to the incompatible combination. Accumulation of this peroxidase in extracellular spaces thus may play a role in the defense response in cultivar Cas 209.

Rice (Oryza sativa L.) is one of the most important cereal crops worldwide, sustaining more than one-half of the global population (20). Like many plant species, rice employs a diverse array of defenses that minimize losses during pathogen attack. For example, melanin deposition and coordinated increases in peroxidase activities and phenolic compounds are associated with the colonization of rice leaves by Magnaporthe grisea, the blast pathogen (26, 30). Phenolic compounds toxic to Xanthomonas oryzae pv oryzae, the causal agent of bacterial blight, also are found in greater amounts in healthy leaves of resistant cultivars compared to healthy leaves of susceptible cultivars (9). Phytoalexins are thought to be produced in response to infection with some strains of X. oryzae pv oryzae (18). Beyond these observations, however, little is known about the physiology of defense responses in this important monocot.

We recently reported (21) that lignin-like polymers accumulated in inoculated leaves during the incompatible interaction between rice cv Cas 209 and race 2 of X. oryzae pv oryzae. The spatial and temporal patterns of phenolic polymer deposition were correlated with a decrease in bacterial multiplication rates and the onset of bacteriosis in the incompatible interaction. This response may be controlled through the effect of the gene Xa-10, which confers resistance in cv Cas 209 to races 2 and 5 of the pathogen. When this cultivar was infected with race 1 strains, to which it is susceptible, lignin-like materials did not accumulate. The bacteria in the compatible interaction continued to multiply, leading to the death of the leaf and usually the entire seedling.

Lignin and other phenolic polymers serve as physical barriers and, as such, are thought to prevent fungal penetration of host cells (22, 28). However, bacterial plant pathogens such as X. oryzae pv oryzae are found primarily in the vascular tissues or extracellular spaces; they do not penetrate host cells. Consequently, cell wall lignification probably would not be an effective defense against these organisms unless lignified materials prevented bacterial spread by blocking vessels or filling extracellular spaces. Nevertheless, the biosynthetic process itself could be an important component of the defense response. That is, bacterial multiplication and movement may be inhibited by toxic phenolic compounds (9, 33), phenolic free radicals, or activated oxygen (3), all of which are associated with lignification (8).

PO\textsuperscript{3} (EC 1.11.1.7, donor: H\textsubscript{2}O\textsubscript{2}, oxidoreductase) have been implicated in the last enzymic step of lignin biosynthesis, that is, the oxidation of hydroxy cinnamyl alcohols into free radical intermediates, which subsequently are coupled into the lignin polymer (8). Although a role for POs in defense responses has not been clearly demonstrated, increases in PO activity have been correlated with infection in many species, including cotton (16, 33), cucurbits (25), rice (26, 30), and wheat (5, 17, 23, 24). For example, after infection of cotton

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\textsuperscript{2}Present address: Division of Plant Pathology, The International Rice Research Institute, P.O. Box 933, Manila 1099, The Philippines.

\textsuperscript{3}Abbreviations: PO, peroxidase; AEC, 3-amino-9-ethylcarbazole; APS, ammonium persulfate; CA, comiferal alcohol; G6P-D, glucose-6-phosphate dehydrogenase; IEF, isoelectric focusing; PO-A, anionic peroxidase; PO-C, cationic peroxidase; pl, isoelectric point; anti-HP, anti-horseradish peroxidase.
by *X. campestris pv malvacearum*, an increase in PO activity was observed only in the incompatible interaction (33). This was accompanied by a decline in the number of bacteria recovered from the inoculated tissue and the accumulation of brown materials in the inoculation site. The PO-containing extract from cotton catalyzed *in vitro* the oxidation of catechin, an abundant phenol in cotton tissues, to highly toxic products that were bactericidal to *X. campestris pv malvacearum* (33).

To determine if increases in PO activity correlated with resistance to *X. oryzae pv oryzae*, we monitored the PO activity and characterized the isozyme profile in extracellular extracts of rice leaves during compatible and incompatible interactions. We report herein that the incompatible reaction in cultivars carrying the *Xa-10* gene for bacterial blight resistance involves increases in the extracellular activities of one cationic and two anionic POs.

**MATERIALS AND METHODS**

**Rice Cultivars, Bacterial Strains, and Inoculation**

Seeds of rice (*Oryza sativa*) cvs Cas 209, IR-BB10, and IR24, provided by T. Mew and G. Khush of The International Rice Research Institute (Los Baños, The Philippines), were cultivated in growth chambers as described (21). Cas 209 carries gene *Xa-10* for resistance to races 2 and 5 of *Xanthomonas oryzae pv oryzae*, whereas IR24 carries no known resistance genes to the strains of the pathogen used in this study. IR-BB10 is near-isogenic to IR24, but carries the gene *Xa-10* from Cas 209.

Strains PXO61<sup>sm</sup> and PXO86<sup>rd</sup> of *X. oryzae pv oryzae* were prepared for inoculation of rice plants as described (21). The second leaf of seedling rice plants was infiltrated with either sterile distilled deionized water or approximately 3 to 5 μL of a bacterial suspension (10<sup>6</sup> colony forming units/mL) in water with a syringe as described (21). Multiple sites, 3 to 4 mm in diameter and approximately 2 mm apart, were infused along the leaf from the base to the tip. After inoculation, growth chamber lighting was adjusted from the normal 12 h day/night cycle to continuous light for 24 h. At the end of that light period, the normal photoperiod was resumed.

**Extraction of Leaf POs**

POs were extracted from rice leaves at 0.5, 8, 16, 24, 36, 48, and 72 h after infiltration. Duplicate pots were randomized by treatment and sampling time. Eight leaves from plants in one pot were cut into 2- to 3-cm segments and immersed in buffer (pH 6.0) that contained 100 mM Mes (free acid), 50 mM Tris (free base), 50 mM CaCl<sub>2</sub>, and 0.5% (w/v) Triton X-100. Air in the extracellular spaces was evacuated from the submerged leaves by placing the tube in a vacuum chamber at 200 Pa for 2 min. Upon return to atmospheric pressure, the leaves became permeated with buffer. The leaves were removed from the buffer, blotted dry, and placed vertically inside a 1.5-mL microfuge tube with a hole punctured in the bottom. The tube was placed inside a second 1.5-mL tube, and both were placed in a 15-mL Corex tube. The extracellular washing fluid was removed by centrifugation at 30,000 g for 15 min. The extract was stored on ice for further analysis.

**PO Assay**

PO activity was assayed with a modification of the procedure of Maehley and Chance (15). Extracellular fluids were diluted with extraction buffer such that when 10 μL of diluted extract was used per assay, the PO reaction rate under the specified conditions resulted in an absorbance change of between 0.2 and 1.0 unit after 3 min. Assays were performed in a 1-mL reaction volume that contained 100 mM sodium phosphate (pH 6.0), 18 mM guaiacol, and 0.03% H<sub>2</sub>O<sub>2</sub> at 30°C. The reference cuvette contained all reaction components except H<sub>2</sub>O<sub>2</sub>. The oxidation of guaiacol to tetraguaiacol was monitored at 470 nm for 3 min with a Hitachi 3200 double-beam spectrophotometer. Relative activity was described in terms of the change in absorbance per min per 10 μL of extract. (One leaf yielded approximately 20 μL of extract.) Each extract was assayed twice and the results were averaged to obtain one treatment value. The treatments were replicated and the entire experiment was performed twice. The means of the treatment values from the two experiments were averaged and plotted by time.

**G6P-D Assay**

G6P-D (EC 1.1.1.49) activity was determined in samples extracted from leaves at 0.5, 24, and 48 h after infiltration with either water or a suspension of *X. oryzae pv oryzae* strain PXO86<sup>rd</sup> (incompatible). Samples were prepared either by extraction of the extracellular fluids, as described for PO experiments, or by homogenization of whole leaves in 50 mM potassium phosphate buffer (pH 6.5) followed by centrifugation at 27,000g for 10 min. G6P-D activity was measured by a modification of the procedure of Koijima and Conn (12). The reaction mixture contained 100 μM Tricine buffer (adjusted to pH 8.0 with NaOH), 2 μM MgCl<sub>2</sub>, 6 mM glucose-6-phosphate, 0.6 mM NADP<sup>+</sup>, and extract (50 μL) in a final volume of 1 mL. The increase in optical density at 340 nm due to the reduction of NADP<sup>+</sup> was monitored at 30°C. Samples from each treatment were assayed once, and the experiment was performed twice.

**Electrophoresis**

Isoperoxidases in rice leaf extracts were separated by IEF in precast polyacrylamide gels (LKB, Uppsala, Sweden) that contained 2.4% (w/v) carrier ampholytes (pH 3.5–9.5). Electrode wick solutions were 1 mM H<sub>2</sub>PO<sub>4</sub> (anode) and 1 M NaOH (cathode). Gels were prefocused for 30 min at 25 mA, and samples (10–15 μL of extract, diluted 1:1 with distilled, deionized H<sub>2</sub>O to less than 100 mM salt) were applied to the anodic side of the gel with filter paper applicators or a plastic template. Focusing was performed for 2.5 h at limits of 25 mA and 25 W using a power supply with automatic crossover and a 2000-V maximum for 0.1 × 24.5 × 11 cm gels. If filter paper applicators were used, they were removed 1 h after sample application. Gels were prepared by comparison with the following standards obtained from Sigma Chemical Co.: amyloglucosidase, pl 3.6; glucose oxidase, pl 4.2; β-lactoglobulin A, pl 5.1; carbonic anhydrase II, pl 5.9; carbonic anhydrase I, pl 6.6; myoglobin, pl 6.8 and 7.2; l-lactic dehydrogenase, pl 8.3, 8.4, and 8.6; trypsinogen, pl 9.3.
Nondenaturing cathodic PAGE (27) was accomplished with a tank buffer (pH 6.8) of 22 mM Mops in 100 mM histidine (free base). The stacking gel was made with 2.5% (w/v) acrylamide and 0.63% (w/v) bisacrylamide in 50 mM KOH and 63 mM Mops, pH 8.0, and 0.13% (v/v) N,N,N',N'-tetramethylethlenediamine and 0.13% (w/v) APS as polymerization catalysts. The resolving gel was made with 15% (w/v) acrylamide and 0.1% (w/v) bisacrylamide in 50 mM KOH and 213 mM MOPS pH 6.8, with 0.1% (v/v) N,N,N',N'-tetramethylethlenediamine and 0.03% (w/v) APS. The sample (0.2–0.6 µg of protein) was loaded as a 3:1 (extract:sample buffer) dilution with a final buffer concentration identical to that of the stacking gel. The sample also contained 10% glycerol and 0.001% (w/v) methyl green (tracking dye). Electrophoresis was performed at a constant current of 15 mA for 9 to 10 h for 0.15 × 18 × 12 cm long resolving gels, or 20 mA for 2.75 h for 0.15 × 10 × 5.5 cm long resolving gels.

PO activity was detected by incubating gels in 100 mM sodium phosphate (pH 6.0) with 18 mM guaiacol (15), 0.01% AEC (7), and 0.03% H2O2 at 30°C for 15 to 20 min, or until bands appeared to the desired intensity. Alternatively, to determine if CA could serve as a substrate for the POs, gels were immersed in 100 mM sodium phosphate (pH 6.0) with 1.2 mM CA and 0.1% H2O2 at 30°C for 30 min (5). Substrate stocks were prepared in 95% ethanol (1.8 mM guaiacol), dimethylformamide (2% w/v AEC), or methoxethanol (600 mM CA). Stained gels were photographed with Kodak electrophoresis duplicating paper.

Samples for SDS-PAGE were prepared by fractionating leaf extracts by native cathodic PAGE. Portions of the cathodic gel that contained PO activity were excised, and the native proteins were denatured by boiling in 2% (w/v) SDS and 5% (v/v) 2-mercaptoethanol. After electrophoresis in 12% SDS-polyacrylamide gels (13), the proteins were transferred to nitrocellulose membranes by electroblotting (29), and the blots were immunostained (11) with a commercial preparation of antibodies against horseradish PO (Sigma).

Protein Assays

Extracts were assayed for protein content by the method of Bradford (1) with reagent supplied by Bio-Rad Laboratories. BSA dissolved in extraction buffer was used as the standard. Each sample was assayed in triplicate and the results were averaged to obtain the estimate of protein concentration.

RESULTS

Time Course of Leaf PO Accumulation

Rice leaves of cv Cas 209 (carrying gene Xa-10 for resistance to race 2 of X. oryzae pv oryzae) were inoculated with X. oryzae pv oryzae and examined for the appearance of extracellular POs (Fig. 1). Between 0.5 and 16 h after infiltration, extracellular extracts from the water-infiltrated control and both bacterial interactions (PX061<sup>sm</sup> [race 1], compatible and PX086<sup>sm</sup> [race 2], incompatible) contained about the same amount of total PO activity (Fig. 1). By 24 h after inoculation, however, the total PO activity in extracts from the incompatible interaction rose sharply to about twice the activity observed in the water control and the compatible interaction. This trend continued until 48 h after inoculation; by that time, total PO activity in the incompatible treatment was three times more than that in the water control. Some variability was observed in the total PO level after its initial increase, as reflected in the larger SE in the later sample times (Fig. 1). In contrast to the trend seen in the incompatible interaction, total PO activity in extracts from the compatible interaction remained stable during the first 36 h after treatment, but doubled by 48 h. Levels of total PO activity in extracts from water-infiltrated controls remained stable during the first half of the experiment, and then increased gradually from 36 to 72 h after treatment.

The changes in extracellular PO activity occurred without a major change in total extracellular protein. Protein content of the extracellular extracts did not change significantly after inoculation (0.29 ± 0.02 µg/leaf [mean ± se]). Similarly, G6PD activity remained constant over time in extracellular extracts (11.2 ± 2.3 [mean ± se] nmol NADPH/min/leaf) and in homogenates of leaves (4764.5 ± 1043.6 nmol NADPH/min/leaf).

Characterization of PO Isoenzyme Profile

At least seven isoperoxidases are known in rice (4). To determine which isozyme(s) contributed to the rise in total PO activity, IEF was used to resolve the isoperoxidases extracted from Cas 209, IR-BB10, and IR24 during a time course experiment. The activities of several POs, detected with a mixture of nonspecific substrates (guaiacol and AEC), changed during interactions with X. oryzae pv oryzae (Fig. 2).
At least three isoperoxidases, one cationic and two anionic, concomitantly increased in activity between 16 and 24 h after infiltration in the incompatible interaction. PO-C1, a cationic PO with a pl of 8.64 ± 0.03, usually was not detected in samples up to and including 16 h after infiltration. By 24 h after infiltration, however, the activity of PO-C1 had increased dramatically. The activity corresponding to this isoenzyme was not seen in compatible interactions until 36 h after infiltration. A highly cationic PO was observed at the cathode in most gels in all treatments. There was little or no change apparent in the activity of this PO during the experiment.

The activities of most of the PO-As changed little during the course of the experiment. However, the activities of two isoenzymes with similar pl (PO-A1 and PO-A2), which were usually detectable in all samples, increased markedly during the incompatible interaction between 16 and 24 h after infiltration relative to extracts from the water-infiltrated control and the compatible interaction. The activity of a third isoperoxidase, PO-A11, seemed to change with the diurnal cycle, but peaked at 24 h after infiltration and then decreased again in extracts 48 and 72 h after infiltration.

**Time Course of Extracellular PO-C Accumulation**

PO-C1 was characterized further because its activity increased most dramatically with the onset of the defense response. Native cathodic PAGE (27) was used to resolve isoperoxidases of pIs greater than 6.8 (Fig. 3). In addition to the nonspecific substrates used to detect isoperoxidases in the IEF gels, the lignin precursor CA also was used (5). The major band observed in these gels was a slowly migrating isoperoxidase that later was determined to be identical to PO-C1 (see below). The numerous rapidly migrating PO-Cs such as PO-C2, PO-C3, PO-C4, and PO-C5 (Fig. 3) were later found to migrate as the single highly cationic PO band observed under the cathode of broad range IEF gels (Fig. 2). All five cationic isoperoxidases utilized CA as a substrate, as evidenced by a white precipitate in the gel, although the highest activity was exhibited by PO-C1 (data not shown). In the samples from the incompatible interaction, PO-C1 activity increased dramatically between 16 and 24 h after infiltration (Fig. 2). This increase paralleled the increase in total PO activity (Fig. 1), as well as the increase observed in the PO-C1 band in IEF gels (Fig. 2).

The rapid increase in PO-C1 activity in incompatible interactions involving strain PXO86Rd also was observed in rice cv IR-BB10, which contains the gene Xa-10 for resistance to X. oryzae pv oryzae. Conversely, extracellular extracts from IR24, the near-isogenic parent to IR-BB10 that lacks Xa-10, did not contain elevated levels of PO-C1 activity relative to water controls until 48 h after infiltration with either PXO86Rd or PXO61Sm (Fig. 4).

**Figure 2.** Profiles of PO isoenzymes in extracellular extracts from rice cv Cas 209 with X. oryzae pv oryzae. Rice leaves were infiltrated with strain PXO61Sm (compatible, C), PXO86Rd (incompatible, I), or water (W). Samples of extract (15 μL/well) were applied to a broad range IEF gel and, after electrofocusing, isoperoxidase profiles were detected with a mixture of the substrates guaiacol and AEC.

**Figure 3.** Cationic isoperoxidases in leaf extracts from rice cv Cas 209 infiltrated with X. oryzae pv oryzae. Extracts were from leaves infiltrated with strain PXO61Sm (compatible, C), PXO86Rd (incompatible, I), or water (W). Proteins (0.6 μg/lane) were separated in a nondenaturing, cathodic polyacrylamide gel using previously described buffering conditions (27). PO activity was detected with a mixture of the substrates guaiacol and AEC.

**Figure 4.** Accumulation of PO-C1 in extracellular extracts from rice cvs IR24, IR-BB10, and Cas 209 at 24 and 48 h postinfiltration. From left to right, extracts are from leaves infiltrated with water (W) or strains PXO61Sm or PXO86Rd of X. oryzae pv oryzae at 24 and 48 h after infiltration. PXO61Sm is compatible (C) with all three cvs. PXO86Rd is compatible (C) on IR24 and incompatible (I) on IR-BB10 and Cas 209. Proteins (0.2 μg/lane) were separated in a nondenaturing, cathodic polyacrylamide gel with previously described buffering conditions (27). PO activity was detected with a mixture of the substrates guaiacol and AEC.
Electrophoretic Properties of PO-C1

The electrophoretic properties of PO-C1 were defined further with both native (cathodic and IEF) and denaturing PAGE. Crude extract from the incompatible interaction (Cas 209 infiltrated with race 2) 48 h after infiltration was subjected to native cathodic PAGE. A gel slice that comprised one lane was removed and placed on an IEF gel horizontal to the electric field. A two-dimensional PO profile was observed after the enzymes in the native gel slice were resolved by IEF. The slowly migrating PO-C from the cathodic gel slice migrated to the same position (corresponding to pH 8.6) on the IEF gels as did PO-C1 from crude extracts (data not shown). Furthermore, PO-C1-containing fractions from IEF migrated to the same position in native cathodic gels as did PO-C1 from crude extracts (data not shown).

The size of PO-C1 was estimated by SDS-PAGE. Proteins in extracellular extracts from leaves 48 h after infiltration with water or strain PXO86Rf of X. oryzae pv oryzae (incompatible) were separated first by native cathodic PAGE. Although the activity of PO-C1 was detected easily in the native gels, the protein itself was barely detectable even after silver staining (19). To detect the PO, gels were electroblotted to nitrocellulose and immunostained with commercially available anti-HRP antibody. Anti-HRP antiserum cross-reacted with proteins in western blots from either native or SDS gels. The immunoreactive bands in western blots corresponded only to bands in adjacent lanes of the native gels that stained for PO activity, suggesting specificity for the POs.

Because several rice P0s apparently cross-reacted with the anti-HRP antiserum, gel pieces that corresponded to the portion of native cathodic gels that contained PO-C1 (detected by staining an adjacent lane for PO activity) were excised and subjected to SDS-PAGE. After immunostaining, protein with an apparent Mr of 43,000 was detected only in gel pieces from leaves undergoing an incompatible interaction (Fig. 5). Regions on the blot corresponding to gel pieces from water-infiltrated leaves did not contain PO-C1.

**DISCUSSION**

A dramatic increase in PO activity was observed in extracts from rice leaves during the incompatible interaction with race 2 of X. oryzae pv oryzae in cv Cas 209 (Fig. 1). This increase, which occurred between 16 and 24 h after infiltration of the pathogen, was correlated with several changes in the PO isoenzyme profile, including the appearance of PO-C1 with a pl of 8.6 (Fig. 2). Later during the interactions, total PO activities increased in both compatible (cv Cas 209 infiltrated with race 1) and control (Cas 209 infiltrated with water) treatments, but the final activities were less than those observed in the incompatible combination (Fig. 1). Similarly, PO-C1 was detected in all three treatments by 48 h after infiltration, but at reduced levels in compatible and water-infiltrated control treatments relative to the incompatible combination (Fig. 3).

During the hypersensitive response both in rice to Magnaporthe grisea (26, 30) and in cotton to X. campestris pv malbacearum (33), total PO activity increased. Furthermore, in both of these cases, increases in PO activities were correlated not only with the accumulation of brown materials and toxic phenolic compounds, but also with the death or restriction in movement or multiplication of the invading pathogen in the incompatible combination. In our studies, total PO activity increased during the incompatible interaction between rice and X. oryzae pv oryzae, as brown pigments and lignin were forming in leaves of cvs carrying gene Xa-10 for resistance to bacterial blight (21, J.E. Leach, R. Hammerschmidt, unpublished).

Although numerous reports relate increases in PO activity to infection, total PO activity did not precisely characterize the process. This is because the total PO activity is the combination of activities of several different isoperoxidases both in intra- and extracellular locations (14). In our studies, POs were extracted from extracellular spaces of intact leaves. Low levels of G6P-D in extracellular extracts compared to whole cell extracts indicated that little cytoplasmic contamination occurred. Furthermore, although PO activity in the extracellular extracts increased significantly over time during the experiment, there was no increase in extracellular G6P-D activity.

To determine if increases in the activity of specific isoenzymes accounted for the increase in total PO activity, the isoenzyme profile of rice leaf POs was monitored by IEF gel electrophoresis during time course experiments. The number of bands with PO activity was higher than the seven previ-
Peroxisomal activity may be involved in the defense response. In fact, bactERICidal activity can be generated in vitro by PO in the presence of phenolic compounds and H2O2 (31). In cotton leaves, toxic oxidation products accumulate to bactERICidal levels as a result of reactions catalyzed by PO. Oxidized lignin precursors with bactERICial activity are also found in leaves of healthy, bacterial blight-resistant rice cultivars (9). Perhaps such compounds accumulate to higher levels during the incompatible interaction as a result of the increase in PO activity.

The peroxidase activity increased during the interval in which lignin accumulation peaked in tissues undergoing the resistant response (21). Although it has not been demonstrated that PO-C1 is involved in lignin synthesis in vivo, the lignin precursor CA was utilized as a substrate by this isoenzyme in gel assays. This result suggests that lignin precursors in rice leaves may be converted to free radical intermediates by PO-C1. There is similar evidence from a dicot species for the involvement of a cationic isoperoxidase in lignification (2). PO-C1 also may be involved in generating the greater quantities of H2O2 necessary for lignification to proceed, as has been described for PO-Cs from tobacco (14).

The PO-C1 isoenzyme also reached high levels of activity in the compatible treatments (race 1 in Cas 209 or IR-BB10; race 1 or race 2 in IR24), but this accumulation occurred approximately 24 h later than in the incompatible combination (Fig. 4). The host cells that actually produced the PO-C1 activity were not identified. However, host cells within the infiltrated site appeared to be dead at this time, based on their inability to retain the dye fluorescein (P.J. Reimers and J.E. Leach, unpublished). If PO-C1 was produced by healthy cells near the infiltrated site of the compatible interaction, it had little effect on defense, because these cells later succumbed to the infection (21).

Alternatively, if PO-C1 was released from the dying host cells during the compatible interaction, this apparently occurred too late for any potential contribution to the defense response. Lignin was not detected within the site of the compatible interaction (21), even though the PO-C began to accumulate in extracts from those tissues 36 h after infiltration. Low levels of PO-C1 also were detected in extracts from control (water-infiltrated) leaves, so it is clear that this PO was produced by rice tissues and not by X. oryzae pv oryzae.

An increase in PO activity has been associated with infection in numerous studies (5, 6, 17, 26, 33), and some attribute this to a general resistance response (16, 23–25, 30). However, only a few reports link the accumulation of PO isoenzymes to race-specific resistance (17, 26, 33). We monitored changes in the PO isoenzyme profile over time during a race-specific interaction between X. oryzae pv oryzae and rice. Our results indicated that the incompatible interaction between race 2 of X. oryzae pv oryzae and rice cultivars containing bacterial blight resistance gene Xa-10 involved a rapid appearance or increase in the activities of one cationic and two anionic PO isoenzymes.

Although the evidence suggests that gene Xa-10 influences the accumulation of specific PO isoenzymes in extracellular spaces, the significance of this phenomenon is not known. Furthermore, it is not clear how the resistance gene may cause this effect. PO accumulation simply may be a consequence of, but not a determinant in, the primary defense response (17, 24). Our working hypothesis is that PO isoenzymes (such as PO-C1, PO-A1, and PO-A2) catalyze the synthesis of lignin within rice leaves, perhaps generating a weakly bactERICial condition in uninfected host tissues. At elevated levels, such as those observed early in the incompatible interaction, lignification and associated reactions escalate, perhaps producing highly bactERICial conditions that lead to the restriction of bacterial multiplication (21).

Our current efforts in molecular characterization of PO-C1
may provide insight into the significance of this isoperoxidase to the defense response in rice. This, in turn, may clarify our understanding of the physiological and molecular bases of host-parasite interactions in bacterial diseases of monocots such as rice.

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LITERATURE CITED