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Multiple Periplasmic Catalases in Phytopathogenic Strains of *Pseudomonas syringae*†

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Phytopathogenic strains of *Pseudomonas syringae* are exposed to plant-produced, detrimental levels of hydrogen peroxide during invasion and colonization of host plant tissue. When *P. syringae* strains were investigated for their capacity to resist H_2O_2, they were found to contain 10- to 100-fold-higher levels of total catalase activity than selected strains belonging to nonpathogenic related taxa (*Pseudomonas fluorescens* and *Pseudomonas putida*) or *Escherichia coli*. Multiple catalase activities were identified in both periplasmic and cytoplasmic fluids of exponential- and stationary-phase *P. syringae* cells. Two of these activities were unique to the periplasm of *P. syringae* pv. glycinea. During the stationary growth phase, the specific activity of cytoplasmic catalases increased four- to eightfold. The specific activities of catalases in both fluids from exponential-phase cells increased in response to treatment with 0.25 to 10 mM H_2O_2 but decreased when higher H_2O_2 concentrations were used. In stationary-growth phase cultures, the specific activities of cytoplasmic catalases increased remarkably after treatment with 0.25 to 50 mM H_2O_2. The growth of *P. syringae* into stationary phase and H_2O_2 treatment did not induce synthesis of additional catalase isozymes. Only the stationary-phase cultures of all of the *P. syringae* strains which we tested were capable of surviving high H_2O_2 stress at concentrations up to 50 mM. Our results are consistent with the involvement of multiple catalase isozymes in the reduction of oxidative stress during plant pathogenesis by these bacteria.

The species *Pseudomonas syringae* consists of at least 40 host range variants or pathovars that cause diseases in characteristic host plant species but induce defense reactions in other plants (6). Critical to the development of plant disease during compatible interactions is the ability of *P. syringae* to multiply and colonize plant tissue (20). Oxidative stress induced by active oxygen species (·OH, H_2O_2, O_2^-) may be part of the defensive strategy which plants use to limit colonization by invading pathogens (5, 33), such as *P. syringae* (1, 16–18, 21a). One initial response of invaded plant tissue during both compatible and incompatible interactions with *P. syringae* is elevated production of H_2O_2 (17), whereas the levels of superoxide and hydroxyl radicals increase during later phases of incompatible interactions in which disease fails to develop (1, 16, 21a). Multiplication of *P. syringae* in planta can be significantly enhanced by prior infiltration of tissue with agents that reduce free active oxygen, such as catalase, superoxide dismutase, or Fe-chelating and hydroxyl radical-quenching agents (1, 16, 18, 21a). The same agents also inhibit development of the hypersensitive response (1, 5, 16, 18, 21a), a rapid necrosis that is generally associated with the induction of defense reactions during incompatible interactions (20).

The capacity of phytopathogenic bacteria to multiply in plant tissue may be due, in part, to the ability of these organisms to detoxify H_2O_2. In contrast to other active oxygen species, H_2O_2 can penetrate through membranes to affect a variety of cellular processes. A likely candidate to modulate H_2O_2 is catalase, which enzymically converts H_2O_2 to H_2O and O_2. Although it has been known for many years that strains of *P. syringae* have catalase activity (6), little is known about the physical and genetic properties of the catalases of these bacteria. Exogenous catalase or crude extracts of *P. syringae* pv. phaseolicola which contain catalase activity have been shown to decrease plant isoperoxidase activity and active oxygen-dependent senescence during pathogenesis (30). *Pseudomonas aeruginosa* appears to contain multiple catalases, some of which are inducible (11). Katsuwon and Anderson (15) have reported that the catalase activity of the saprophytic soil bacterium *Pseudomonas putida* increases after contact with legume roots. The critical role of specific isozymes in the survival of this bacterium in the presence of H_2O_2 has been demonstrated recently (21).

The only gram-negative bacterium whose catalase complement has been studied in depth is *Escherichia coli* (3, 4, 6, 9, 12, 14, 22–27, 32). *E. coli* is known to contain two hydroperoxidases, HPI and HPII, which are encoded by noncontiguous loci and exhibit distinct physical and enzymic properties (3, 22, 25–27, 32). HPII, a monofunctional atypical catalase that is restricted to the cytoplasm (12, 22), is a hexamer of 93-kDa subunits (23). HPII levels increase substantially in stationary-phase cultures, but do not respond to H_2O_2 (9). The bifunctional hydroperoxidase, HPI, is a tetramer of 78-kDa subunits that is induced by H_2O_2 and is associated with the plasma membrane in *E. coli* (4, 12, 25, 26). The *E. coli* catalases exhibit only limited homology with catalases from other bacteria belonging to the family *Enterobacteriaceae* (34). Three distinct catalase isozymes have been described in *Bacillus subtilis* (24).

In this study we characterized the catalase complements of phytopathogenic strains of *P. syringae* to examine the role of the catalases during tissue colonization. We observed that *P. syringae* strains have multiple bands of catalase activity, which suggests that there are multiple catalase isozymes. Unique to *P. syringae*, some of these activities were detected only in the periplasmic fluids. Moreover, in contrast to other bacterial species, the strains belonging to different *P. syringae* pathovar groups exhibited an unexpected diver-

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TABLE 1. Bacterial strains and plasmids used in this study

Strain	Relevant characteristics and plant host (if pathogenic)	Reference or source
E. coli strains		
HB101	F' *hsd-20 recA13 thr leu thi pro* Sm^r	31
NM522(pAM*katE72*)	Ap^r *recA supE* Δ(*lac-proAB*) *hsd5* (F' *proAB lacI9 lacZ*Δ*15*)	27
P. putida 2440		T. Kosuge
P. fluorescens 55	Nal^r	13
P. syringae pv. glycinea race 4	Ap^r, soybean	N. T. Keen
P. syringae pv. glycinea race 6	Soybean	N. T. Keen
P. syringae pv. syringae 61	Nal^r, bean	13
P. syringae pv. phaseolicola AN201	Nal^r, bean	A. Novacky
P. syringae pv. savastanoi EW2009	Nal^r, olive	4a
P. syringae pv. lachrymans AN101	Nal^r, cucumber	18
P. syringae pv. pisi ATCC 11055	Nal^r, pea	18
P. syringae pv. tabaci ATCC 11528	Nal^r, tobacco	A. Novacky

sity in their catalase isozyme patterns and in their compartmentation.

MATERIALS AND METHODS

Culture conditions for bacteria. The bacterial strains used in this work and their characteristics are shown in Table 1. *E. coli* and *Pseudomonas* strains were grown to different growth phases in LB broth at 37°C and in King medium B at 28°C, respectively (19, 31). Autoclaved media were supplemented with filter-sterilized antibiotics when they were required at the following concentrations: ampicillin, 50 μg/ml; and nalidixic acid, 25 μg/ml. Chemicals were obtained from the Sigma Chemical Co., St. Louis, Mo., except where indicated otherwise. To study survival after exposure to exogenous H_2O_2, bacteria were harvested in 5-ml aliquots at different population densities by centrifugation at 5,000 × *g* for 10 min, washed with 10% (vol/vol) glycerol, and resuspended in 5 ml of fresh medium in 14-ml culture tubes. The cultures were then exposed for 15 min to H_2O_2 (final concentrations, 0.25 to 50 mM), which was added to the culture tubes from a 30% (vol/vol) stock solution. The bacteria in the H_2O_2-treated and control samples were washed with fresh growth solution and used for further analysis (fractionation or survival studies).

Fractionation of bacteria. Bacteria were harvested from cultures in the logarithmic growth phase (2×10^8 cells per ml) or the stationary growth phase (2×10^9 cells per ml) by centrifugation at 5,000 × *g* for 10 min and then washed with buffer I (10 mM Tris HCl, 30 mM $MgCl_2$; pH 7.3) prior to further manipulation. To prepare crude lysates, the washed bacteria were suspended in buffer II (50 mM potassium phosphate buffer, pH 7.5) and frozen at −20°C. Thawed cells were lysed by sonication and filtered, and the filtrate was clarified by centrifugation at 15,000 × *g* and 4°C for 60 min. For fractionation into periplasmic, cytoplasmic, and membrane fractions, the washed bacteria from 25 ml of culture solution were suspended in 1 ml of buffer I, and 15 μl of chloroform was added (7). After 15 min at 4°C, an additional 1 ml of ice-cold buffer I was added, and the solution was clarified by centrifugation at 10,000 × *g* for 10 min at 4°C. The resulting supernatant was used as the periplasmic fraction. The pelleted cells were lysed by sonication and separated into cytoplasmic and membrane fractions by centrifugation at 35,000 × *g* for 60 min at 4°C. The periplasmic and cytoplasmic fluids were filter sterilized (pore size, 0.22 μm; msi, Westborough, Mass.), and all fractions were stored at <5°C.

Protein and enzyme activity assays. The protein contents of the fractions were measured by using the procedure of Bradford (2) at 596 nm, a Hewlett-Packard model HP8452A diode array spectrophotometer, and bovine serum albumin as the standard.

Glucose-6-phosphate dehydrogenase activity was determined by measuring the increase in A_{340} in an assay solution containing 10 μl of a fraction, 2.7 mM $NADP^+$, and 18 mM glucose 6-phosphate in 50 mM Tris HCl buffer (pH 8.9).

The total catalase activity was measured polarographically as oxygen evolution (29) by using a biological oxygen monitor (Yellow Springs Instrument Co., Yellow Springs, Ohio) equipped with a Clark type of electrode in buffer II (pH 6.8) containing 0.083% (vol/vol) H_2O_2; 1 U of activity was equivalent to 1 μmol of O_2 evolved per min. Buffer II was adjusted to other pH values by using saturated KOH or H_3PO_4. The kinetic properties of catalase were determined by adding 0.01 to 0.2% H_2O_2 to the assay medium to initiate the reaction. The absolute and specific activities were calculated, and substrate dependence was monitored by using Lineweaver-Burk equations.

Visualization of catalase activity on gels. Bacterial lysates were prepared as described above and were fractionated in discontinuous, nondenaturing, 6% polyacrylamide gels by using the procedure of Hedrick and Smith (11). After polyacrylamide gel electrophoresis (PAGE), the gels were washed with distilled water three times (20 min each) to remove surface-attached buffer ions and were treated with 0.003% H_2O_2 for 10 min (35). Activity was then visualized by transferring the gels to a solution of 1% (wt/vol) ferric chloride-potassium ferricyanide until the gel background was stained green. Since this staining procedure is only qualitative (35), the intensities of the bands could not be used for correlation with the catalase activities loaded onto the gels.

Survival studies. After the treatments, the numbers of colony-forming units were determined by dilution plating the preparations onto King medium B agar plates supplemented with the appropriate antibiotics. The levels of survival for H_2O_2-treated bacteria were expressed as the percentages of colony-forming units recovered compared with untreated control samples.

RESULTS AND DISCUSSION

Crude cell lysates from several *P. syringae* strains were surveyed polarographically (29) for catalase activity. In lysates of exponential-phase *P. syringae* cells, substantial

TABLE 2. Catalase activities in lysates of *P. syringae* strains during different growth phases and in non-phytopathogenic bacterial strains

Strain	Catalase activity (U/10^9 cells) in bacterial lysates obtained from cultures during[a]:	
	Exponential growth	Stationary growth
P. syringae pv. glycinea race 4	131 ± 4	1,141 ± 62
P. syringae pv. glycinea race 6	168 ± 19	593 ± 38
P. syringae pv. syringae 61	138 ± 15	681 ± 27
P. fluorescens 55	2 ± <1	13 ± 4
P. putida 2440	13 ± 2	6 ± 2
E. coli HB101	<1	55 ± 7
E. coli NM522(pAM*katE72*)	<1	516 ± 91

[a] Values are means ± standard deviations of data from three separate experiments.

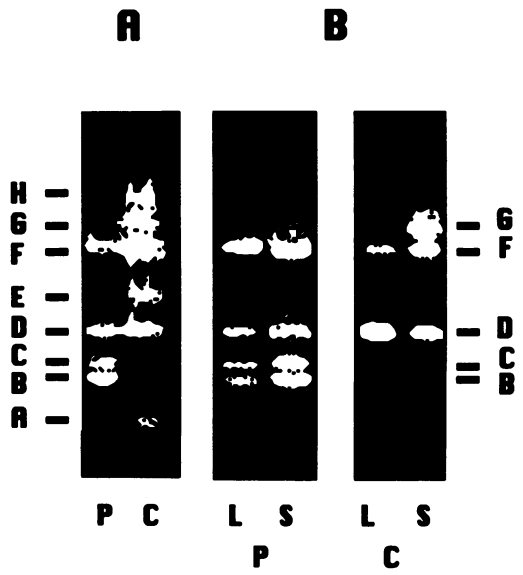

FIG. 1. Catalase activity bands on gels after discontinuous nondenaturing electrophoresis (native PAGE) of periplasmic and cytoplasmic fluids from *P. syringae* pv. glycinea race 4 at different growth phases. (A) Low-current, long-term native PAGE of periplasmic (P) and cytoplasmic (C) fluids from stationary-phase cells. (B) High-current, short-term native PAGE (Minigel) of periplasmic (P) and cytoplasmic (C) fractions from cultures in logarithmic phase (L) or stationary phase (S). The positions of activity bands A through H are indicated on the sides (see text).

levels of catalase activity were detected (Table 2). The total catalase activities in *P. syringae* strains were 10- to 100-fold higher than the activities in selected strains of nonpathogenic fluorescent pseudomonads, such as *Pseudomonas fluorescens* 55 and *P. putida* 2440, or *E. coli*. *P. syringae* catalase activity also depended on the growth phase of the culture, as has been reported previously for *E. coli* (9), *P. putida* (15, 21), and *P. aeruginosa* (11). The total activity in *P. syringae* lysates increased four- to eightfold as the cultures entered the stationary phase (Table 2).

Nonlinear substrate dependence during kinetic analyses of catalase activity in crude lysates of *P. syringae* suggested that *P. syringae* strains may contain multiple catalase species (data not shown). To determine the number of catalase isozymes in *P. syringae* strains, crude lysates were fractionated by native PAGE. Visualization of catalase activity on gels demonstrated that there were multiple bands of catalase activity in all of the *P. syringae* lysates which we tested. For example, eight catalase activity bands, bands A through H, were apparent in lysates of *P. syringae* pv. glycinea race 4 (Fig. 1A). Whole-cell lysates from other *P. syringae* strains produced two (*P. syringae* pv. phaseolicola), three (*P. syringae* pv. lachrymans and *P. syringae* pv. tabaci), four (*P. syringae* pv. pisi), five (*P. syringae* pv. savastanoi), or six (*P. syringae* pv. glycinea race 6 and *P. syringae* pv. syringae 61) catalase activity bands on the gels (data not shown).

Periplasmic, cytoplasmic, and membrane fractions from *P. syringae* strains were prepared to localize the apparent catalase isozymes in the bacterial cells. Periplasmic fractions were obtained by using the chloroform extraction method that was originally developed for *E. coli* strains (7). The periplasmic fluids extracted from *P. syringae* contained less than 3% of the total glucose-6-phosphate dehydrogenase activity (data not shown). Since glucose-6-phosphate dehydrogenase is thought to be exclusively cytoplasmic (28), the chloroform extraction method could be used to work with *P. syringae* strains. Much to our surprise, phytopathogenic strains of *P. syringae* contained considerable catalase activity in their periplasmic fractions. During logarithmic growth, the catalase activity in *P. syringae* pv. glycinea race 4 was equally distributed between the periplasmic and cytoplasmic fractions (Table 3). Similar results were obtained with strains of *P. syringae* pv. glycinea race 6, *P. syringae* pv. syringae 61 (Table 3), *P. syringae* pv. lachrymans, *P. syringae* pv. phaseolicola, *P. syringae* pv. pisi, *P. syringae* pv. tabaci, and *P. syringae* pv. savastanoi (data not shown). The periplasmic catalase activities in the *P. syringae* strains were greater than the activities detected in the total lysates of the strains of *E. coli* which we surveyed, including strains HB101 (Table 3), CC118, TB1, and DH5α (data not shown). Low levels of periplasmic catalase activity were also detected in other fluorescent pseudomonads, such as *P. fluorescens* 55, *P. putida* 2440 (Table 3), and *P. putida* Corvallis (21), but not in *E. coli* HB101, DH5α, or NM522 (pAM*katE72*). Strain NM522(pAM*katE72*) is a genomic Cat^- mutant which carries a plasmid-borne *katE* gene that overproduces the strictly cytoplasmic isozyme HPII (12, 23, 27). Screening experiments to determine peroxidase activity by using guaiac alcohol yielded negative results, in agreement with the results of Anderson (1a).

After native PAGE, distinct catalase activity bands were obtained from periplasmic and cytoplasmic fractions. The addition of thioglycolate to the upper tank buffer during native PAGE did not change the number of activity bands (data not shown). Therefore, the number of activity bands did not appear to reflect protein degradation caused by the electrophoresis conditions. The eight catalase activity bands of *P. syringae* pv. glycinea race 4 were used to tentatively designate *P. syringae* catalase isozymes (Fig. 1A). Catalase activity bands B and C were unique to the periplasmic fluids from *P. syringae* pv. glycinea races 4 and 6 and *P. syringae* pv. savastanoi. Comigrating bands were present in the periplasmic and cytoplasmic fluids of *P. syringae* pv. syringae 61 but were not detected in either fluid of any other strain (Fig. 2). Band D (Fig. 1A) appeared to be present in both fractions of all strains except *P. syringae* pv. syringae 61 at equivalent levels. Bands E, G, and H were primarily cytoplasmic and unique to *P. syringae* pv. glycinea race 4. Bands E and H appeared only after native PAGE performed under

TABLE 3. Catalase activities in fractions of *P. syringae* strains and other bacterial strains

Strain	Catalase activity (U/10^9 cells) obtained from cultures during[a]:							
	Exponential growth				Stationary growth			
	Total lysate	Periplasmic fluid	Cytoplasmic fluid	Membrane fraction	Total lysate	Periplasmic fluid	Cytoplasmic fluid	Membrane fraction
P. syringae pv. glycinea race 4	133	64	54	15	1,131	34	667	430
P. syringae pv. glycinea race 6	182	74	102	6	570	40	325	205
P. syringae pv. syringae 61	150	45	93	12	611	31	350	230
P. fluorescens 55	2	<1	<1	<1	13	3	3	7
P. putida 2440	13	5	4	3	7	3	2	2
E. coli HB101	<1	<1	<1	<1	60	<1	50	10
E. coli NM522(pAM*katE72*)	<1	<1	<1	<1	460	<1	451	5

[a] The values are one set of results from three experiments which yielded similar results (standard error, ≤7%).

long-term, low-constant-current conditions (Fig. 1A) and not after short-term (higher-current; Minigel) PAGE (Fig. 1B); thus, these bands may represent active, natural degradation products of the cytoplasmic catalase activities in bands D, F, and G (Fig. 1). The intensity of band A increased with the age of the preparation concomitantly with a loss of activity band F intensity, and therefore, band A may represent a degradation product of the activity in band F. It has not been established whether catalase activity band F represents separate periplasmic and cytoplasmic isozymes or whether an isozyme, CatF, is present in both compartments. A comparison of the activity patterns of the two fractions (Fig. 1) suggested that periplasmic CatF is most likely not a contaminant from the cytoplasm. Furthermore, partial purification of the periplasmic catalase isozymes from *P. syringae* pv. glycinea race 4 by using a combination of hydroxylapatite, ion-exchange, and sizing column chromatography revealed that there were three discrete isozymes, CatF, CatD, and CatC, which had different physical and enzymic properties (21b). The results of these experiments suggested that bands B and C represent two isoforms of isozyme CatC that have different enzymatic stabilities. From the results of an analysis of electrophoretic mobilities in native polyacrylamide gels that had different monomer contents (9), as well as the results of gel filtration, we predicted the following holoenzyme masses: CatF, 196 kDa; CatD, 170 kDa; $CatC_I$, 158 kDa; and $CatC_{II}$, 143 kDa (21b). These apparent masses are substantially smaller than those of *E. coli* hydroxyperoxidases HPI and HPII (23, 27) but are similar to the apparent mass of catalase KpA from *Klebsiella pneumoniae* (8).

We detected considerable variation among the periplasmic catalase isozymes in the *P. syringae* strains (Fig. 2). CatD and CatF were present in most of the *P. syringae* strains which we investigated. However, CatC was missing in several *P. syringae* strains, such as *P. syringae* pv. phaseolicola (Fig. 2, lane e), *P. syringae* pv. pisi (lane f), *P. syringae* pv. tabaci (lane g), and *P. syringae* pv. lachrymans (lane h). It has not been established yet whether unique catalase

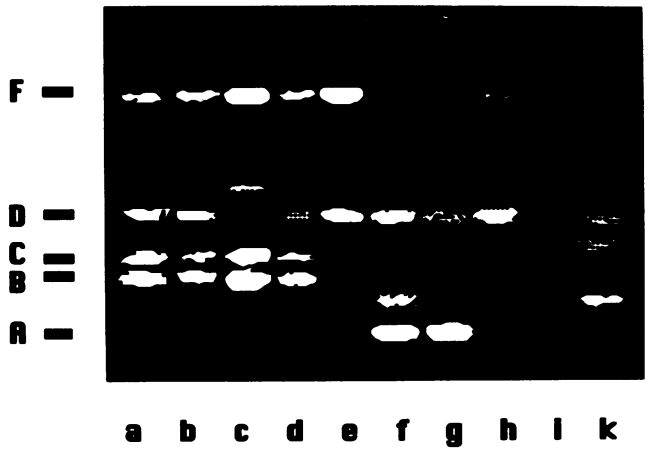

FIG. 2. Periplasmic catalase isozyme activities of selected *P. syringae* strains after low-current, long-term native PAGE. The lanes contained periplasmic fractions from *P. syringae* pv. glycinea race 4 (lane a) and race 6 (lane b), *P. syringae* pv. syringae 61 (lane c), *P. syringae* pv. savastanoi (lane d), *P. syringae* pv. phaseolicola (lane e), *P. syringae* pv. pisi (lane f), *P. syringae* pv. tabaci (lane g), and *P. syringae* pv. lachrymans (lane h). For comparison, periplasmic fluids from *E. coli* HB101 (lane i) and *P. fluorescens* 55 (lane k) were included.

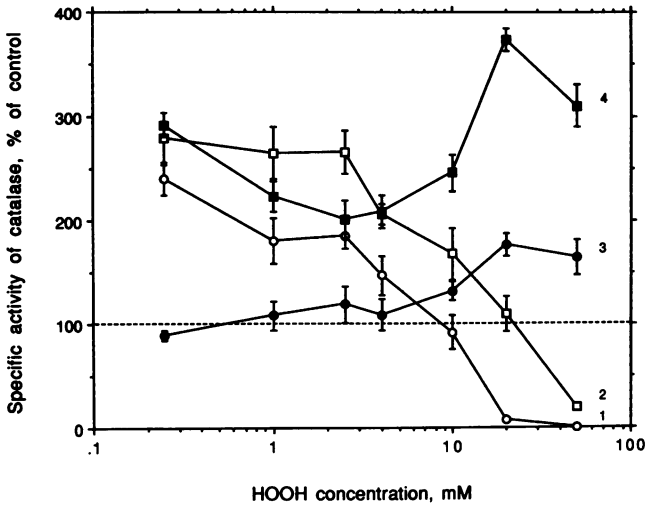

FIG. 3. Catalase activities in periplasmic (circles) and cytoplasmic (squares) fluids from logarithmic-phase (open symbols) and stationary-phase (solid symbols) cultures of *P. syringae* pv. glycinea race 4 which were prepared after 15 min of exposure of the bacteria to different concentrations of H_2O_2. The values are percentages of the values for untreated controls (defined as 100%). The specific activities of the catalases from untreated bacteria were as follows: line 1, $0.72 \times 10^3 \pm 0.031 \times 10^3$ U/mg; line 2, $2.91 \times 10^3 \pm 0.068 \times 10^3$ U/mg; line 3, $1.282 \times 10^3 \pm 0.019 \times 10^3$ U/mg; and line 4, $4.744 \times 10^3 \pm 0.117 \times 10^3$ U/mg.

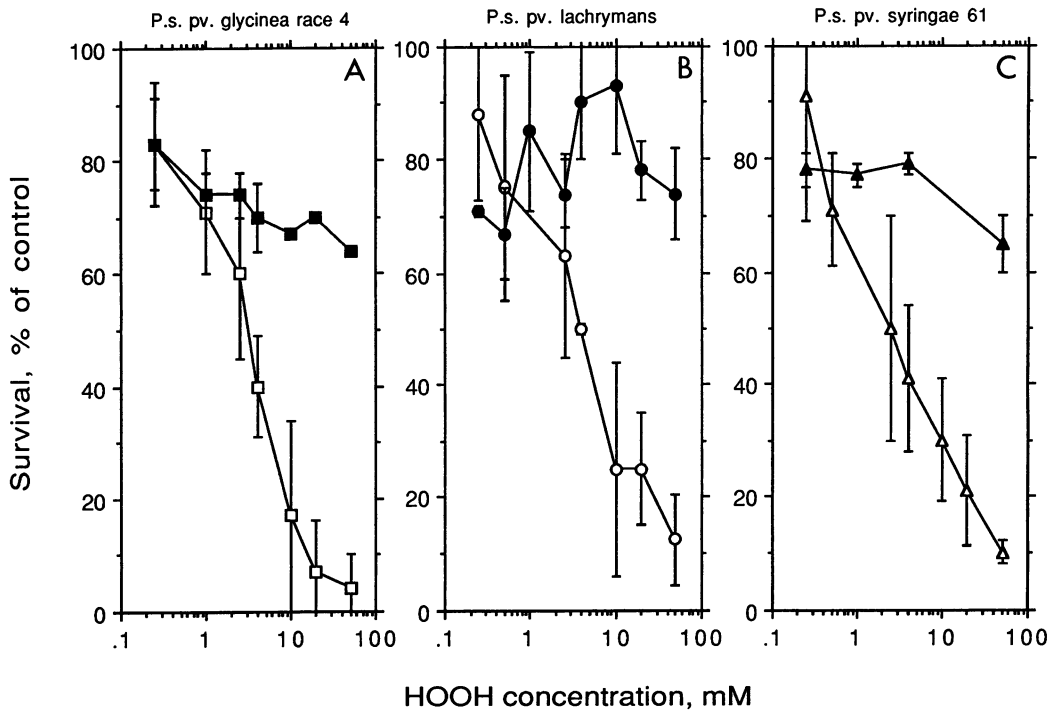

FIG. 4. Survival of *P. syringae* pv. glycinea race 4 (A), *P. syringae* pv. lachrymans (B), and *P. syringae* pv. syringae 61 (C) during exponential (open symbols) and stationary (filled symbols) growth phases after 15 min of exposure to HOOH. Treated and control cultures were washed and dilution plated onto antibiotic-supplemented agar plates. The values are percentages of the control values and are means ± standard deviations from three independent experiments in which we performed four determinations of the number of colony-forming units for two different dilution steps.

activities present in *P. syringae* pv. pisi and *P. syringae* pv. tabaci are derivatives of CatC or CatF or are distinct isozymes. The lower activity band co-migrated with band A from *P. syringae* pv. glycinea race 4. The catalase isozyme pattern of *P. fluorescens* 55 was distinct from the isozyme patterns of the *P. syringae* strains, but was similar to the isozyme patterns of other strains belonging to the *P. fluorescens-P. putida* group, as reported previously by Katsuwon and Anderson (15). The activity of HPI and HPII in *E. coli* lysates did not comigrate on gels with any of the apparent catalase isozymes of the fluorescent pseudomonads. The observed variation in catalase isozymes among the strains which we tested may be a useful diagnostic feature for plant-associated bacterial strains. The assay is relatively quick (about 3 h) and inexpensive and could be used as a marker for specific bacterial strains.

The levels of the cytoplasmic isozymes were affected by the growth phases of the cultures. The catalase specific activities in cytoplasmic fractions increased three- to eightfold during growth into stationary phase (e.g., from 1,400 U/mg at a density of 3×10^8 cells per ml to 7,600 U/mg at a density of 3×10^9 cells per ml). The total catalase activity in the cytoplasmic fraction increased more than 10-fold during this period (Table 3). Periplasmic catalase activity declined during late logarithmic growth and remained relatively stable during the transition to the stationary phase (data not shown). The high catalase activities observed in lysates from stationary-phase cultures of *P. syringae* pv. glycinea race 4 appeared to be caused predominantly by the increased activity of CatF in the cytoplasm (Fig. 1B).

Treatment of the *P. syringae* cultures for 15 min with 0.25 to 50 mM HOOH did not induce the synthesis and accumulation of additional catalase isozymes. On the other hand, we observed significant increases in the total and specific activities of catalases in the periplasmic and cytoplasmic fluids of the *P. syringae* strains which we tested, such as *P. syringae* pv. glycinea race 4 (Fig. 3). This organism and other strains were also used to study the susceptibility of *P. syringae* to exogenous H_2O_2 during the logarithmic and stationary growth phases (Fig. 4). Logarithmic-growth phase cultures of *P. syringae* pv. glycinea race 4, *P. syringae* pv. syringae 61, and *P. syringae* pv. lachrymans (Fig. 4) were susceptible to H_2O_2 concentrations of more than 3 mM. Similar exogenous H_2O_2 concentrations (1 to 3 mM) have been reported to be critical for the initiation of killing "mode-one" in *E. coli* by Imlay and Linn (14). In contrast, stationary-growth phase cultures of *P. syringae* pv. glycinea race 4, *P. syringae* pv. syringae 61, and *P. syringae* pv. lachrymans (Fig. 4) tolerated up to 50 mM H_2O_2 with a survival rate of >60%. Similar results were obtained with *P. syringae* pv. phaseolicola and *P. syringae* pv. pisi strains (21). These results correlate with the much higher catalase activity observed during growth into stationary phase and the activation of catalase after H_2O_2 treatment (Fig. 3).

The results of our initial study of the catalase complements of phytopathogenic *P. syringae* strains suggest that a unique arsenal of catalase isozymes operates to combat oxidative stress. The high catalase activities, multiple isozymes, and unique periplasmic catalase activities correlate with the potential of these bacteria to colonize plant tissues. The catalase isozymes could serve to minimize oxidative stress caused by the H_2O_2 burst as observed during compatible interactions and, therefore, may be virulence factors that are necessary for plant pathogenesis by *P. syringae*.

There are currently studies under way at the molecular and enzymic levels to support this hypothesis.

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