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Screening of Nonfilamentous Bacteria for Production of Cutin-Degrading Enzymes

W. F. FETT,* H. C. GERARD, R. A. MOREAU, S. F. OSMAN, AND L. E. JONES

Plant Science Research Unit, Eastern Regional Research Center, Agricultural Research Service, U.S. Department of Agriculture, Philadelphia, Pennsylvania 19118

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Two hundred thirty-two nonfilamentous bacterial strains, including saprophytes, plant pathogens, and opportunistic plant and human pathogens, were screened for the ability to produce cutinases (cutin-degrading esterases). Initially, esterase activity of culture filtrates of strains grown in nutrient broth-yeast extract medium supplemented with 0.4% apple or tomato cutin was determined by a spectrophotometric assay utilizing the model substrate *p*-nitrophenyl butyrate. The culture filtrates of the 10 *Pseudomonas aeruginosa* strains tested exhibited the highest esterase activity, with values of >500 nmol/min/ml. Of these 10 strains, 3 (K799, 1499A, and DAR41352) demonstrated significant induction (10-fold or above) of esterase activity by addition of cutin to nutrient broth-yeast extract medium. The ability of culture filtrates of the three strains to cause release of apple cutin monomers was confirmed by a novel high-performance liquid chromatography technique. Monomer identification was confirmed by gas chromatography-mass spectroscopy analyses. Addition of the nonionic detergent *n*-octylglucoside stimulated cutinase activity of culture filtrates from strains K799 and DAR41352, but not that of filtrates from strain 1499A. Time course studies in nutrient broth-yeast extract medium supplemented with apple cutin indicated maximal levels of cutinase in the culture fluids after cultures entered stationary phase. Incubation temperatures below the optimal temperature for growth (37°C) led to maximal production of cutinase.

Cutin is an insoluble plant biopolyester composed primarily of C_{16} and C_{18} hydroxy and epoxy fatty acids (13). Cutin comprises the structural component of the plant cuticle and occurs embedded in and covered by cuticular waxes (13). A cuticle of varying thickness and composition covers most aerial parts of plants, including leaves, fruits, flowers, and young stems. The cuticle is considered to function as a protective barrier against desiccation as well as insect and microbial attack and is the major barrier against absorption of foliar applied agrichemicals (12).

Several fungal pathogens of plants produce enzymes, called cutinases, which can hydrolyze cutin, resulting in release of chloroform-methanol-soluble cutin monomers. Many of these fungal cutinases have been purified and characterized, and an important role for cutinase production in penetration of intact plant surfaces by certain fungal pathogens has been demonstrated by the use of anti-cutinase antibodies or chemical inhibitors (for a review, see reference 13) as well as by molecular cloning techniques (6). Purified cutinase has been demonstrated to increase the permeability and decrease the mechanical strength of isolated cutin membranes (1).

In contrast to the growing body of literature on fungal cutinases, very little is known about the ability of bacteria to degrade cutin. There are published reports of bacterial cutinase production involving a total of three bacterial strains. Two bacterial cutinases have been purified and characterized. One cutinase producer is the potato tuber pathogen *Streptomyces scabies* (14). The cutinase produced by *S. scabies* is similar to fungal cutinases in molecular weight, amino acid composition, and carbohydrate content. The other well-characterized bacterial cutinase is produced by a strain of the saprophytic bacterium *Pseudomonas putida* isolated from the phylloplane (17, 18). The cutinase produced by this strain of *P. putida* differs from fungal cutinases in several respects, including amino acid composition and an increased heat stability. Both bacterial cutinases are similar to fungal cutinases in that they belong to a class of enzymes called serine hydrolases (14, 18). Bashan et al. (2) also reported evidence of cutinase production by the tomato pathogen *P. syringae* pv. tomato during infection of tomato leaves, but purification and characterization of this putative bacterial cutinase have not been reported.

The purpose of this study was to screen a large number of both gram-positive and gram-negative nonfilamentous bacteria for ability to produce cutinase. The bacteria screened included a large number of plant-associated bacteria, either saprophytes or pathogens, as this group of bacteria would seem to afford an excellent opportunity for isolation of new bacterial cutinases. For determination of cutinase activity, we utilized a novel, sensitive high-performance liquid chromatography (HPLC) technique developed in our laboratory (9). This technique precluded the previous requirement for the generation of radioactive cutin to use as a substrate. Results of a screening program with filamentous bacteria will be reported in a separate publication (8).

MATERIALS AND METHODS

Bacterial strains. The bacteria tested and the number of strains of each (given in parentheses) were as follows: *Bacillus brevis* (5), *B. cereus* (3), *B. pumilus* (2), *B. sphaericus* (5), *B. subtilis* (4), *Corynebacterium michiganense* (2), *C. sepedonicum* (5), *Cytophaga johnsonae* (8), *Erwinia amylovora* (3), *E. ananas* (3), *E. carotovora* subsp. *atroseptica* (1), *E. chrysanthemi* (2), *E. cypripedii* (3), *E. herbicola* (10), *E. mallotovora* (1), *E. nigrifluens* (2), *E. rhapontici* (3), *E. rubrifaciens* (1), *E. stewartii* (1), *E. tracheiphila* (2), *E. uredovora* (3), *Pseudomonas aeruginosa* (10), *P. cepacia*

* Corresponding author.

TABLE 1. Source and origin of strains of *P. aeruginosa*

Strain	Source	Origin
813	A. Linker	Clinical
1499A	A. Linker	Clinical
K799	R. E. W. Hancock	Clinical
AK1012	R. E. W. Hancock	Clinical
PAO1.AK.957	R. E. W. Hancock	Clinical
DAR41352	E. J. Cother	Diseased onion bulb
DAR41353	E. J. Cother	Diseased onion bulb
DAR41354	E. J. Cother	Soil (field of infected onions)
DAR41355	E. J. Cother	Diseased onion bulb
DAR41360	E. J. Cother	Diseased onion bulb

(7), *P. chlororaphis* (1), *P. cichorii* (1), *P. fluorescens* (21), *P. marginalis* (7), *P. putida* (10), *P. syringae* pv. glycinea (7), *P. syringae* pv. lachrymans (1), *P. syringae* pv. morsprunorum (1), *P. syringae* pv. papulans (5), *P. syringae* pv. phaseolicola (7), *P. syringae* pv. pisi (2), *P. syringae* pv. syringae (18), *P. syringae* pv. tagetis (1), *P. syringae* pv. tabaci (1), *P. syringae* pv. tomato (12), *P. putida* (11), *P. viridiflava* (23), *Pseudomonas* spp. (2), *Xanthomonas campestris* pv. campestris (3), *X. campestris* pv. cucurbitae (1), *X. campestris* pv. glycines (3), *X. campestris* pv. malvacearum (2), *X. campestris* pv. pelargonii (1), *X. campestris* pv. pruni (2), *X. campestris* pv. raphani (1), *X. campestris* pv. vesicatoria (1), and *X. campestris* pv. vignicola (1). The source and origin of the strains of *P. aeruginosa* are shown in Table 1.

Bacteria were maintained on Difco *Pseudomonas* agar F (fluorescent pseudomonads), yeast extract-dextrose-calcium carbonate agar (xanthomonads) (21), or Difco (Difco Laboratories, Detroit, Mich.) nutrient agar supplemented with Difco dextrose (10 g/liter) and Difco yeast extract (5 g/liter) (all others) at 4°C with monthly transfer. For long-term storage, bacteria were suspended in Trypticase soy broth (BBL Microbiology Systems, Cockeysville, Md.) containing 15% (wt/vol) glycerol and kept at −80°C.

Preparation of cutin. Cutin was prepared from mature fruits of apple (*Malus domestica* Borkh.) cv. Golden Delicious and of tomato (*Lycopersicon esculentum* L.) by a modification of the procedure of Walton and Kolattukudy (20), as described previously (9).

General screening for esterase and cutinase activity. All bacterial strains were screened for esterase production in nutrient broth-yeast extract (NBY) medium (Difco nutrient broth, 0.8% [wt/vol], and Difco yeast extract, 0.2% [wt/vol]) containing 0.4% (wt/vol) cutin (17). In addition, strains of *P. syringae* pv. tomato were also tested, using Difco nutrient broth supplemented with Difco dextrose (10 g/liter) and Difco yeast extract (5 g/liter) (NDYB) containing 0.4% cutin and a completely defined medium (GS) containing K_2HPO_4 (40 mM), KH_2PO_4 (15 mM), $MgSO_4 \cdot 7H_2O$ (8 mM), $(NH_4)_2SO_4$ (8 mM), $CaCl_2 \cdot 2H_2O$ (1 mM), and glycerol (0.02%, wt/vol), pH 7.2.

Culture tubes (20 by 150 mm) with 5 ml of broth medium containing cutin were inoculated with 0.1 ml of an overnight bacterial culture grown up in the same broth medium without cutin. Cultures were incubated with shaking (250 rpm) at room temperature, and culture fluids were periodically removed (100 μl for esterase assays, 1.5 ml for cutinase assays) during the incubation period. The fluids were clarified by centrifugation and assayed immediately or were stored at −20°C until used.

Esterase activity was determined by a spectrophotometric assay utilizing *p*-nitrophenyl butyrate (PNB) as substrate (17). The assay mixture (1-ml total volume) contained 2.3 mM PNB (Sigma Chemical Co., St. Louis, Mo.) and 0.2% (vol/vol) Triton X-100 (Sigma) in 50 mM potassium phosphate buffer, pH 8.0. From 1 to 50 μl of culture fluid was added, and the change in A_{405} was monitored for 1 min, during which time active preparations showed a linear increase in A_{405}. The rate of reaction was determined by reference to a standard curve prepared by adding various amounts of *p*-nitrophenol (the colored reaction product) to the assay mixture. Standard curves were prepared daily.

Cutinase activity was determined by adding 0.5 ml of culture fluid to 30 mg of apple cutin and 0.5 ml of 0.1 M potassium phosphate buffer, pH 8.0, contained in a screw-cap glass tube; a glass bead (3 mm) was added to facilitate mixing. For some assays, the detergent *n*-octylglucoside was added to a final concentration of 35 mM. Tubes were shaken (125 oscillations per min) for 18 h in a water bath held at 27°C. After incubation, released cutin monomers were extracted with acidified chloroform-methanol as described previously (9). The organic soluble material was collected and dried under a stream of nitrogen and taken up in 1 ml of chloroform-methanol (85:15, vol/vol). The samples were filtered through glass wool, dried, and again taken up in 1 ml of chloroform-methanol. Controls consisted of substituting uninoculated media, after removal of cutin by centrifugation, or buffer alone for culture fluid in the assays.

Released cutin monomers were separated and quantified by a new HPLC method developed in our laboratory (9). Quantification was based on a standard curve of area units versus weight for 9,10,18-trihydroxyoctadecanoic acid, which constituted approximately 15% of the monomers in our apple cv. Golden Delicious cutin preparations (9). The identification of the major released cutin monomers was confirmed by gas-liquid chromatography-mass spectroscopy (GC/MS). Individual peak materials were collected after HPLC, pooled and converted to the methyl esters by reaction with boron trifluoride in methanol in accordance with the manufacturer's (Pierce Chemical, Rockford, Ill.) instructions. Hydroxyl groups of the methyl esters were silylated with *N,O*-bis(trimethylsilyl)acetamide (Pierce) for 30 min at room temperature. The silylated methyl esters were examined by GC/MS on a Hewlett-Packard model 5990B instrument fitted with an Ultra 1 methyl silicone 12 M capillary column (Hewlett-Packard, Avondale, Pa.), using temperature programming from 150 to 250°C at 4°C/min.

Kinetics of esterase and cutinase production. Time course experiments were run in sterile 500-ml flasks containing 40 ml of NBY with or without addition of apple cutin (0.4%). For inoculation, 0.8 ml of an overnight bacterial culture grown in NBY alone was added, and cultures were incubated at 37°C with shaking (250 rpm). Growth was determined by removing 1-ml samples of culture fluid, letting the samples sit for a short time to allow any cutin present to settle to the bottom of the cuvette and then determining the optical density at 600 nm. Esterase and cutinase activities of the culture fluids were determined as stated above.

Effect of incubation temperature on esterase and cutinase production. Time course experiments were run in sterile 125-ml flasks containing 10 ml of NBY with apple cutin (0.4%). For inoculation, 0.2 ml of an overnight bacterial culture grown in NBY alone was added, and cultures were incubated at 22, 28, or 37°C with shaking (250 rpm). Duplicate flasks of inoculated media were incubated at each temperature, and esterase activity was determined as stated above. The experiment was repeated once.

Assay for lipase activity. The *P. aeruginosa* strains were

TABLE 2. Maximum esterase activity of *P. aeruginosa* during growth in NBY broth amended with apple cv. Golden Delicious cutin

Strain	Maximum esterase activity (nmol/min/ml)[a]	
	− Cutin	+ Cutin
813	1,200	866
1499A	577	7,200
K799	125	56,875
AK1012	1,200	1,100
PAO1.AK.957	350	1,300
DAR41352	780	26,650
DAR41353	980	900
DAR41354	1,000	780
DAR41355	640	530
DAR41360	1,200	880

[a] Cultures were incubated for 7 days at 28°C.

screened for the ability to produce lipase on solid media containing Tween 80 by the method of Sierra (19). *P. chloroaphis* ATCC 17813 was used as a positive control and *P. cichorii* P36 was used as a negative control (15). Plates were incubated at 37 or 28°C or both.

RESULTS

Esterase production. The production of esterase in broth media supplemented with cutin is easily followed by monitoring the ability of culture fluids to hydrolyze the ester bond present in the colorless substrate PNB, resulting in the release of *p*-nitrophenol, which absorbs at 405 to 410 nm. This ability is presumptive evidence of the presence of cutinase (16) as all reported cutinases, with one exception (3), can hydrolyze this substrate.

Two hundred thirty-two nonfilamentous strains of bacteria were tested for production of esterase in NBY supplemented with tomato cutin (strains of *P. syringae* pv. tomato) or apple cutin (all others). This collection contained both gram-negative and gram-positive bacteria which are saprophytic, plant pathogenic, or opportunistic plant and human pathogens. Of these strains, only the 10 strains of *P. aeruginosa* demonstrated esterase activities of >500 nmol/min/ml during the 7-day incubation period in NBY supplemented with cutin (Table 2). Most of the other strains gave values for esterase of <50 nmol/min/ml. Weak esterase production (≤100 nmol/min/ml) was exhibited by all 12 strains of *P. syringae* pv. tomato in NBY, NDYB, and GS media supplemented with tomato cutin. Of these three cutin-containing media, NDYB supported the best growth of *P. syringae* pv. tomato. The two pseudomonads, ATCC 21808 and ATCC 21809, listed as lipase producers in the ATCC catalogue also exhibited very low esterase activity when grown in NBY with apple cutin.

Of the 10 *P. aeruginosa* strains exhibiting high esterase activity in NBY with apple cutin, only 3 strains (1499A, K799, and DAR41352) demonstrated significant induction of esterase production (10-fold or above) when esterase values were compared in NBY broth with or without apple cutin (Table 2).

Confirmation of cutinase activity. To determine whether the three strains of *P. aeruginosa* which were high in cutin-inducible esterase production were also capable of cutin degradation, culture fluids were collected when esterase activity was at or near its maximum in NBY supplemented with apple cutin. These culture fluids were then

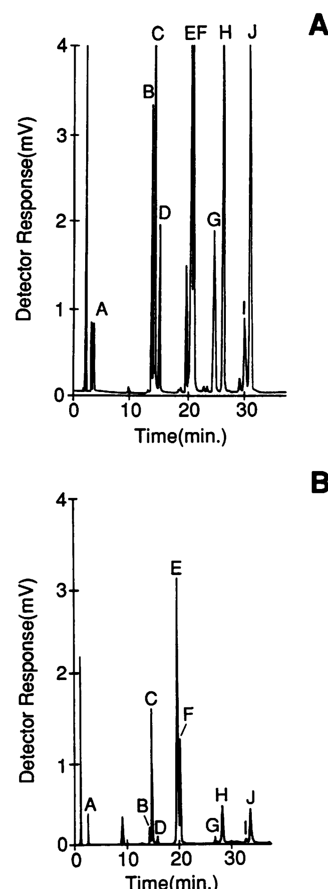


FIG. 1. Comparison of HPLC chromatograms of monomers released from apple cv. Golden Delicious cutin treated with either acidified methanolic KOH (A) or culture filtrate of *P. aeruginosa* DAR41352 grown in NBY supplemented with 0.4% apple cutin (B). Identity of cutin monomers: A, hexadecanoic plus octadecanoic acids; B, 18-hydroxyoctadeca-9,12-dienoic acid; C, 18-hydroxyoctadeca-9-enoic acid; D, 16-hydroxyhexadecanoic acid; E and F, 9,10-epoxy-18-hydroxyoctadecanoic acid and 9,10-epoxy-18-hydroxyoctadeca-12-enoic acid; G, 9,18-dihydroxyoctadecanoic acid; H, 10,18-dihydroxyoctadecanoic acid; I, 9,10,18-trihydroxyoctadeca-12-enoic acid; J, 9,10,18-trihydroxyoctadecanoic acid.

tested for their ability to release cutin monomers from apple cutin by HPLC. In preliminary experiments, the addition of *n*-octylglucoside to the assay mixture was found to be stimulatory towards cutin monomer release by culture fluids of strains K799 and DAR41352, but inhibitory for release by fluids of strain 1499A. For all subsequent studies, the detergent was added to the assay mixtures containing culture fluids from strains K799 and DAR41352. The culture fluids of all three strains catalyzed the release of monomers characteristic of apple cv. Golden Delicious cutin as determined by HPLC analysis (9, 11). Chromatograms of monomers released by standard chemical hydrolysis and enzymatic hydrolysis are shown in Fig. 1. Identification of the five major monomers present in apple cutin was confirmed by GC/MS analyses (Fig. 2) (11).

Because of the reported ability of *P. syringae* pv. tomato to produce cutinase (2), culture fluids of all strains of this pathovar grown in NDYB plus tomato cutin were also tested for cutinase activity by the HPLC technique even though the culture filtrates were low in esterase activity. None of the *P.*

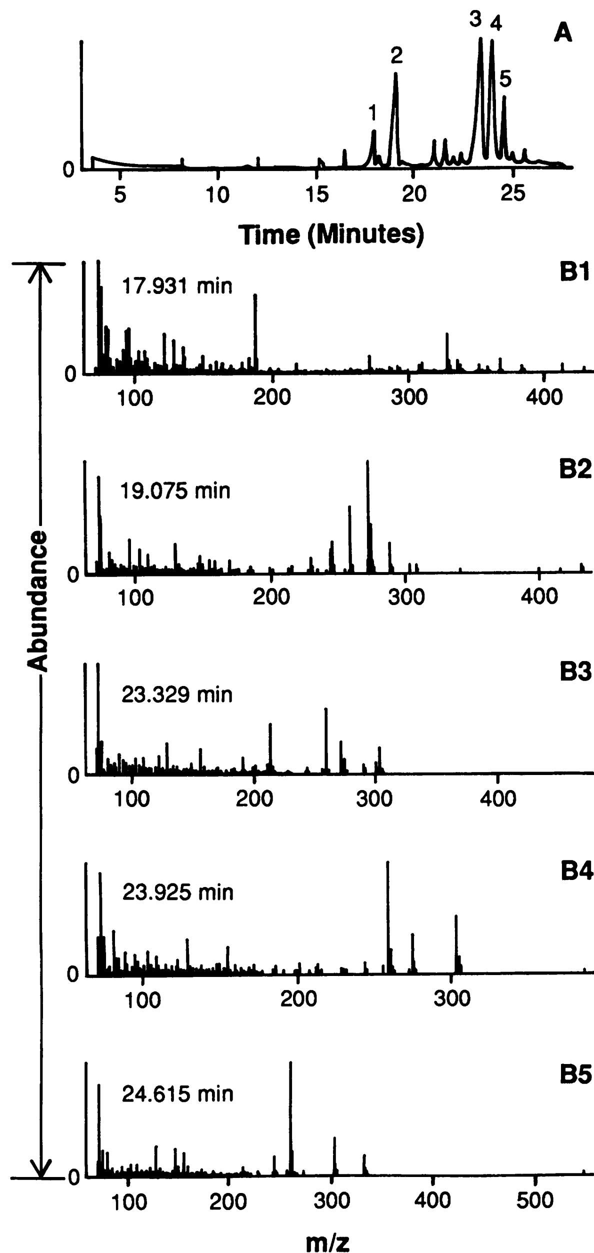


FIG. 2. GC profile (A) and GC/MS (B) of methylated cutin monomers released from apple cutin after incubation with culture fluid of *P. aeruginosa* K799. Identity of cutin monomers: 1, 18-hydroxyoctadeca-9,12-dienoic acid; 2, 10,16-dihydroxyhexadecanoic acid; 3, methoxyhydrins derived from 9,10-epoxy-18-hydroxyoctadeca-12-enoic acid; 4, methoxyhydrins derived from 9,10-epoxy-18-hydroxyoctadecanoic acid; 5, 9,10,18-trihydroxyoctadecanoic acid.

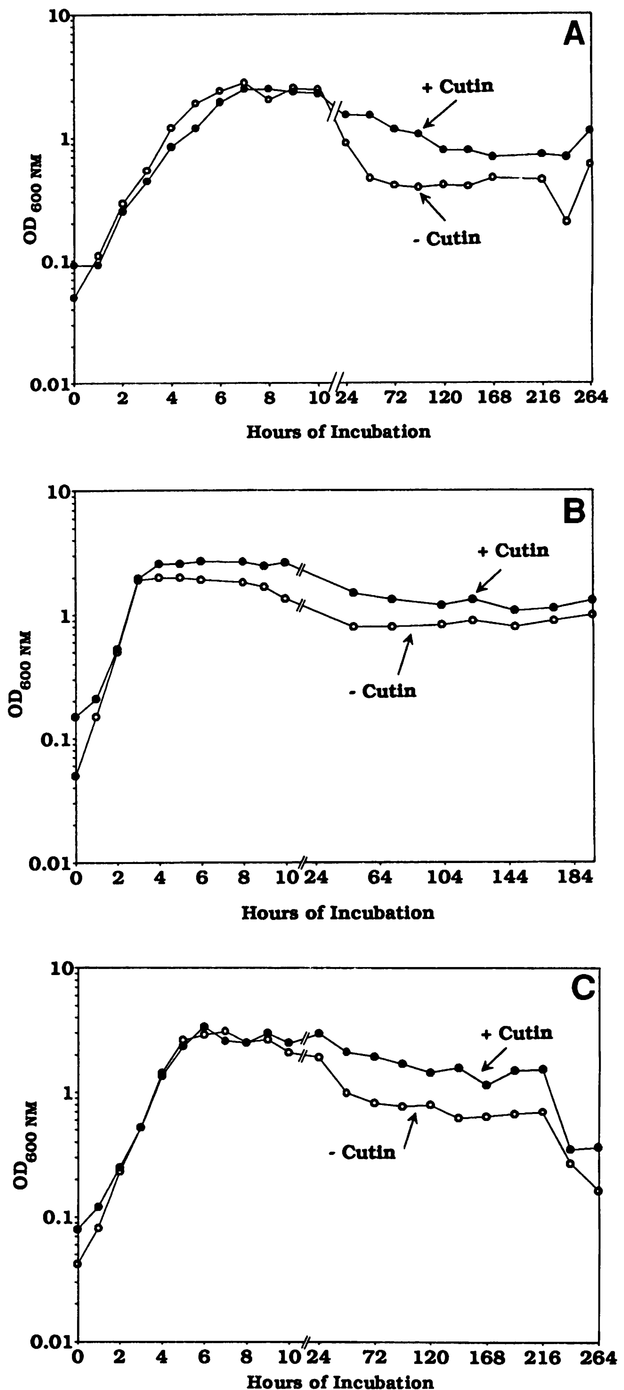


FIG. 3. Growth of *P. aeruginosa* K799 (A), DAR41352 (B), and 1499A (C) in NBY medium with or without the addition of apple cutin (0.4%) at 37°C (with shaking at 250 rpm). OD_{600}, optical density at 600 nm.

syringae pv. tomato culture filtrates demonstrated cutinase activity.

Kinetics of esterase and cutinase production. The production of esterase and cutinase along with bacterial growth in NBY with or without apple cutin was determined. Growth rates exhibited by strains K799, DAR41352, and 1499A in both media were similar, with doubling times of approximately 0.5 to 1 h during log phase (Fig. 3). However, slightly higher optical densities for all three strains were maintained after the end of log phase in media with cutin (Fig. 3). Esterase activity of culture fluids was relatively low during the first 9 h of incubation during log phase and into early stationary phase in media with or without cutin in the medium (Fig. 4). In media supplemented with cutin, a significant increase in esterase activity occurred between 9 and 24 h, with maximum activity seen between 24 and 48 h

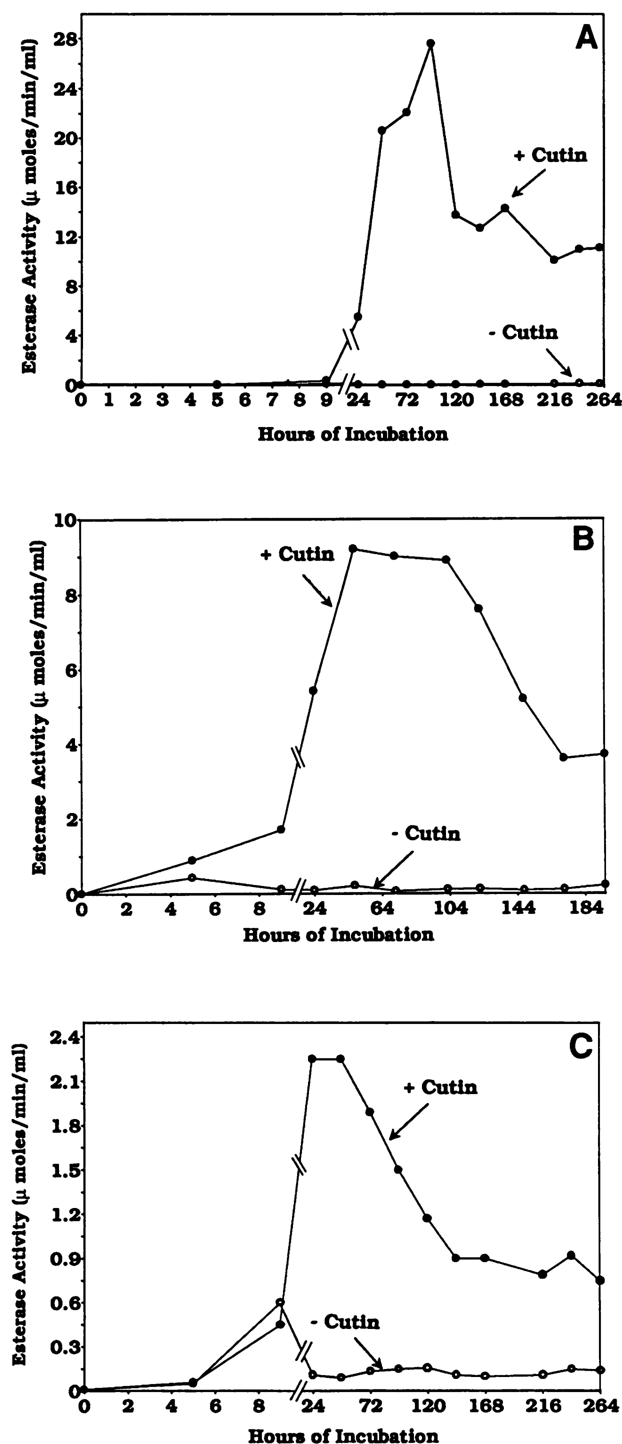


FIG. 4. Extracellular esterase activity of culture fluids of *P. aeruginosa* K799 (A), DAR41352 (B), and 1499A (C) grown in NBY medium supplemented with apple cutin (0.4%) at 37°C (with shaking at 250 rpm). Esterase activity was determined by spectrophotometric assay, using PNB as the substrate.

for strain 1499A, at 48 h for DAR41352, and at 96 h for strain K799, at which points the cultures were well into stationary phase. In media without cutin, no significant increase in esterase activity occurred. Maximum esterase activity represented a 33- to 600-fold increase over levels achieved without cutin in the medium.

For strains K799 and DAR41352, the ability of culture fluids to cause release of cutin monomers indicative of cutinase activity showed a pattern similar, but not identical, to that for the ability to hydrolyze PNB (Fig. 5). No or very little cutinase activity was present in culture fluids resulting from bacterial growth in media without cutin. For strain 1499A, cutinase activity was very erratic, relatively low, and not dependent on the presence of cutin in the medium (not shown).

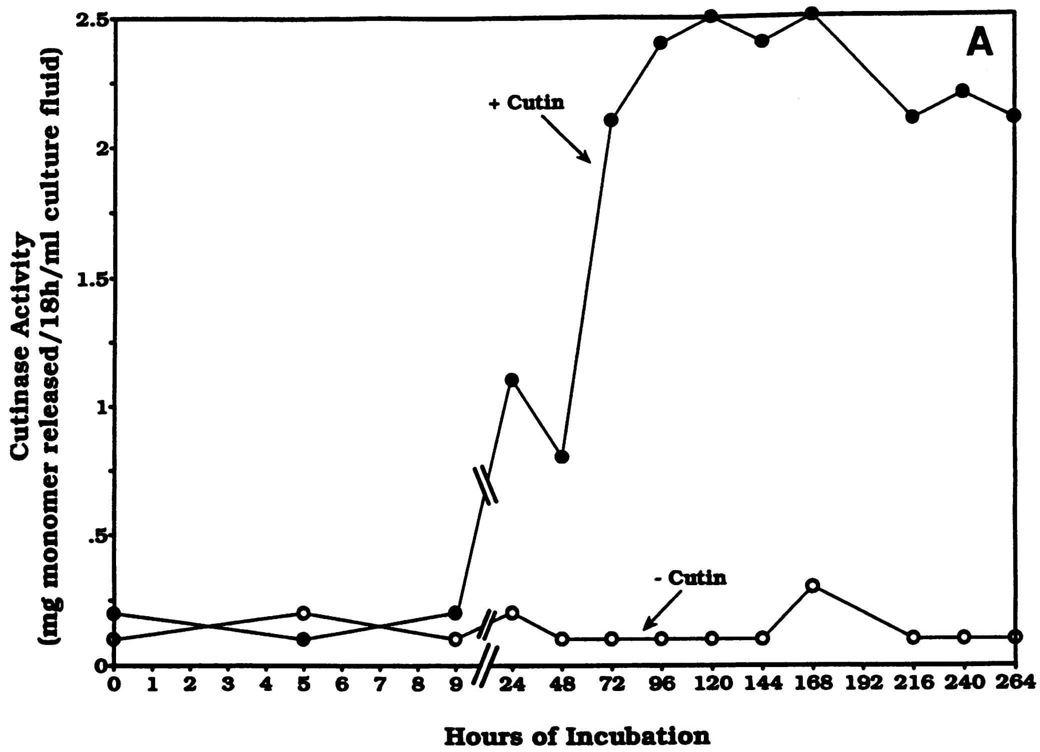
Temperature effects. The effect of incubation temperature on esterase and cutinase activities is shown in Fig. 6. Results of the two experiments were similar, and the data shown are average values from one experiment. Enzyme production was most rapid at 28 and 37°C for strains K799 and DAR41352, with highest levels achieved at 28°C for K799 and 22°C for DAR41352. Enzyme activity for strain 1499A was relatively low at all three temperatures, with highest activities achieved at 22 and 28°C.

General lipase activity. All 10 strains of *P. aeruginosa* exhibited lipase activity, as indicated by the appearance of opaque zones around bacterial colonies grown on Tween 80 agar medium for 2 days at 37 or 28°C. No relationship between the rapidity of formation of opaque zones around the bacterial colonies and the ability to produce cutinase was noted.

DISCUSSION

The availability of a simple spectrophotometric assay for general esterase activity coupled with the development of a novel HPLC technique (9) for detection, separation, and quantification of cutin monomers in our laboratory allowed us to conveniently screen a large collection of bacteria for extracellular cutinase production. Our results indicate that the ability to hydrolyze the plant biopolyester cutin is not widespread among the nonfilamentous bacteria under the growth conditions tested, as only 3 strains of *P. aeruginosa* of the 232 bacterial strains tested were found to be positive for this trait. Most of the strains tested were either saprophytes commonly found on plant surfaces (i.e., *P. fluorescens* and *E. herbicola*) or plant pathogens (i.e., *P. syringae* pathovars). We chose to concentrate our screening efforts on plant-associated bacteria since all three of the previously reported cutinase-producing bacteria were plant associated (2, 14, 17). NBY medium supplemented with 0.4% cutin was used since this was the optimal medium for cutinase production by *P. putida* (17). It is possible that additional cutinase producers might be identified in our collection of bacteria if other media and cutin sources were tested. Both medium composition and the source of cutin have been reported to affect bacterial cutinase production (2, 17). Interestingly, the cutinase-producing strain of *P. putida* was taxonomically classified as such even though it reportedly has the ability to grow at 41°C (17), a trait solely characteristic of *P. aeruginosa* among the fluorescent pseudomonads (15). Under the growth conditions tested, the previous report of cutinase production by the tomato pathogen *P. syringae* pv. tomato (2) could not be confirmed.

In time course experiments, the kinetics of extracellular esterase production were similar, but not identical, to extracellular cutinase production by *P. aeruginosa* K799 and DAR41352. It is not known whether the enzyme(s) is actively secreted or is present in culture fluids due to cell lysis. The possibility exists that a non-cutin-degrading esterase as



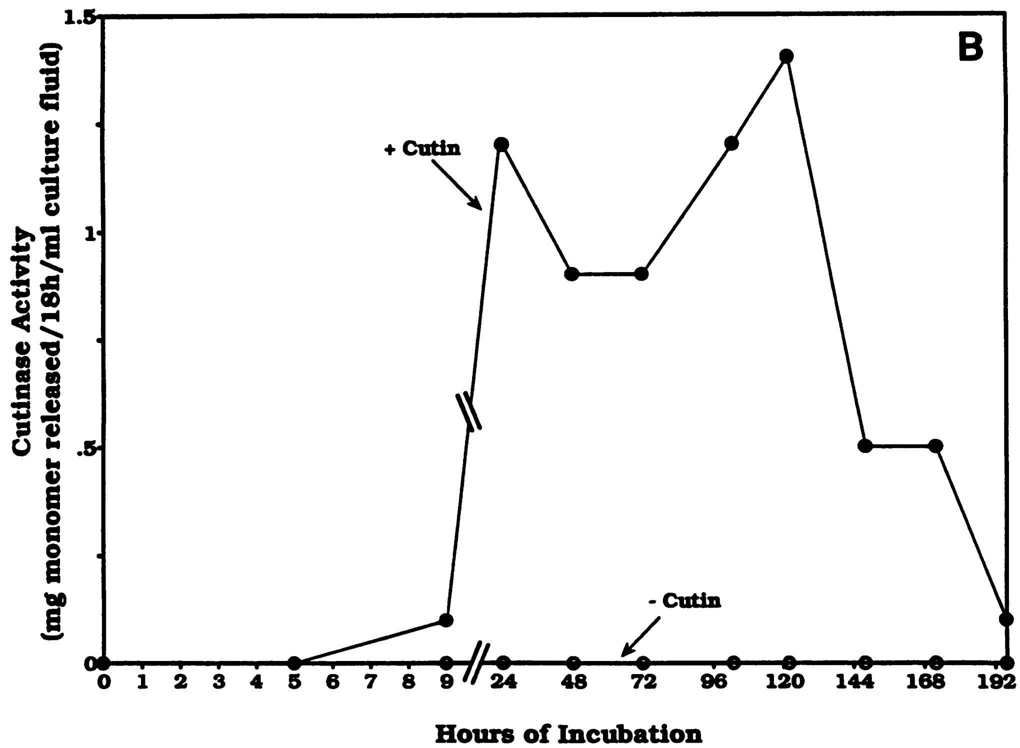


FIG. 5. Release of apple cutin monomers (as determined by HPLC analyses) after incubation of apple cutin with culture fluids of *P. aeruginosa* K799 (A) and DAR41352 (B) grown in NBY medium supplemented with apple cutin (0.4%) at 37°C with shaking (250 rpm).

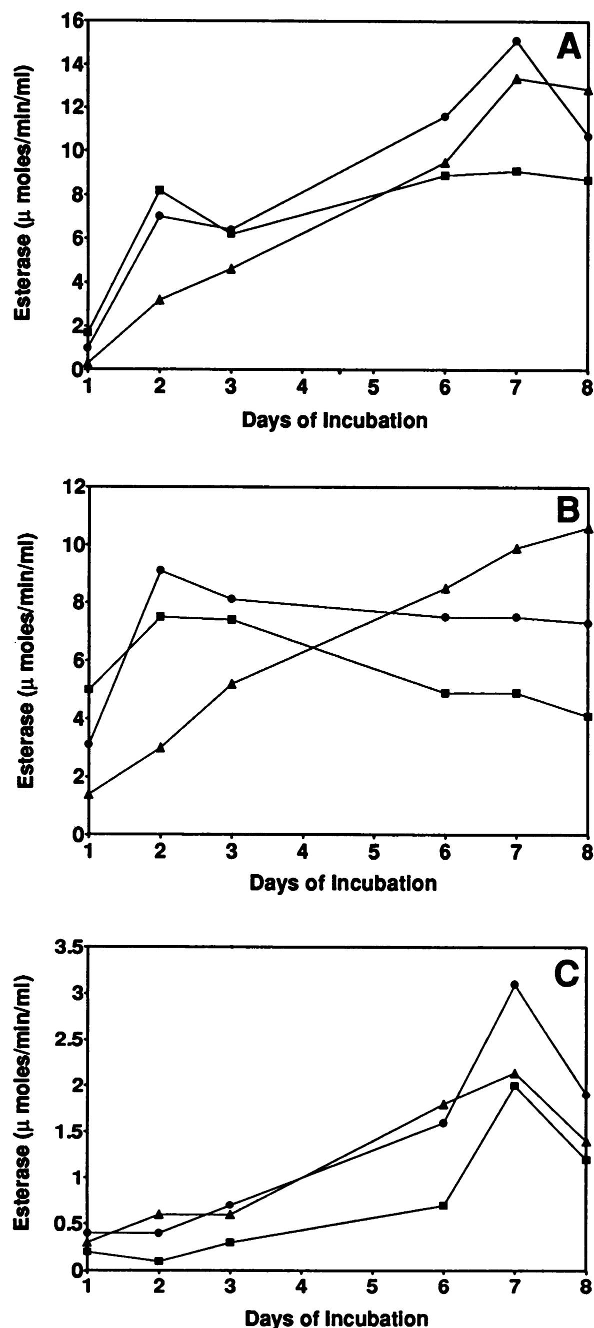


FIG. 6. Effect of incubation temperature on esterase and cutinase activities of culture filtrates of *P. aeruginosa* K799 (A), DAR41352 (B), and 1499A (C) grown in NBY medium supplemented with apple cutin (0.4%), with shaking (250 rpm) and at 22 (▲), 28 (●), or 37°C (■) as determined by spectrophotometric assay, using PNB as the substrate.

well as a cutinase was present in the culture filtrates. The non-cutin-degrading esterase may be a lipase, as all 10 strains of *P. aeruginosa* were found to be capable of lipase production on the basis of hydrolysis of Tween 80 and since *P. aeruginosa* is well known to be capable of lipase production (for a review, see reference 10).

Two of the three cutinase-producing strains of *P. aeruginosa* reported here were originally isolated from diseased humans, while the third was isolated from a diseased onion bulb (5). The common soil inhabitant *P. aeruginosa* is known to be an opportunistic plant as well as human pathogen and is occasionally isolated as a plant epiphyte (4). Whether these two clinical strains are able to cause plant disease or to survive on plant surfaces is not known.

Similar to *P. putida* (18), cutinase production by *P. aeruginosa* K799 and DAR41352 was highly inducible by addition of cutin to the medium. The highest levels of cutinase and esterase activities in the culture fluids of *P. aeruginosa* K799 and DAR41352 were obtained after the cultures had entered stationary phase. For strain 1499A, esterase production was induced by the presence of cutin in the medium, but cutinase was not. Cutinase production was much lower than that by K799 and DAR41352, and levels of activity in culture fluids were quite erratic.

The cutinase produced by strain 1499A differs from the cutinases produced by strains K799 and DAR41352 in that it does not require addition of the detergent *n*-octylglucoside for activity. Activity of the well-characterized cutinase of *Fusarium solani* f. sp. *pisi* (13) is inhibited by this detergent (unpublished data).

The role of cutinase production in the ecology and plant pathogenic capability of *P. aeruginosa* is not known. The ability to degrade cutin may favor survival and multiplication of *P. aeruginosa* as a saprophyte in soils containing plant debris and as an epiphyte on plant surfaces, as has been proposed for the cutin-degrading strain of *P. putida* (17). To date, there is no evidence of a role for cutinase in plant pathogenesis of any plant pathogenic bacterium. However, cutinase production by the potato pathogen *S. scabies* was proposed to be involved in pathogenesis (17) due to the ability of fungal cutinase (and presumably bacterial cutinase) to hydrolyze the plant polymer suberin found in the potato tuber periderm layer (7).

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REFERENCES

1. **Baker, C. J., S. I. McCormick, and D. F. Bateman.** 1982. Effects of purified cutin esterase upon the permeability and mechanical strength of cutin membranes. Phytopathology **72:**420–423.
2. **Bashan, Y., Y. Okon, and Y. Henis.** 1985. Detection of cutinases and pectic enzymes during infection of tomato by *Pseudomonas syringae* pv. *tomato*. Phytopathology **75:**940–945.
3. **Bonnen, A. M., and R. Hammerchmidt.** 1989. Cutinolytic enzymes from *Colletotrichum lagenarium*. Physiol. Mol. Plant Pathol. **35:**463–474.
4. **Bradbury, J. F.** 1986. Guide to plant pathogenic bacteria. C.A.B. International, Slough, United Kingdom.
5. **Cother, E. J., and V. Dowling.** 1986. Bacteria associated with internal breakdown of onion bulbs and their possible role in disease expression. Plant Pathol. **35:**329–336.
6. **Dickman, M. B., G. K. Podla, and P. E. Kolattukudy.** 1989. Insertion of cutinase gene into a wound pathogen enables it to infect intact host. Nature (London) **342:**446–448.
7. **Fernando, G., W. Zimmerman, and P. E. Kolattukudy.** 1984. Suberin-grown *Fusarium solani* f. sp. *pisi* generates a cutinase-like esterase which depolymerizes the aliphatic components of suberin. Physiol. Plant Pathol. **24:**143–155.
8. **Fett, W. F., H. C. Gerard, R. A. Moreau, S. F. Osman, and L. E. Jones.** Cutinase production by *Streptomyces* spp. Curr. Microbiol., in press.
9. **Gerard, H. C., S. F. Osman, W. F. Fett, and R. A. Moreau.** Separation, identification and quantification of monomers from

cutin polymers by high-performance liquid chromatography and evaporative light-scattering detection. Phytochem. Anal., in press.

10. **Godtfredsen, S. E.** 1990. Microbial lipases, p. 255–274. *In* W. M. Fogarty and C. T. Kelly (ed.), Microbial enzymes and biotechnology, 2nd ed. Elsevier Applied Science, New York.
11. **Holloway, P. J.** 1982. The chemical composition of plant cutins, p. 45–85. *In* D. F. Cutler, K. L. Alvin, and C. E. Price (ed.), The plant cuticle. Academic Press, Inc., New York.
12. **Hull, H. M.** 1970. Leaf structure as related to absorption of pesticides and other compounds. Residue Rev. **31:**1–155.
13. **Kolattukudy, P. E.** 1985. Enzymatic penetration of the plant cuticle by fungal pathogens. Annu. Rev. Phytopathol. **23:**223–250.
14. **Lin, T. S., and P. E. Kolattukudy.** 1980. Isolation and characterization of a cuticular polyester (cutin) hydrolyzing enzyme from phytopathogenic fungi. Physiol. Plant Pathol. **17:**1–15.
15. **Palleroni, N. J.** 1984. Genus I. *Pseudomonas*, p. 141–191. *In* N. R. Krieg and J. G. Holt (ed.), Bergey's manual of systematic bacteriology, vol. 1. The Williams & Wilkins Co., Baltimore.
16. **Purdy, R. E., and P. E. Kolattukudy.** 1973. Depolymerization of a hydroxy fatty acid biopolymer, cutin, by an extracellular enzyme from *Fusarium solani* f. *pisi*: isolation and some properties of the enzyme. Arch. Biochem. Biophys. **159:**61–69.
17. **Sebastian, J., A. K. Chandra, and P. E. Kolattukudy.** 1987. Discovery of a cutinase-producing *Pseudomonas* sp. cohabiting with an apparently nitrogen-fixing *Corynebacterium* sp. in the phyllosphere. J. Bacteriol. **169:**131–136.
18. **Sebastian, J., and P. E. Kolattukudy.** 1988. Purification and characterization of cutinase from a fluorescent *Pseudomonas putida* bacterial strain isolated from the phyllosphere. Arch. Biochem. Biophys. **263:**75–85.
19. **Sierra, G.** 1957. A simple method for the detection of lipolytic activity of microorganisms and some observations on the influence of the contact between cells and fatty substrates. Antonie van Leeuwenhoek J. Microbiol. Serol. **23:**15–22.
20. **Walton, T. J., and P. E. Kolattukudy.** 1972. Determination of the structures of cutin monomers by a novel depolymerization procedure and combined gas chromatography and mass spectrometry. Biochemistry **11:**1885–1897.
21. **Wilson, E. E., F. M. Zeitoun, and D. L. Fredrickson.** 1967. Bacterial phloem canker, a new disease of Persian walnut trees. Phytopathology **57:**618–621.