Postharvest Discoloration of the Cultivated Mushroom *Agaricus bisporus* Caused by *Pseudomonas tolaasii*, *P.* 'reactans', and *P.* 'gingeri'

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This study was made possible and supported in part, through a cooperative agreement with the USDA, by Sylvan Foods, Inc., Worthington, PA; J-M Farms, Inc., Miami, OH; Campbell Institute for Research and Technology, Napoleon, OH; Franklin Mushroom Farms, North Franklin, CT; Highline Produce Ltd., Leamington, Ontario, Canada; and Wilson Mushroom Co., Avondale, PA.

Mention of brand or firm names does not constitute an endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

Accepted for publication 17 June 1996.

ABSTRACT

Wells, J. M., Sapers, G. M., Fett, W. F., Butterfield, J. E., Jones, J. B., Bouzar, H., and Miller, F. C. 1996. Postharvest discoloration of the cultivated mushroom *Agaricus bisporus* caused by *Pseudomonas tolaasii*, *P.* 'reactans', and *P.* 'gingeri'. Phytopathology 86:1098-1104.

A postharvest discoloration of the cultivated mushroom *Agaricus bisporus* in Pennsylvania was associated with three pathotypes of fluorescent pseudomonads. Pathotype A strains caused pitted, dark-brown blotches on mushroom caps, formed precipitates in agar ('white line' reactions) with *Pseudomonas tolaasii* strain ATCC 14340 (reclassified as *P.* 'reactans'), were phenotypically like *P. fluorescens* biovar V, and fit descriptions of *P. tolaasii*. Pathotype B strains caused pitted, yellow-brown, sometimes slimy lesions on mushrooms, formed no 'white line' reactions, were phenotypically like *P. fluorescens* biovars III and V, and fit descriptions of *P.* 'gingeri'. Pathotype C strains caused mild, light-brown discoloration on mushrooms with little tissue collapse, formed 'white line' reactions with *P. tolaasii* ATTC 33618 (type strain), had *P. fluorescens* biovar III and V phenotypes, and fit descriptions of *P.* 'reactans'. Isolations from mushroom casing material yielded all pathotypes including both pathogenic and nonpathogenic strains of *P.* 'reactans' with distinguishing phenotypic or chemical characteristics. Cellular fatty acid analysis suggested pathogenic strains of *P.* 'reactans' were more similar to *P. tolaasii* and *P.* 'gingeri' than to saprophytic strains of *P. fluorescens*, and nonpathogenic strains were more similar to saprophytic *P. fluorescens* than to *P. tolaasii* or *P.* 'gingeri'.

Additional keywords: fatty acids.

Pseudomonas tolaasii is known primarily as the cause of brown blotch of the cultivated mushrooms *Agaricus bisporus*, *A. bitorquis*, *Pleurotus ostreatus*, and *Psalliota edulis* (6,19,22). It was first described in St. Paul, MN, by Tolaas (28). *P. tolaasii* is commonly isolated from mushrooms and associated growing media and is considered a normal constituent of the microbiota (4,24). Brown blotch disease occurs during mushroom production and generally begins as surface discoloration on caps and stipes, developing into dark-brown, sunken, or pitted lesions. *P. tolaasii* has physiological properties similar to those of *P. fluorescens* biovar V, but can be distinguished from it and from other fluorescent pseudomonads by its pathogenicity to mushrooms and by a 'white line' test (9,11,20,23,31,32). Strains of *P. tolaasii* form a white precipitate on Pseudomonas agar F (PAF) (Difco Laboratories, Detroit) when grown next to certain (uncharacterized) fluorescent pseudomonads known as *P.* 'reactans' (22,31). The reaction is associated with the production of two hydrophobic lipodepsipeptides, tolaasin from *P. tolaasii* and white line inducing principle (WLIP) from *P.* 'reactans' (7,15,16,21).

A disease of cultivated mushrooms etiologically similar to brown blotch but known as ginger blotch was first reported in Northcumberland, England, by Wong et al. (30) and later in Australia by Cutri et al. (2). Ginger blotch lesions on mushroom caps are yellow-brown instead of dark brown and generally not sunken or pitted. The causal bacterium, known as *P.* 'gingeri', was reported as similar to *P. tolaasii*, but was mucoid on PAF and negative for the white line test with *P.* 'reactans' (2).

Recent outbreaks of an unfamiliar postharvest discoloration on mushrooms from Pennsylvania prompted an investigation of bacteria that might be associated with the condition. Mushrooms of good quality and color, harvested, and washed or marketed dry occasionally developed dark blotches within several days of packaging. Early stage lesions tended to be brown to purple with a slight surface depression, but not noticeably sunken. As lesions became older, they were sunken and darker. The lesions were not initially recognized as being associated with bacterial blotch disease, known to be caused by *P. tolaasii*, because of the purple discoloration, lack of sheen, and absence of pitting.

This report describes the bacteria found to cause the postharvest discoloration of mushrooms and their relationship to several known taxonomic groups including *P.* 'gingeri' and *P.* 'reactans', based on three experimental parameters: pathogenicity, physiological properties, and composition of their cellular fatty acids.

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Publication no. P-1996-0815-01R

MATERIALS AND METHODS

Isolation and culture of bacteria. Fluorescent bacteria were isolated from two sources: discolored lesions on caps of packaged cultivated mushrooms, *A. bisporus* (Lange) Imbach (26 strains), and casing material composed of peat and lime used in commercial mushroom cultivation (218 strains). Mushroom tissue sections were smeared directly, without surface sterilization, onto PAF. After 48 h of incubation at 25°C, individual fluorescent colonies were subcultured and streaked on PAF agar. One-gram samples of casing material from the mushroom rhizosphere were agitated for 15 min in 100 ml of sterile distilled water, serially diluted, spread on PAF agar plates, and incubated for 48 h at 25°C. Single fluorescent colonies were subcultured and streaked on PAF agar. Two additional strains, listed as *P. tolaasii* ATCC 33618^T and ATCC 14340, were obtained from the American Type Culture Collection (ATCC, Rockville, MD). ATCC 14340 (= NCPPB 387) has been characterized and redesignated *P.* 'reactans' by Wong and Preece (31). Strains were subcultured on PAF agar every 4 to 6 weeks and stored in vials at –90°C in Trypticase soy broth (Difco Laboratories) with 5% dimethylsulfoxide.

Pathogenicity tests. Bacteria were bioassayed for pathogenicity to mushrooms within 5 days of isolation, because of the tendency of *P. tolaasii* to undergo phenotypic variation including loss of pathogenicity upon subculturing (2,3,7). Freshly harvested mushrooms were inoculated with 24-h bacterial cultures using sterilized wooden toothpicks for gently smearing bacteria on unwounded surfaces and for wound-puncturing directly into cap tissues. Actual inoculum on toothpicks was about 0.5 to 5×10^9 bacterial cells, and the area smeared was about 1 cm in diameter. Each isolate was tested on three different mushrooms, each by the smear and puncture technique. Inoculated mushrooms were stored in lidded glass dishes at 4°C, the normal storage temperature for mushrooms, and observed daily up to 7 days for lesion development. Wound punctures that developed water-soaked lesions with darkened margins were considered positive. Smear lesions that became slightly discolored but not sunken were considered weak reactions. Darkened and sunken lesions were rated as severe. Wounded but uninoculated checks were included in each test, as well as positive checks inoculated with *P. tolaasii* ATCC 33618^T.

White line test. Bacteria were tested for the white line reaction by streaking isolates about 2 cm apart on PAF. Within 2 to 3 days of growth at 25°C, a white precipitate formed between positive cultures, generally intensifying upon further storage at 4°C. All isolates were tested against *P. tolaasii* strains ATCC 14340 and ATCC 33618^T.

Physiological and biochemical tests. Colony morphology and color of fluorescence under long-wave UV light were observed on bacteria grown on PAF. Growth at 41°C, levan production, catalase, pectinase, arginine dihydrolase, lecithinase, nitrate reductase, gelatinase, and carbon source utilization were tested in agar tubes or plates using methods described by Hildebrand et al. (10). Levan production by mucoid strains was confirmed by gas chromatographic analysis of acetate derivatives of exopolysaccharides using procedures previously described (18). Oxidase reactions were tested on cells grown for 24 h at room temperature on PAF and also on nutrient agar plus 1% glucose as recommended by Hildebrand et al. (10). Lipase was tested on an agar medium that included 10 g of proteose peptone, 5 g of NaCl, 0.1 g of $CaCl_2 \cdot 2H_2O$, and 15 g of agar/liter, with 5 g of Tween 80 added after sterilization, and incubated for 5 days at room temperature.

Carbon sources at 0.1% final concentration were tested on plates using 1% purified Noble agar (Difco Laboratories) supplemented with the mineral salts used for the medium of Ayers, Rupp, and Johnson (10). Carbon sources, selected on the basis of their diagnostic value for fluorescent pseudomonads (20), included adonitol, L-alanine, L-arabinose, 2-ketogluconate, propylene glycol, L(+)tartaric acid, D-trehalose, L-rhamnose, D-sorbitol, and sucrose (Sigma Chemical Co., St. Louis). Growth data were taken after 3 days at 25°C and again after 7 days. Since many isolates grew to a limited extent on agar without an added carbon source because of volatile and residual carbohydrates (3), mineral salts-agar controls were included in each test. Eight strains from each pathotype were also tested twice for utilization of 95 carbon sources with the GN Microplate System of Biolog Inc. (Hayward, CA) by procedures described previously (12). Dendograms were prepared by the method of unweighted pair-groups with averages.

Whole cell fatty acid analyses. Bacterial cells grown on PAF for 24 h at 25°C were analyzed for fatty acids by methods previously described (29). Methylated fatty acid concentrates were analyzed with a Model 3700 gas chromatograph (Varian, Sunnyvale, CA) equipped with a flame ionization detector and a 15-m × 0.25-mm capillary glass column coated with SPB-1 (Supelco, Inc., Bellefonte, PA) as a nonpolar stationary phase. Eluted peaks in each sample were integrated and quantified as percent total peak area with a Model 4270 integrator (Varian). Chromatograms with a minimum of 40 individual components were obtained from each sample. Each isolate was tested once, except for the ATCC strains that were grown and tested five times to determine experimental variability. Fatty acid components were identified by cochromatography with reference standards, by chemical confirmation, and by comparison of retention times expressed as equivalent chain lengths with those of published reports (26,29).

Percent values for fatty acids in each chromatogram were entered into an Apple III microcomputer programmed with an Omnis 3 database manager (Blythe Software Inc., San Mateo, CA) that generated classifications of individual fatty acids by chemical class, profiles for individual strains, average profiles (models) for selected groups of strains, coefficients of similarity (i.e., proximities) for any two profiles, and dendrograms. Proximities were calculated as 100% minus total percentage differences for all fatty acids in the profile

TABLE 1. Properties of fluorescent pseudomonads pathogenic to mushrooms isolated from discolored lesions on commercially cultivated mushrooms after harvest and of two strains from American Type Culture Collection (ATCC)

	Pathogenicity			White line reaction[v] with:		Phenotype		
Strain designations[u]	Pathotype	Lesion color[w]	Tissue collapse[w]	ATCC 33618[x]	ATCC 14340[y]	Mucoid[z]	*P. fluorescens* biovar	Tentative identification
P2, P3a, P3b, Pf28, Pf29, Pf30, ATCC 33618	A	Dark	+	–	+	–	V	*P. tolaasii*
Pf31	B	Brown	+	–	–	+	V	*P.* 'gingeri'
Pf3, Pf11, Pf13	B	Yellow	+	–	–	+	III	*P.* 'gingeri'
Pf6, Pf14	B	Yellow	+	–	–	–	III	*P.* 'gingeri'
Pf2, Pf9	B	Yellow	+	–	–	+	V	*P.* 'gingeri'
Pf1, Pf8	C	Brown	–	+	–	–	III	*P.* 'reactans'
ATCC 14340	C	Brown	–	+	–	–	V	*P.* 'reactans'

[u] Deposited with ATCC: *P. tolaasii* P3 = ATCC 51309 and Pf28 = ATCC 51310; *P.* 'gingeri' Pf9 = ATCC 51311 and Pf31 = ATCC 51312.
[v] A white line precipitate formed after 48 h at 25°C in the agar between strains grown on Pseudomonas agar F (PAF) medium.
[w] Within 24 h after wound inoculations with pure bacteria.
[x] ATCC 33618 = type strain of *P. tolaasii*.
[y] ATCC 14340 designated *P. tolaasii*, but characterized as *P.* 'reactans' by Wong and Preece (31).
[z] After growth on PAF medium for 5 days at 25°C.

being compared. Statistical comparisons of groups of means were by an omnibus F test followed by a multiple t test on all pairs of means (1). Analyses were confirmed by Duncan's multiple range test for data with equal samples sizes and by Tukey's wholly significant difference test modified by the Tukey-Kramer generalization for data with unequal samples sizes (1). Dendrograms were based on average proximities of selected strains to model profiles of each group and constructed by pair-weighted cluster analysis (Statpro, Wadsworth Professional Software Inc., Boston).

RESULTS

Pathogenicity. Of 26 fluorescent pseudomonads isolated from discolored mushroom caps, 16 were pathogenic (Table 1). Three pathogenic types could be distinguished and were given the provisional designations A, B, and C. Six pathotype A strains produced distinct, dark, pitted lesions on inoculated mushroom caps; eight pathotype B strains produced distinct, yellow-brown, sometimes slimy lesions with tissue collapse; and two pathotype C strains produced faint to distinct, dark- to light-brown blemishes with little if any tissue collapse. Pathogenicity of *P. tolaasii* strains ATCC 33618[T] and ATCC 14340 corresponded to those of pathotypes A and C, respectively. Inoculation by smearing bacteria on mushroom caps reproduced symptoms typical of the pathotype within 24 h. Caps inoculated with nonpathogenic strains, or wounded without bacteria (checks), occasionally developed slightly discolored depressions or scratches caused by the inoculating instrument, but no generalized discoloration. Wound punc-

TABLE 2. Strains of fluorescent pseudomonads isolated from casing material in rhizospheres of commercial mushroom beds, classified by phenotype and pathogenicity

		Strains classified by phenotype						Strains[w] classified by pathogenicity				
		Pseudomonas fluorescens biovar						Pathotype			Nonpathogenic white line[x]	
Grower lot	Total strains isolated	I	II	III	IV	V	*P. putida*	A	B	C[y]	Positive[z]	Negative
B	27	0	0	22	0	1	4	0	0	1	0	22
C	24	0	0	6	0	10	8	2	0	5	2	7
D	24	0	2	18	0	2	2	0	0	2	0	20
G	24	0	0	13	0	9	2	1	0	1	0	20
H	24	0	0	16	0	8	0	0	0	2	2	20
I	24	0	0	4	0	13	7	0	0	3	8	6
J	24	0	1	7	0	16	0	0	0	1	1	22
K	24	0	0	5	0	17	2	0	2	2	0	18
L	24	1	0	11	0	11	1	0	0	5	1	17
Total	219	1	3	102	0	87	26	3	2	22	14	152

[w] Excluding strains of *P. putida*. Pathotypes A (*P. tolaasii*) = dark sunken lesions, white line reaction with ATCC 14340 (*P.* 'reactans' according to Wong and Preece [31]); B (*P.* 'gingeri') = yellow-brown, mucoid sunken lesions, no white line reactions; and C (*P.* 'reactans') = weakly pathogenic, brown discoloration, no tissue collapse, white line reaction with ATCC 33816 (*P. tolaasii* type strain).
[x] White line reaction with ATCC 33816 (*P. tolaasii*). All *P. fluorescens* biovar V phenotype.
[y] Pathogenic *P.* 'reactans' strains deposited with ATCC: D1 = ATCC 51314, D2 = ATCC 51313; and K15 = ATCC 51315.
[z] By definition, *P.* 'reactans'.

TABLE 3. Physiological properties variable for selected groups of fluorescent pseudomonads isolated from postharvest mushroom lesions and rhizospheres

	Percent positive strains[v] (number of strains tested[w])					
				Nonpathogenic		
	Pathotype[x]			White line-positive[y]	White line-negative	
Phenotypic property[u]	A (10)	B (10)	C (25)	Biovar V (14)	Biovar V (50)	Biovar III (50)
White line reaction[z]	100	0	100	100	0	0
Fluorescence						
Blue	80	60	28	36	38	52
Yellow-green	20	40	48	21	14	56
Weak	0	0	24	43	32	6
Mucoid	0	80	20	7	10	26
Oxidase, reaction with cells grown on nutrient agar + glucose	10	90	88	86	100	100
Lipase	100	100	96	57	62	20
Adonitol	100	100	88	100	84	88
L-arabinose	10	100	84	100	96	96
2-ketogluconate	100	100	100	88	96	96
Propylene glycol	0	70	54	56	60	94
L(+)tartrate	0	70	52	14	14	86
D-trehalose	50	100	100	100	86	100
L-rhamnose	0	60	68	86	80	80
D-sorbitol	100	100	100	79	96	98
Sucrose	0	60	52	36	28	38

[u] All strains positive for catalase, oxidase (on glucose-free medium), arginine dihydrolase, and gelatinase; and negative for levan, pectic enzymes, and growth at 41°C.
[v] Weak or delayed reactions considered positive.
[w] Including strains shown in Tables 1 and 2 for all groups except white line-negative (*Pseudomonas fluorescens*) biovars V and III, each represented by 50 randomly selected strains.
[x] Pathotypes A typical of *P. tolaasii*, B typical of *P.* 'gingeri', and C typical of *P.* 'reactans'.
[y] By definition, *P.* 'reactans.'
[z] White line reactions: pathotype A strains reactive with ATCC 14340; pathotype C and nonpathogenic strains reactive with ATCC 33618.

tures remained clear and free of marginal discoloration or lesions, unless the bacterial strain was pathogenic. Pathogenic strains caused 1 to 2 mm of marginal tissues to become discolored or sunken within 24 h.

Isolation of bacteria from casing material yielded 26 strains of *P. putida* and 193 strains with properties similar to *P. fluorescens*, the majority of which were biovar III and V phenotypes. Twenty-seven of the 193 strains were pathogenic to mushroom, 22 of them pathotype C (Table 2). In addition, 14 of the saprophytic, nonpathogenic strains produced white line reactions with *P. tolaasii* ATCC 33618^{T} (pathotype A), but not with ATCC 14340 (pathotype C).

White line reactions. All pathotype A strains, as typical of *P. tolaasii* (31), formed white line precipitates with ATCC 14340 and with all strains of pathotype C, but not with each other. Pathotype B formed no white line reactions, as typical of *P.* 'gingeri' (2). Pathotype C (and the 14 nonpathogenic strains) formed white line reactions with ATCC 33618^{T} and with all strains of pathotype A, but not with each other, typical of *P.* 'reactans' (31).

Phenotypic tests. Strains of pathotype A, presumptive *P. tolaasii*, were phenotypically similar to *P. fluorescens* biovar V based on negative levan and denitrification reactions and on positive reactions for arginine dihydrolase and oxidase (PAF-grown cells). Oxidase reactions for nine of the 10 strains, including ATCC 33618^{T}, were negative with cells grown on nutrient agar plus 1% glucose, evidence of inhibition of oxidase enzyme activity by glucose (14). As a group, these strains were phenotypically homogeneous with the exception of some diversity in the color of fluorescence (Table 3).

Strains of pathotype B, presumptive *P.* 'gingeri', were similar to either biovar V or III phenotypes, depending on the nitrate reductase reaction. All were negative for levan, all positive for oxidase and arginine dihydrolase, and most were mucoid (Table 3).

Heterogeneity was also noticeable among strains of pathotype C, presumptive *P.* 'reactans', and the nonpathogenic, white line-positive strains. Most were phenotypically *P. fluorescens* biovar V, but some were biovar III, based on positive nitrate reductase reactions. A significant proportion were weakly fluorescent, and some were mucoid. All were positive for oxidase on PAF, but 86 to 88% were positive on nutrient agar plus 1% glucose (Table 3).

Of 152 strains of white line-negative saprophytic *P. fluorescens*, 64% (98 strains) were biovar III and 33% (50 strains) were biovar V

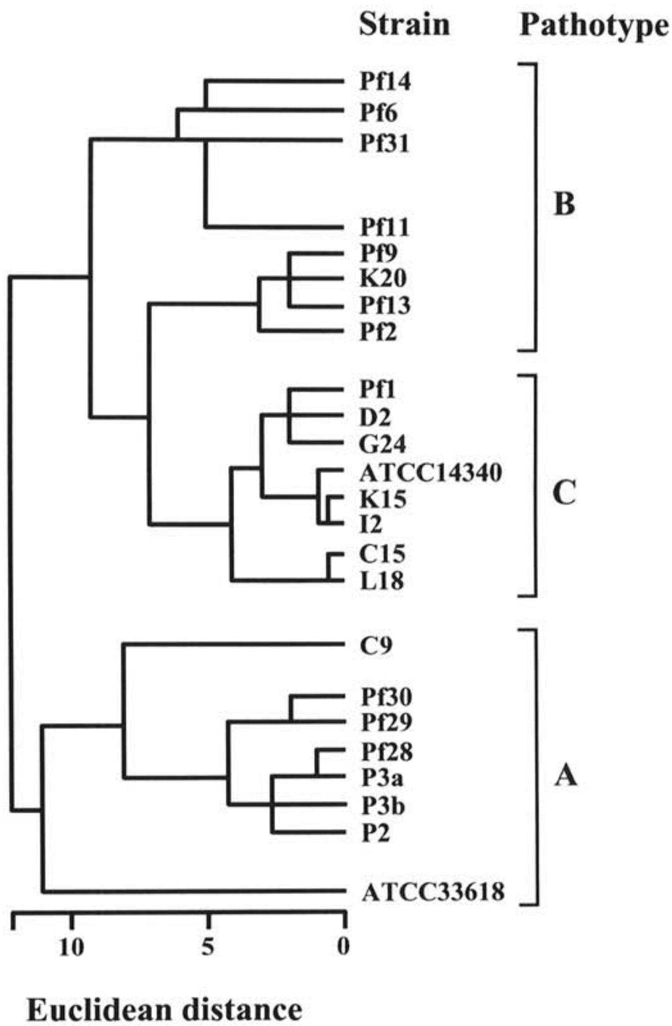

Fig. 1. Dendrogram based on Biolog GN microplate tests of eight strains of each pathotype of fluorescent pseudomonads isolated from mushrooms or casing material or obtained from the American Type Culture Collection (ATCC). Pathotype A = *Pseudomonas tolaasii*, pathotype B = *P.* 'gingeri', pathotype C = *P.* 'reactans'. ATCC 14340 = *P.* 'reactans', as classified by Wong and Preece (31); ATCC 33618 = *P. tolaasii*, type strain.

TABLE 4. Class analysis of cellular fatty acid composition of selected groups of fluorescent pseudomonads isolated from mushroom lesions and rhizospheres

	Mean percent of the total[v] (number of strains tested[w])					
				Nonpathogenic strains		
	Pathogenic strains by pathotype[x]			White line-positive[y]	White line-negative	
Fatty acid parameter	A (10)	B (10)	C (25)	Biovar V (8)	Biovar V (9)	Biovar III (11)
Saturated even-carbon straight-chain acids	31.2 a	31.5 a	32.8 ab	35.6 b	36.5 b	36.3 b
Saturated odd-carbon straight-chain acids[z]	0.6	0.4	0.4	0.4	0.3	0.5
Unsaturated straight-chain acids	53.7 a	52.2 a	51.5 ab	47.3 b	45.0 b	38.6 c
Hydroxy-substituted fatty acids	9.3 b	8.8 b	8.4 bc	8.2 bc	7.3 c	11.2 a
Saturated branched-chain fatty acids	0.7 a	0.7 a	0.9 a	1.0 a	0.9 a	1.6 b
Unsaturated branched-chain fatty acids[z]	0.1	0.1	0.1	0.1	0.1	0.1
Cyclopropane fatty acids	4.1 a	5.9 ab	5.4 ab	7.3 b	9.1 b	9.6 b
Unidentified components[z]	0.4	0.4	0.6	0.6	0.9	2.0
Ratio of saturated to unsaturated acids	0.60 a	0.62 a	0.66 ab	0.75 b	0.84 b	0.99 c

[v] Means based on one analysis per strain using cells grown on Pseudomonas agar F medium for 24 h at 25°C. Means in each row not followed by the same letter are statistically different at the 95% level of significance ($P = 0.05$), based on Tukey's wholly significant difference multiple range test for data with unequal sample sizes (1).

[w] Analysis based on all available strains of pathotype A (P2, P3a, P3b, Pf series 28, 29, and 30, C8, C9, G1, and ATCC 33618), pathotype B (K20, K23, and Pf series 2, 3, 6, 9, 11, 13, 14, and 31), and pathotype C (Pf1, Pf8, B3, C4, C7, C11, C13, C15, D1, D2, G24, H18, H23, I1, I2, I10, J15, K15, K16, L4, L5, L11, L12, L18, and ATCC 14340); on eight nonpathogenic white line-positive strains (H3, H22, I3, I5, I9, I16, I19, and J20); nine strains of *P. fluorescens* biovar V (G4, G18, H19, J4, J13, K1, K13, L3, and L16); and 11 strains of *Pseudomonas fluorescens* biovar III (B7, G10, G21, H2, I14, J14, K11, L6, L10, 362, and 3928).

[x] Pathotypes A = *P. tolaasii*, B = *P.* 'gingeri', and C = *P.* 'reactans'.

[y] By definition, *P.* 'reactans'.

[z] Means in this row not significantly different.

based on negative levan, positive arginine dihydrolase and oxidase, and variable nitrate reductase reactions. One strain was biovar I (positive for levan and negative for nitrate reductase), and three were biovar II (positive levan and nitrate reductase). About a third were weakly fluorescent, and oxidase reactions were positive.

Lipase reactions suggested a distinction between pathogenic and nonpathogenic strains. Ninety-eight percent of pathogens (pathotypes A, B, and C) were positive for lipase, while 61% of nonpathogenic strains were positive. General patterns in carbohydrate utilization between pathogenic and nonpathogenic strains were not distinguishable, because of variability within each group. Pathotype A was distinct on the basis of low percentages of strains utilizing L-arabinose, propylene glycol, L(+)tartrate, D-trehalose, rhamnose, and sucrose compared with other groups (Table 3).

Pathotypes A, B, and C were homogeneous for 77, 80, and 89% of 95 carbon sources tested on Biolog GN Microplate tests. Pathotype A could be distinguished from B and C by positive succinamic acid and hydroxy L-proline reactions (data not shown). None of the carbon sources completely separated pathotype B from pathotype C. A dendrogram suggested a dichotomy between pathotype A and strains of pathotypes B and C, which constitute a diverse group (Fig. 1).

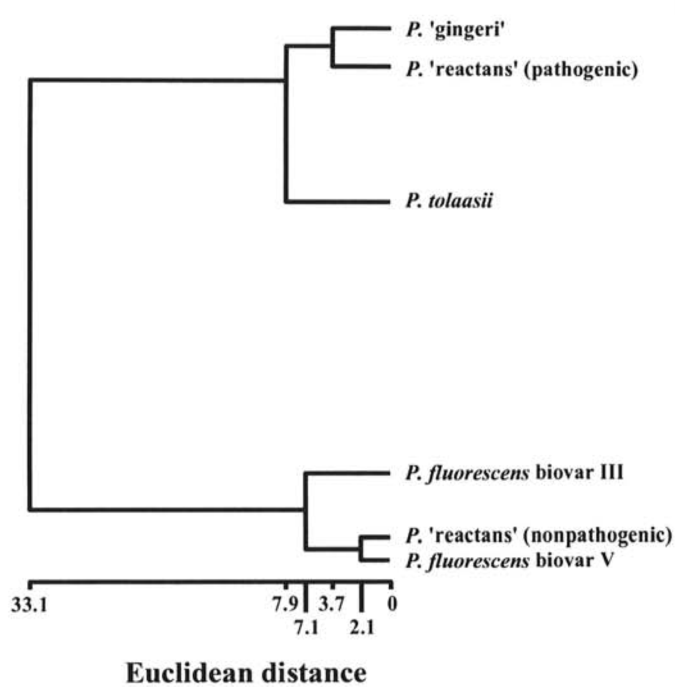

Fig. 2. Dendrogram derived from cellular fatty acid profiles of 25 pathogenic strains of *Pseudomonas* 'reactans' compared to model profiles of six *Pseudomonas* groups (Table 4). Profile differences for each strain, expressed as percent proximities (Table 5), were entered into the dendrogram program (Statpro, Wadsworth Professional Software). Euclidean distances for each group are based on averages for the 25 strains. Strains listed in footnote of Table 4.

Fatty acid composition. The fatty acid composition of strains of *P. tolaasii* and *P. fluorescens* agreed, in general, with profiles described for those species by previous authors (11,27,29). Characteristics of *P.* 'gingeri', *P.* 'reactans', and nonpathogenic white line-positive strains have not been previously published, but their profiles were consistent with general characteristics of the fluorescent pseudomonads. One analysis per strain was considered representative, since similarity coefficients (i.e., proximities) for five replicates of each of two test strains ranged from 90.9 to 97.3% (data not shown).

The pathogenic strains (*P. tolaasii*, *P.* 'gingeri', and *P.* 'reactans') belonged to the same statistical group based on class analysis and on ratios of saturated/unsaturated fatty acids (Table 4). The cluster was confirmed by similarity (i.e., proximity) of *P. tolaasii* strains to model profiles of the pathogenic groups (Table 5) and by a dendrogram based on *P.* 'reactans' proximity values (Fig. 2). As with the physiological data (Fig. 1), the dendrogram suggested a dichotomy between *P. tolaasii* (pathotype A) and a group composed of *P.* 'gingeri' (pathotype B) and the pathogenic strains of *P.* 'reactans' (pathotype C). Similar separations were obtained in dendrograms with different cluster algorithms, i.e., when proximities of *P. tolaasii* or *P.* 'gingeri' strains were compared with group models (data not shown).

P. fluorescens biovar III could be distinguished from biovar V by four of the six fatty acid parameters with statistical differences between groups (Table 4). Mean percent proximities of biovar V strains to the biovar III and biovar V profiles were also statistically different (Table 5). Dendrograms showed a significant dichotomy (Euclidean distance of more than 6) between the two biovars (data not shown).

Class analysis of individual fatty acids indicated no significant differences between pathogenic (pathotype C) and nonpathogenic (white line-positive, biovar V) strains of *P.* 'reactans' at the 95% level of probability (Table 4). Differences were evident, however, when profiles of nonpathogenic strains were compared with group models: average proximities to the nonpathogenic and pathogenic models were 89.6 and 86.2%, respectively, the difference significant at the 95% level (Table 5). The nonpathogenic strains belonged to the same statistical group as *P. fluorescens* biovar V. A dendrogram also showed that nonpathogenic strains of *P.* 'reactans' belonged to the *P. fluorescens* side of a dichotomy, separated from the pathogenic groups (Fig. 2).

TABLE 5. Mean percent proximities of fatty acid profiles of individual bacterial strains to model profiles of each of six groups of fluorescent pseudomonads isolated from mushroom lesions and rhizospheres

Bacteria		Mean percent proximity to model profiles of:[x]					
		Pathogenic strains by pathotype[y]			Nonpathogenic strains		
					White line-positive[z]	*Pseudomonas fluorescens*	
Group	Number of strains[z]	A	B	C	Biovar V	Biovar V	Biovar III
P. tolaasii (pathotype A)	10	88.0 a	86.8 a	85.9 a	82.6 b	78.9 c	73.0 d
P. 'gingeri' (pathotype B)	10	88.1 b	91.3 a	88.5 b	86.8 b	82.9 c	75.8 d
P. 'reactans' (pathotype C)	25	84.3 b	85.1 ab	87.3 a	85.3 ab	82.5 b	76.7 c
Nonpathogenic white line-positive	14	82.8 c	85.9 bc	86.2 b	89.6 a	87.6 ab	82.0 c
P. fluorescens biovar V	9	80.3 b	83.5 ab	83.7 ab	88.2 a	88.4 a	81.6 b
P. fluorescens biovar III	11	73.2 c	75.4 c	76.4 bc	79.5 abc	81.0 ab	84.6 a

[x] Proximities calculated as 100% minus the sum total percentage of deviations of individual fatty acids of each strain to those of the model profile. Model profiles derived from average of profiles of all strains in that group. Means in each row not followed by the same letter are statistically different at the 95% level of significance ($P = 0.5$), based on the Duncan's multiple range test (1).

[y] Strains included in analysis are in the footnotes of Table 4.

[z] By definition, *P.* 'reactans'.

DISCUSSION

Postharvest symptoms observed on mushrooms appeared to be caused by each of three phenotypic groups of fluorescent pseudomonads. Severe infections with darkened or yellowed lesions were because of strains of pathotypes A or B, respectively. Mild infections with superficial discoloration were because of pathotype C. Each pathotype corresponded to one or several mushroom-related pseudomonads reported in the literature: pathotype A = *P. tolaasii*, pathotype B = *P.* 'gingeri', and pathotype C = *P.* 'reactans' (3,4,22,30,31). ATCC strains 33618^T and 14340 were typical pathotypes A and C, respectively. These data confirm earlier observations, among them Olivier et al. (17), that *P. tolaasii* and *P.* 'gingeri' can cause postharvest symptoms on mushrooms. We were unable, however, to find previous reports of the pathogenicity of *P.* 'reactans' or of the incidence of *P.* 'gingeri' in the United States.

P. 'reactans' is a term for those fluorescent pseudomonads that react by the white line test to any strain of *P. tolaasii* (31). While previously understood to be nonpathogenic saprophytes, 22 of 36 strains we isolated from casing material were pathogenic to mushrooms. Two of 16 pathogenic strains of fluorescent pseudomonads isolated from discolored mushroom lesions were also *P.* 'reactans'. Pathogenic strains of *P.* 'reactans' were more closely related phenotypically to *P.* 'gingeri' than to *P. tolaasii*, as suggested by dendrograms, while nonpathogenic strains of *P.* 'reactans' clustered with strains of saprophytic *P. fluorescens*. This dichotomy suggests a subdivision of *P.* 'reactans' or at least significant heterogeneity in biological and physiological properties. The difference may relate to the structure or function of the single dominant character defining *P.* 'reactans', i.e., the WLIP.

Physiological data presented in this report are generally in agreement with previously published descriptions of fluorescent pseudomonads, particularly those pathogenic to mushrooms. Fahy (3) noted overlapping clusters of virulent and avirulent strains similar to *P. tolaasii*. Goor et al. (6) placed *P. tolaasii*, *P.* 'gingeri', and the avirulent white line-positive strains in separate phena, but separated their four strains of *P.* 'gingeri' from the *P. tolaasii* group based on diagnostic features not observed in our study. They reported *P.* 'gingeri' negative for L-arabinose, L-rhamnose, 2-ketogluconate, and D-tartrate utilization. In our substrate utilization tests, confirmed by Biolog GN, 100% of the strains were weakly positive or positive for arabinose, tartrate, and 2-ketogluconate. Positive (or weak) rhamnose reactions were found in 60% of the strains by the utilization test and in 25% of the strains by Biolog GN.

Lipase reactions in published data also differed from results in our study. *P.* 'gingeri' has twice been reported as lipase negative (8,30). We found, as suggested by the data of Fahy (3), that *P.* 'gingeri' strains were lipase positive. The discrepancy may be in methodology. We evaluated lipase plates after 2, 3, and 5 days of incubation, rating late-developing reactions as positive. Biolog GN tests for lipase using both Tween 40 and Tween 80 substrates were also positive for all *P.* 'gingeri' strains tested.

P. tolaasii is oxidase positive, but the oxidase reaction was inhibited in nine of the 10 strains tested (including ATCC 33618^T) when cells were grown on media containing glucose, as recommended in some manuals (10). Glucose inhibition is among the precautions necessary when testing bacteria for oxidase (14), but can be of diagnostic value in the case of *P. tolaasii*. Glucose inhibition was observed in only one of the 10 P. 'gingeri' strains (Pf31 = ATCC 51312) and in three of the 25 pathogenic *P.* 'reactans' strains (among them, strain D1 = ATCC 51314).

Most strains pathogenic to mushrooms were phenotypically similar to *P. fluorescens* biovar V. Of 45 pathogenic strains listed in Tables 1 and 2, only four from pathotype B and five from pathotype C were biovar III phenotypes. Fatty acid profiles of these nine strains, however, bore closer resemblance to *P. fluorescens* strains of biovar V with 85.3% mean percent proximity than to biovar III with 79.2% proximity (data not shown). If significance is attached to fatty acid analysis, the biovar III strains of pathotypes B and C may actually be variants of biovar V, variable for the nitrogen reductase reaction, their one distinguishing characteristic. Lelliott et al. (13), in the determinative scheme for fluorescent pseudomonads, although including only two strains of *P. tolaasii*, considered *P. tolaasii* to belong to the *P. fluorescens* biovar V complex and recognized it as heterogeneous (9).

One mucoid strain, K15 (ATCC 51315), was initially classified as biovar II because of ambiguous levan reactions, later proven negative by confirmatory tests, and reclassified as biovar III. This strain may have been similar to the one used by Palleroni (20) in his provisional placement of *P. tolaasii* in biovar II. The preponderance of biovar V isolates in our study is consistent with previous observations that such strains are common in soil and on surfaces of mushrooms and foodstuffs (5,25,32).

LITERATURE CITED

1. Bogartz, R. S. 1994. An Introduction to the Analysis of Variance. Praeger Publishers, Westport, CT.
2. Cutri, S. S., Macauley, B. J., and Roberts, W. P. 1984. Characteristics of pathogenic non-fluorescent (smooth) and non-pathogenic fluorescent (rough) forms of *Pseudomonas tolaasii* and *Pseudomonas gingeri*. J. Appl. Bacteriol. 57:291-298.
3. Fahy, P. C. 1981. The taxonomy of the bacterial plant pathogens of mushroom culture. Pages 293-312 in: Mushroom Science XI: Proc. Congr. Cultivation Edible Fungi, 11th. Part 2. Australian Mushroom Growers Association, Sydney, N.S.W.
4. Fletcher, J. T., White, P. E., and Gaze, R. H. 1986. Mushrooms—Pest and Disease Control. Intercept Ltd., Ponteland, Newcastle upon Tyne, United Kingdom.
5. Gennari, M., and Dragotto, F. 1992. A study of the incidence of different fluorescent *Pseudomonas* species and biovars in the microflora of fresh and spoiled meat and fish, raw milk, cheese, soil and water. J. Appl. Bacteriol. 72:281-288.
6. Goor, M., Vantomme, R., Swings, J., Gillis, M., Kersters, K., and de Ley, J. 1986. Phenotypic and genotypic diversity of *Pseudomonas tolaasii* and white line reacting organisms isolated from cultivated mushrooms. J. Gen. Microbiol. 132:2249-2264.
7. Grewal, S. I. S., Han, B., and Johnstone, K. 1995. Identification and characterization of a locus which regulates multiple functions in *Pseudomonas tolaasii*, the cause of brown blotch disease of *Agaricus bisporus*. J. Bacteriol. 177:4658-4668.
8. Grewal, S. I. S., and Rainey, P. B. 1991. Phenotypic variation of *Pseudomonas putida* and *P. tolaasii* affects the chemotactic response to *Agaricus bisporus* mycelial exudate. J. Gen. Microbiol. 137:2761-2768.
9. Haynes, W. C. 1957. Genus I: *Pseudomonas* Migula 1894. Pages 89-152 in: Bergey's Manual of Determinative Bacteriology. 7th ed. E. G. Breed, E. G. D. Murray, and E. F. Smith, eds. Williams & Wilkins, Baltimore.
10. Hildebrand, D. C., Schroth, M. N., and Sands, D. C. 1988. *Pseudomonas*. Pages 60-80 in: Laboratory Guide for Identification of Plant Pathogenic Bacteria. N. W. Schaad, ed. The American Phytopathological Society, St. Paul, MN.
11. Janse, J. D., Derks, J. H. J., Spit, B. E., and van der Tuin, W. R. 1992. Classification of fluorescent soft rot *Pseudomonas* bacteria, including *P. marginalis* strains, using whole cell fatty acid analysis. Syst. Appl. Microbiol. 15:538-553.
12. Jones, J. B., Chase, A. R., and Harris, G. K. 1993. Evaluation of the Biolog GN Microplate System for identification of some plant-pathogenic bacteria. Plant Dis. 77:553-558.
13. Lelliott, R. A., Billings, E., and Hayward, A. C. 1966. A determinative scheme for the fluorescent plant pathogenic pseudomonads. J. Appl. Bacteriol. 29:470-489.
14. MacFadden, J. F. 1976. Biochemical Tests for Identification of Medical Bacteria. Williams & Wilkins, Baltimore.
15. Mortishire-Smith, R. J., Nutkins, J. C., Packman, L. C., Brodey, C. L., Rainey, P. B., Johnstone, K., and Williams, D. H. 1991. Determination of the structure of an extracellular peptide produced by the mushroom saprotroph, *Pseudomonas reactans*. Tetrahedron 47:3645-3654.
16. Nutkins, J. C., Mortishire-Smith, R. J., Packman, L. C., Brodey, C. L., Rainey, P. B., Johnstone, K., and Williams, D. H. 1991. Structure determination of tolaasin, an extracellular lipodepsipeptide produced by the mushroom pathogen *Pseudomonas tolaasii* Paine. J. Am. Chem. Soc. 113:2621-2622.

17. Olivier, J. M., Guillaumes, J., and Martin, D. 1978. Study of a bacterial disease of mushroom caps. Pages 903-916 in: Proc. Int. Conf. Plant Pathogenic Bact., 4th. Vol. 2. Station de Pathologie Végétale et Phytobactériolgoie, Angers, France.
18. Osman, S. F., Fett, W. F., and Fishman, M. L. 1986. Exopolysaccharides of the phytopathogen *Pseudomonas syringae* pv. *glycinea*. J. Bacteriol. 166:66-71.
19. Paine, S. G. 1919. Studies on bacteriosis: II. A brown blotch disease of cultivated mushrooms. Ann. Appl. Biol. 5:206-219.
20. Palleroni, N. J. 1984. Genus I. *Pseudomonas* Migula, 1984. Pages 141-199 in: Bergey's Manual of Systematic Bacteriology, Vol. 1. 8th ed. N. R. Krieg and J. G. Holt, eds. Williams & Wilkins, Baltimore.
21. Rainey, P. B. 1993. Identification of a gene cluster encoding three high molecular weight proteins which is required for synthesis of tolaasin by the mushroom pathogen *Pseudomonas tolaasii*. Mol. Microbiol. 8:643-652.
22. Rainey, P. B., Brodey, C. L., and Johnstone, K. 1992. Biology of *Pseudomonas tolaasii*, cause of brown blotch disease of cultivated mushroom. Pages 95-118 in: Advances in Plant Pathology, Vol. 8. J. H. Andrews and I. Tommerup, eds. Academic Press, Inc., New York.
23. Rhodes, M. E. 1959. The characterization of *Pseudomonas fluorescens*. J. Gen. Microbiol. 21:221-263.
24. Royse, D. J., and Wuest, P. J. 1980. Mushroom brown blotch: Effects of chlorinated wastes on disease intensity and bacterial populations in casing soil and or pilei. Phytopathology 70:902-905.
25. Sands, D. C., and Rovira, A. D. 1971. *Pseudomonas fluorescens* biotype G, the dominant fluorescent pseudomonad in South Australian soils and wheat rhizospheres. J. Appl. Bacteriol. 34:261-275.
26. Sasser, M. 1991. Identification of bacteria through fatty acid analysis. Pages 199-204 in: Methods of Phytobacteriology. F. Klement, K. Rudolf, and D. C. Sands, eds. Akademiai Kiado, Budapest.
27. Stead, D. E. 1992. Grouping of plant-pathogenic and some other *Pseudomonas* spp. by using cellular fatty acid profiles. Int. J. Syst. Bacteriol. 42:281-295.
28. Tolaas, A. G. 1915. A bacterial disease of cultivated mushroom. Phytopathology 5:51-54.
29. Wells, J. M., Butterfield, J. E., and Revear, L. G. 1993. Identification of bacteria associated with postharvest diseases of fruits and vegetables by cellular fatty acid composition: An expert system for personal computers. Phytopathology 83:445-455.
30. Wong, W. C., Fletcher, J. T., Unsworth, B. A., and Preece, T. F. 1981. A note on ginger blotch, a new bacterial disease of the cultivated mushroom, *Agaricus bisporus*. J. Appl. Bacteriol. 52:43-48.
31. Wong, W. C., and Preece, T. F. 1979. Identification of *Pseudomonas tolaasii*: The white line in agar and mushroom tissue block rapid pitting tests. J. Appl. Bacteriol. 47:401-407.
32. Zarkower, P. A., Wuest, P. J., Royse, D. J., and Myers, B. 1983. Phenotypic traits of fluorescent pseudomonads causing bacterial blotch of *Agaricus bisporus* mushrooms and other mushroom-derived fluorescent pseudomonads. Can. J. Microbiol. 30:360-367.