Identification of Bacteria Associated with Postharvest Diseases of Fruits and Vegetables by Cellular Fatty Acid Composition: An Expert System for Personal Computers

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Accepted for publication 15 December 1992.

ABSTRACT

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.

The cellular fatty acid composition of 190 bacterial strains representing six genera associated with postharvest diseases of fruits and vegetables was statistically analyzed and was used as the basis of an expert system of identification. The expert system was built with off-the-shelf hardware and software, i.e., a commercially available, database management program and personal computer. The database included fatty acid profiles of *Bacillus, Clostridium, Cytophaga, Xanthomonas,* and the species: *Erwinia amylovora, E. ananas, E. herbicola, E. carotovora* subsp. *carotovora* and *E. carotovora subsp. atroseptica, E. chrysanthemi, E. rhapontici, Pseudomonas cepacia, P. gladioli, P. aeruginosa, P. cichorii, P. fluorescens, P. putida, P. syringae,* and *P. viridiflava.* A total of 78 fatty acids were detected by gas-liquid chromatography, and mean percentages (of the total) were analyzed statistically for each fatty acid and chemical class. Genera were differentiated by a class analysis. *Clostridium* had the highest mean percentage (63.90%) of saturated, straight-chain, even-carbon fatty acids (class A), significantly different from all genera except *Erwinia* (40.89%). *Cytophaga* was unique, with a high mean percentage (9.44%) of saturated, straight-chain, odd-carbon fatty acids (class B). Mean percentages for class C, saturated, straight-chain fatty acids, were significantly higher for *Erwinia* (42.11%) and *Pseudomonas* (fluorescent, 53.10%, and nonfluorescent, 35.33%) than for *Bacillus* (8.91%), *Clostridium* (16.31%), and *Cytophaga* (19.98%). In class D, hydroxy-substituted acids, mean percentages for *Bacillus* (1.06%) and *Clostridium* (2.28%) were significantly lower than for *Cytophaga* (11.00%) and the nonfluorescent pseudomonads (13.97%). In class E, saturated, branched-chain fatty acids, mean percentages for *Erwinia* and *Pseudomonas* were less than 1.5% compared to over 11% in other genera. In class F, unsaturated, branched-chain fatty acids, mean percentages for the pseudomonads were less than 0.2%, significantly lower than in any other genus. The ratio of class C to class D was useful in differentiating over 90% of the fluorescent pseudomonads (<3.5) from the nonfluorescent pseudomonads (>3.5). Of 61 fatty acids identified in *Erwinia* and *Pseudomonas*, the mean percentages of nine differed significantly in *Erwinia* species, and 23 differed significantly in the pseudomonads. "Rules" based on a profile of percentage ranges for each fatty acid and each class total differentiated each genus and species in the computer expert system. Fatty acid data from analyzed samples were compared with profile rules by a series of "if/then" (true/false) statements. The expert system correctly identified all strains in the database, with the exception of one strain of *P. viridiflava* that also matched the profile of *P. syringae* and one strain of *E. rhapontici* that also matched the profile of *E. herbicola.* The system also calculated a covariance factor for each strain, measuring its similarity to profiles of any selected group.

Postharvest pathologists working with bacterial diseases must identify a variety of pathogenic bacteria as well as bacteria that indirectly affect the postharvest quality and availability of fruits and vegetables. Pathogens include species of soft-rotting *Erwinia, Pseudomonas, Xanthomonas, Cytophaga,* and *Bacillus* (7,14–17). In addition, *E. ananas, P. aeruginosa, P. pseudoalcaligenes,* and *Enterobacter* and *Clostridium* spp. can directly cause postharvest losses (3,5,17,22,28). Phytopathogenic bacteria, such as *E. amylovora* and *X. campestris* pv. *citri,* on commodities in interstate or international commerce are subject to quarantine restrictions (29,31). Potential biocontrol agents for postharvest diseases include *B. subtilis, E. herbicola,* and *P. putida* (11,13,24).

Fatty acid analysis has become a valuable taxonomic tool in identifying plant-pathogenic bacteria (12,20,25). Composition of cellular fatty acids, as a biochemical property of bacteria, generally is stable enough and specific enough to differentiate genera, species, and, in some cases, subspecies (6,26). Information is available on the fatty acid composition of various phytopathogenic bacteria. Profiles of *Clavibacter michiganesis* subsp. *michiganesis, Erwinia, Pseudomonas, Xanthomonas,* and *Xylella* have been published (9,18,30,32–36). In addition, the proprietary Microbial Identification System (MIDI, Newark, DE) identifies plant-pathogenic bacteria by matching computer patterns of fatty acid chromatograms (25). The fatty acid composition of bacteria in the MIDI data base has been reported only in part (26,30).

Cellular fatty acids of plant-pathogenic bacteria generally are analyzed by gas-liquid chromatography (GLC) to yield reproducible profiles that may be composed of more than 70 identifiable components. Some groups of bacteria may have profiles sufficiently different from each other to permit differentiation based on a few calculated parameters, such as percentages of individual fatty acids or classes of fatty acids (6,35). Differentiation of a larger array of taxonomic groups, or species, subspecies, and pathovars with very similar fatty acid profiles, may require simultaneous comparisons of many individual fatty acids and derived parameters. Such multiple comparisons may be practical only with assistance from computer programs.

Pattern matching is one approach to computerizing chromatographic analyses of bacterial fatty acids. Fatty acid chromatograms from bacterial samples are compared with each other or with known standards for identification by pattern recognition. The proprietary MIDI is an application of this method. The system is composed of a GLC unit linked to a minicomputer with specialized software (25,26).

Another approach to computerizing an identification system for bacteria based on fatty acid analyses includes an expert system that uses a PC microcomputer. An expert system processes data through a series of "if/then" decisions to make an identification. We describe such a system, designed and built following generally accepted principles (10,21), which utilizes off-the-shelf hardware and software, i.e., a PC computer and a relational database management system (RDMS).

The purpose of this report is to detail the cellular fatty acid composition of bacteria involved or associated with postharvest

diseases; to construct a simple, direct taxonomic key for primary differentiation of genera by class analysis of fatty acids; and to describe a computer expert system for identification of these bacteria.

MATERIALS AND METHODS

Cultivation and maintenance of bacterial strains. This study was based on a collection of 190 bacterial strains from six different genera: 10 *Bacillus*, nine *Clostridium*, six *Cytophaga*, 70 *Erwinia*, 71 *Pseudomonas*, and 24 *Xanthomonas* (Table 1). Erwinias and pseudomonads included five to 18 strains of each of the species *E. amylovora, E. ananas, E. herbicola, E. carotovora* subsp. *atroseptica* and *E. carotovora* spp. *carotovora, E. chrysanthemi, E. rhapontici, P. aeruginosa, P. cepacia, P. cichorii, P. fluorescens, P. gladioli, P. putida, P. syringae* pv. *syringae,* and *P. viridiflava.* For most species, type strains from the American Type Culture Collection (ATCC) were included. Bacteria selected were associated directly with postharvest diseases, with postharvest quarantine restrictions, or with biocontrol of postharvest diseases. *P. syringae*, although not a postharvest pathogen, was included because of phenotypic similarities to *P. viridiflava* and unconfirmed reports of postharvest pathogenicity (17,23). Air-tolerant species of *Clostridium* were included because of phenotypic resemblances to isolates causing postharvest decay (17; J. M. Wells, *unpublished data*). Bacteria were maintained on King's medium B (KB) agar (Difco Laboratories, Detroit, MI) and were cloned from single colonies three times if they were from original isolations or once if they were received as pure cultures. Prior to fatty acid analysis, cells were cultured on KB agar at 25 C for 24 h, except air-tolerant *Clostridium* strains, which were grown for 72 h under normal aerobic conditions. Strains were stored in Trypticase soy broth plus 5% DMSO at −90 C.

Fatty acid analysis. Bacterial cells were saponified, esterified, and analyzed for fatty acids by a method based on that of Moss (19) and modified by Sasser (26). Approximately 400–500 mg (wet weight) of cells was saponified and esterified by mixing the cells in 1 ml of 1.2 N NaOH in 50% aqueous methanol, heating the mixture for 30 min in a boiling water bath, combining it with 0.5 ml of 6 N HCl and 1 ml of 12% boron trichloride methanol, and heating it for 5 min at 85 C. Methylated acids were extracted with 1 ml of hexane diethyl ether (1:1), were washed with 3 ml of 0.3 N NaOH, and were concentrated to approximately 20–30 μl under a stream of filtered, high-purity nitrogen gas. Two microliters of the concentrate was injected into a Model 3700 gas chromatograph (Varian Associates, Sunnyvale, CA) that included 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. Solvent blanks were periodically tested for impurities. Operating conditions included helium carrier gas flow 30 cc per minute; injector temperature 230 C; detector temperature 250 C; initial column temperature 130 C; final column temperature 230 C; and temperature increase rate 4 C per minute. Chromatograms with a minimum of 30 individual components were obtained from each sample. Generally, each strain was grown and tested once. Strains of special interest (such as type strains) were tested at least three times to establish the extent of experimental variability.

Fatty acids between 8- and 20-carbons long were identified by a combination of cochromatography with reference standards (Matreya, Inc., Pleasant Gap, PA; and Supelco, Inc.), mass spectroscopy, and chemical confirmation tests. Major fatty acid peaks were confirmed with a Finnegan 8230 HR mass spectrometer. Unsaturated fatty acids were confirmed chemically by hydrogenation of methyl esters for 15 min using a method originally described by Moss (19). During hydrogenation, unsaturated acids disappeared, and a corresponding increase was observed in the saturated, straight-chain analogs. Hydroxy-substituted fatty acids were confirmed by trifluoroacetylation, also using the method of Moss (19). During acetylation, the retention times of the diacyl derivatives of these acids shifted when chromatographed. Methylated samples were tested for the presence of cyclopropane fatty acids by the method of Brian and Gardner (2). Known 17:0 and 19:0 cyclic fatty acids (Supelco, Inc., and Applied Science, State College, PA) were used as internal standards. Equivalent (carbon) chain length (ECL) was calculated for each chromatogram peak and provided further confirmation of fatty acid identities by referencing published reports (8,26).

Data handling. Using a Model 4270 integrator (Varian Associates), eluted fractions in each sample were integrated and quantified as a percent of the total peak area. Data from the integrator was entered into an Apple III microcomputer programmed with an Omnis 3 database manager (Blythe Software Inc., San Mateo, CA). Fatty acids were organized into chemical classes based on those described by Asselineau (1), and class subtotals were used to assist in comparative analyses. Class A = saturated, straight-chain, even-carbon fatty acids; class B = saturated, straight-chain, odd-carbon fatty acids; class C = unsaturated fatty acids; class D = hydroxy-substituted acids; class E = saturated, branched-chain fatty acids; class F = unsaturated, branched-chain fatty acids; and class G = cyclopropane fatty acids. Report programs generated fatty acid profiles of each genus and species.

Expert system. An expert system was built into the Omnis 3 RDMS instead of using commercial expert-system "shell" software. This approach permitted direct access to the data set and input mechanism and integrated all features of the system. The expert-system library contained files for approximately 1,000 fields. Menus were linked to system information, instructions, data sets, and data-processing instructions. The knowledge base, the collection of rules that governed the identification process, was based on the profiles of bacterial genera and species (domain expert data). The expert-system generator was provided by Omnis 3. Rule sets for the knowledge base were constructed with three types of alpha-numeric and numeric rules, i.e., process rules that govern processing of domain expert data, control rules that direct processing, program instruction and results, and display rules that activate preprogrannned statements.

Mean percentages and ranges for fatty acids and classes were calculated for each genus, species, and subspecies. Mathematical rules were derived for each group, consisting of true/false statements formulated as Ab < c or Ab > c, in which Ab = fatty acid code (A = chemical class and b = fatty acid), and c = a value based on the percentage range of each fatty acid or class (Table 2). Over 100 rules were used to define the fatty acid composition of a genus or species, after which the rules were entered into 13–18 computer fields, each field holding six to eight rules. When data for a specific sample were entered and tested against genus rules, the program display indicated whether rules in a field were true or false (true = 1 and false = 0). If data agreed with all genus rules for *Erwinia* or *Pseudomonas*, control rules would link data to species rules. A particular sample was identified when data agreed with all rules for one genus and one species.

Statistical analysis. Variations in percentages from sample means were calculated as sample standard deviations (SD) by Statpro statistical software (Wadsworth Professional Software, Inc., Boston, MA), and comparisons among means were carried out using the method of J. W. Tukey as modified by G. Snedecor (27). Coefficients of similarity were generated from either of two subprograms (documentation not shown): one calculated from the number of rules for a particular species matched by the fatty acid composition of the sample, and one derived from a covariance factor measuring the degree of relatedness to the profile of any selected strain or groups of strains. Variation within a strain, after repeated culturing and methylation, was measured as the relative SD, derived as a fraction of the sample mean and expressed as a coefficient of variation (27). The sample mean for these comparisons was the coefficient of similarity, described above.

RESULTS

Statistical analysis of differences in genera. A total of 78 different fatty acids were detected and identified in the six genera of bacteria studied (Tables 3 and 4). The 16:0 and 16:1 *cis* 9

TABLE 1. Strains of *Bacillus, Clostridium, Cytophaga, Erwinia, Pseudomonas*, and *Xanthomonas* associated with postharvest diseases of fruits and vegetables and reference strains used in this study

Bacterium	Strain	Postharvest association	Source or reference[x]
Bacillus polymyxa	ATCC 842	Type strain	ATCC
	ATCC 21551	Pectolytic, potato rot	ATCC (17)
B. subtilus	ATCC 6051	Type strain	ATCC
	85R, D39SR, D60R	Pectolytic, soft rot	D. Thompson
	361A,3911,36266	Saprophyte of *Agaricus*	Original isolates
	B-3	Biocontrol agent	L. Pusey (24)
Clostridium	E,MX,T1	Rot of tomato (unpublished)	Original isolates
C. histolyticum	ATCC 19401	Air-tolerant sp.	ATCC
C. perfringens	1362,1451	Air-tolerant sp.	M. Solberg
	580,8283B,FD16	Air-tolerant sp.	C. Huhtanen
Cytophaga johnsonea	ATCC 17061	Type strain	ATCC
	AJ063	Pectolytic, soft rot	(15)
	CL1,CL2,CL3	Pectolytic, soft rot	C. Liao
	OKEEFE	Pectolytic, soft rot	G. O'Keefe
Erwinia amylovora	ATCC 15580	Type strain	ATCC
	MO15,47,59,69,95	Quarantine target	T. Van Der Zwet
	WC212,218,220,228	Quarantine target	T. Van Der Zwet
	WV250,267,55,RIF-NY	Quarantine target	T. Van Der Zwet
E. ananas	11530,23822,35400	Pineapple rot	ATCC
	CO540,541,542,543	Muskmelon rot	(3)
	X5,X5R,X6,X7R,X9R	Melon rot	(3)
E. carotovora	ATCC 33260	Type strain	ATCC
subsp. *atroseptica*	C2,E15,E18,E25s	Pectolytic, soft rot	(35)
	E27s,E3,E4,E6	Pectolytic, soft rot	(35)
E. carotovora	ATCC 15713	Type strain	ATCC
subsp. *carotovora*	C14,C352,C6,C7s,C9	Pectolytic, soft rot	(35)
	E21,E22,E27,E40,E60	Pectolytic, soft rot	(35)
E. chrysanthemi	ATCC 11663	Type strain	ATCC
	ATCC 27385,27550,29261	Pectolytic	ATCC
	581	Pectolytic	J. Janse
	635	Pectolytic	F. Lukezic
	Ec16	Pectolytic	A. Kelman
E. herbicola	112,133	Biocontrol of *E. amylovora*	S. Beer[y] (12)
	109 (SU 844)	Saprophytic	D. Ritchie[y]
	130 (UCBPP 844)	Saprophytic	M. Schroth[y]
	141 (UG Y193)	Saprophytic	D. Gibbons[y]
	171 (UCB M232A)	Saprophytic	S. Lindow[y]
	181 (E. Malling P7)	Saprophytic	E. Billings[y]
	35A,715,M12	Saprophytic	T. Van Der Zwet
	SP1	Biocontrol (unpublished)	S. Pfister
E. rhapontici	ATCC 29283	Type strain	ATCC
	ATCC 23376,29284,29287	Pectolytic, soft rot	ATCC
	1025,1026	Pectolytic, soft rot	H. Moline
Pseudomonas aeruginosa	ATCC 27853	Type strain	ATCC
	ATCC 14425	Onion rot	ATCC (17)
	1499A,813,K799,AK1012	...	W. Fett
P. cepacia	17616,25416	Type strains	ATCC
	376,378,419	Onion rot	W. Fett (17)
	SP20	Onion rot	S. Pfister
P. cichorii	10857	Type strains	ATCC
	13455,14120,14122	Lettuce rot	ATCC (17)
	P36	...	W. Fett
P. fluorescens			
Biovar I	27	Pectolytic	C. Liao (14)
Biovar II	ATCC 10844	Type strain	ATCC
	CL24,G16,LC51,PF52	Pectolytic	C. Liao
	PU31B,SJ33	Pectolytic	C. Liao
	G13D2,R8Z80	...	W. Fett
	8,129,174,143	Pepper rot	Original isolate[z]
Biovar III	362,3928	Saprophytic	Original isolate[z]
Biovar IV	G21	Pectolytic	C. Liao
Biovar V	29	Pectolytic	C. Liao
P. gladioli	ATCC 10248	Type strain	ATCC
	P21,P32,P33,P34	Pectolytic	W. Fett (17)
P. putida	12633,17391,17527	Type strains	ATCC
	28	Biocontrol of soft rot	C. Liao (13)
	3931A,3926	Saprophytic	Original isolate[z]
P. syringae	ATCC 19310	Type strain	ATCC
pv. *syringae*	18,416,Meyer,Y30	...	W. Fett
	11,54,200,220,246	Saprophytic	Original isolate[z]
P. viridiflava	ATCC 43557	Type strain	ATCC
	F5,6,8,9,13,15	Pectolytic, soft rot	W. Fett
	PJ86A,SF312,SF318,SF53	Pectolytic, soft rot	C. Liao
	62a,147,209,256	Pepper rot	Original isolate[z]
Xanthomonas campestris			
pv. *campestris*	ATCC 33913	Type strain	ATCC
	CJ92,CJ193,TJ71	Pectolytic, soft rot	C. Liao (16)
	XCC1,XCC2,XCC3,XCC5	Pectolytic, soft rot	W. Fett
pv. *citri A*	M174,Xc118,143,59,62, 63,97,98,99	Quarantine target	J. Hartung (29)
pv. *pruni*	ATCC 19316	Type strain	ATCC
	NCCPB 1196	Bacterial spot	NCCPB
	XP1, XP3, XP4, XP28, XP31	Bacterial spot	E. Civerolo (18)

[x] ATCC = American Type Culture Collection, Rockville, MD; E. Civerolo and J. Hartung, USDA, BARC, Beltsville, MD; C. Huhtanen, USDA, ERRC, Philadelphia, PA; J. Janse, Landbouw en Visserij, Wageningen, Netherlands; A. Kelman, Univ. Wisconsin, Madison; F. Lukezic, The Pennsylvania State Univ., University Park, PA; C. Liao, USDA, ERRC; H. Moline, USDA, BARC; G. O'Keefe, Univ. Georgia, Tifton; L. Pusey, USDA, Byron GA: D. Thompson and S. Pfister, The State Univ., Rutgers, New Brunswick, NJ; M. Solberg, The State Univ., Rutgers; T. Van Der Zwet, USDA, Kearneysville, WV.

[y] Via S. Beer, Cornell Univ., Ithaca, NY.

[z] Original isolations from soft rotted bell peppers.

were the only major components that occurred in all genera, present at concentrations of over 1% of the total. The 14:0 was a major component in all genera except the fluorescent pseudomonads, and the 18:1 *cis* + *trans* 9 was a major fatty acid in all genera except *Bacillus, Cytophaga,* and *Xanthomonas*. Thirty of the fatty acids occurred at concentrations of less than 1% of the total in all genera.

Several fatty acids were abundant in some genera but not in others. Hydroxy-substituted 30H-14:0 occurred at minor or trace levels in all genera, except in *Erwinia* and in the nonfluorescent pseudomonads, in which they were major components, with mean percentages of 5.72 and 6.33%, respectively (Table 4). Saturated and unsaturated, branched-chain *iso-* and *anteiso*-15:0 and -17:0, *iso*-16:0, and *iso*-17:1 were major components of *Bacillus* and *Xanthomonas*; in *Cytophaga* all of these, except *iso*-16:0 and -17:0 (mean percentage of 0.37 and 0.77%, respectively), were major components (Table 4). Both cyclopropane 17:0 and 19:0 were major components in the nonfluorescent pseudomonads and were absent or minor components (less than 1% of total) in *Bacillus, Clostridium, Cytophaga,* and *Xanthomonas*.

Three components in *Bacillus* spp. had ECLs of 14.55, 15.33, and 16.33 and could not be identified completely. They were tentatively assigned to class C, the unsaturated, straight-chain fatty acids, because their methyl esters disappeared during hydrogenation, and their saturated, straight-chain analogs (15:0, 16:0, and 17:0, respectively) increased proportionally.

A statistical analysis of class totals indicated there were significant differences among genera in all classes of fatty acids. *Clostridium* had the highest mean percentage of class A (63.90%), significantly different from all except *Erwinia* (40.89%). *Cytophaga* had the highest percentage of class B (9.44%), different from all other genera. Mean percentages in class C for *Erwinia* (42.11%) and *Pseudomonas* (nonfluorescent, 35.33%, and fluorescent, 53.10%) were higher than for *Bacillus* (8.91%), *Clostridium* (16.31%), and *Cytophaga* (19.98%) (Table 3). In class D, *Bacillus* and *Clostridium* had significantly lower mean percentages (1.06 and 2.28%, respectively) than had *Cytophaga* (11.00%) or the nonfluorescent pseudomonads (13.97%). In class E, mean percentages for *Erwinia* and the pseudomonads were less than 1.5%, significantly lower than for all other genera. In class F, mean percentages for the pseudomonads (nonfluorescent, 0.20%, and fluorescent, 0.04%) were lower than in all other genera, and in class G, the mean percentage for nonfluorescent pseudomonads (10.45%) was higher than that for *Bacillus, Clostridium, Cytophaga,* and *Xanthomonas* (Table 4).

Differentiation of genera by class analysis. Genera differences,

TABLE 2. Mathematical rules for computer-assisted differentiation of *Erwinia* and *Pseudomonas*[v] based on the percentage ranges of their fatty acids

Computer code[w]	Fatty acid[x]	*Erwinia*		*Pseudomonas*	
		Low-high range[y]	Rule	Low-high range	Rule
A1	8:0	0–0.60	<1	0–2.62	<3
A2	10:0	0–0.96	<1.4	0–0.49	<.8
A3	12:0	0.18–7.85	>0 <8.5	.05–10.97	>0 <12
A4	14:0	1.10–9.00	>.7 <9.7	0.14–4.36	>0 <4.9
A5	16:0	19.16–41.10	>18 <44	16.09–34.71	>15 <36
A6	18:0	0.06–1.59	>0 <2	0.06–3.00	>0 <3.5
A7	20:0	0–2.34	<2.7	0–0.58	<.9
A	Class total	28.45–53.25	>27 <56	24.06–42.08	>23 <45
B1	9:0	0–0.14	<.3	0–0.90	<1.3
B2	11:0	0–0.26	<.6	0–0.10	<.3
B3	13:0	0–0.75	<1.1	0–0.12	<.3
B4	15:0	0–2.22	<2.6	0–0.46	<.8
B5	17:0	0–2.26	<2.7	0–0.68	<1.1
B6	19:0	0–1.45	<1.8	0–2.39	<2.8
B	Class total	0.12–5.39	>0 <6	.06–2.88	>0 <3.3
C1	11:1 *cis* 5	0–0	<.1	0–0	<.1
C2	12:1 *cis* 5	0–1.02	<1.4	0–0.66	<1.1
C3	12:1 *cis* 11	0–0	<.1	0–0.09	<.2
C4	13:1	0–3.35	<3.8	0–0	<.1
C5	14:1 *cis* 7	0–1.21	<1.6	0–0.14	<.3
C6	14:1 *cis* 9	0–0.59	<.9	0–0.11	<.3
C7	15:1	0–0.69	<1.1	0–0.75	<1.1
C1C[z]	16:1 *cis* 9 + *trans* 9	2.62–40.53	>2.2 <43	11.96–43.94	>10 <46
C10	17:1 *cis* 5	0–2.21	<2.6	0–0.41	<.8
C11	17:1 *cis* 12	0–0	<.1	0–1.87	<2.3
C2C[z]	18:1 *cis* 9 + *trans* 9	6.41–27.09	>5.8 <29	7.89–44.92	>7.2 <47
C15	20:4 *cis* 5	0–0.34	<.7	0–0.36	<.7
C16	20:4 *cis* 8	0–0	<.1	0–0	<.1
C17	20:2	0–0.56	<.9	0–0.65	<1
C	Class total	10:12–63.33	>9.3 <66	24.96–66.07	>23 <69
D1	20H-10:0	0–4.66	<5.2	0–0.49	<.8
D2	30H-10:0	0–1.66	<2.1	0–4.68	<5.2
D3	i30H-11:0	0–0.05	<.2	0–0	<.1
D4	30H-11:0	0–1.06	<1.5	0–3.39	<3.9
D5	20H-12:0	0–1.24	<1.6	0–5.94	<6.5
D6	30H-12:0	0–2.69	<3.1	0–3.47	<4
D7	i30H-13:0	0–0	<.1	0–0	<.1

(*continued on next page*)

[v] Species and strains listed in Table 1.
[w] Code includes letter designating chemical class and number for relative order of elution.
[x] Fatty acids of bacteria grown on King's medium B agar at 25 C for 24 h, and analyzed by gas-liquid chromatography. A = saturated, even-carbon, straight-chain fatty acids; B = saturated, odd-carbon, straight-chain fatty acids; C = unsaturated straight-chain fatty acids; D = hydroxy-substituted fatty acids; E = saturated, branched-chain fatty acids; F = unsaturated, branched chain fatty acids; G = cyclopropane fatty acids; H = unidentified unsaturated fatty acids designated by their equivalent (carbon) chain length. *Cis* and *trans* isomers are indicated and followed by the carbon number of the unsaturation site. *Iso* and *anteiso* branching is indicated by i and a.
[y] Lowest and highest percentage values for strains in each genus.
[z] Combined values for *cis* and *trans* isomers.

with their exclusive percentage ranges, could be incorporated into a dichotomous taxonomic key that differentiates all genera on the basis of class percentages of fatty acids (Table 5). The fluorescent and nonfluorescent pseudomonads could not be differentiated at a 100% degree of probability on the basis of one fatty acid class because of some overlapping of percentage ranges. However, the ratio of class C to class D (saturated to hydroxy fatty acids) was a differentiating factor, with ranges exclusive to each group for over 90% of the strains tested. Ratios for the nonfluorescent pseudomonads ranged from 1.64 to 3.64, and those for the fluorescent pseudomonads ranged from 3.13 to 11.74 (data not shown).

Differentiation of genera by the expert system. Using the rules that defined the respective genera, the computer expert system correctly identified the genus of all bacteria shown in Table 1.

Statistical analysis of species differences in *Erwinia* and *Pseudomonas*. In *Erwinia* and *Pseudomonas* spp. 61 fatty acids were detected, identified, and placed in the database. Eight of these were major components (each averaging over 1% of the total) in at least one species of *Erwinia*: 12:0, 14:0, 16:0, 16:1, and 18:1 *cis* + *trans* 9, 30H-14:0, *iso*-15:1, and cyclopropane 17:0. In the pseudomonads, major components in at least one of the species were 12:0, 14:0, 16:0, 18:0 (nonfluorescent species only), 16:1 and 18:1, 20H-12:0 and 30H-12:0 (fluorescents only), 30H-14:0, 30H-16:0, 30H-17:0, *iso*-19:0, and cyclopropane 19:0 (nonfluorescents only), and cyclopropane 17:0 (see Table 2 for percent ranges and Tables 3 and 4 for averages).

In *Erwinia*, mean percentages of nine of 59 fatty acids identified and of classes A, B, C, E, and G were significantly different at the 99% level of probability in one or more species (Table 6). These nine fatty acids accounted for 67–77% of the total fatty acid content of each species. Ranges for classes A and C in *E. carotovora* and *E. chrysanthemi* were statistically different from those in other species, and the mean percentage for class G in *E. carotovora* (0.09%) was significantly different from *E. amylovora, E. ananas,* and *E. herbicola* (all over 4.7%). Furthermore, statistical differences in five of the nine individual fatty acids, accounting for 59–71% of the total fatty acid content, suggested two groupings: *E. carotovora* and *E. chrysanthemi* in one group and *E. amylovora, E. ananas, E. herbicola,* and *E. rhapontici* in the other. Because of overlapping ranges for fatty acids in the profiles, differentiating *Erwinia* species could be accomplished only with the computer expert system.

Among the eight species of *Pseudomonas* there were significant differences in mean percentages of 23 of 56 fatty acids identified, and in six of the seven class totals (Table 7). The nonfluorescent

TABLE 2. (*continued from preceding page*)

Computer code[w]	Fatty acid[x]	*Erwinia*		*Pseudomonas*	
		Low-high range[y]	Rule	Low-high range	Rule
D8	20H-13:0	0–0	<.1	0–0.77	<1.2
D9	30H-13:0	0–0	<.1	0–1.23	<1.6
D10	20H-14:0	0–1.43	<1.8	0–1.21	<1.6
D11	30H-14:0	0.75–8.91	>.4 <9.6	0–8.29	<9
D12	i30H-15:0	0–1.95	<2.3	0–1.43	<1.8
D13	20H-15:0	0–3.19	<3.6	0–0	<.1
D14	30H-15:0	0–0.19	<.4	0–0	<.1
D15	20H-16:0	0–1.22	<1.6	0–0.78	<1.2
D16	30H-16:0	0–0.52	<.9	0–4.58	<5.1
D17	i30H-17:0	0–0.68	<1.1	0–1.04	<1.4
D18	20H-17:0	0–0	<.1	0–0.06	<.2
D19	30H-17:0	0–4.34	<4.8	0–2.76	<3.2
D	Class total	2.53–12.26	>2.1 <14	5.43–21.88	>4.8 <23
E1	i11:0	0–1.27	<1.7	0–2.53	<2.9
E2	a11:0	0–0	<.1	0–0	<.1
E3	i13:0	0–0.99	<1.4	0–0.72	<1.1
E4	a13:0	0–0.03	<.1	0–0	<.1
E5	i14:0	0–0.24	<.5	0–0.36	<.7
E6	a14:0	0–0.74	<1.1	0–0	<.1
E7	i15:0	0–2.00	<2.4	0–0.16	<.4
E8	a15:0	0–0.62	<1	0–0.25	<.6
E9	i16:0	0–0.51	<.9	0–0.06	<.2
E10	a16:0	0–0	<.1	0–0	<.1
E11	i17:0	0–0.73	<1.1	0–0.34	<.7
E12	a17:0	0–1.56	<2	0–0.40	<.8
E14	a18:0	0–0.72	<1.1	0–0	<.1
E15	i19:0	0–2.52	<2.9	0.01–1.73	>0 <2.1
E16	a19:0	0–0.34	<.7	0–0.84	<1.2
E	Class total	0.06–5.77	>0 <6.4	0.16–3.30	>0 <3.8
F1	i15:1	0.44–2.65	>0<3	0–0	<.1
F2	a15:1	0–0	<.1	0–0	<.1
F3	i16:1	0–0	<.1	0–0	<.1
F4	a16:1	0–0	<.1	0–0	<.1
F5	i17:1	0–5.19	<5.8	0–0.25	<.6
F6	a17:1	0–0.13	<.3	0–0.30	<.7
F7	i18:1	0–0.07	<.2	0–0	<.1
F	Class total	0.44–7.29	>0 <7.8	0–0.48	<.8
G1	*cyclo* 17:0	0–18:83	<20	0–15	<16
G2	*cyclo* 19:0	0–2.85	<3.2	0–4.72	<5.2
G	Class total	0–21.68	<23	0–18.97	<20
H1	ECL 13.33	0–0	<.1	0–0.11	<.3
H2	ECL 14.55	0–.02	<.1	0–0	<.1
H3	ECL 15.33	0–0	<.1	0–0	<.1
H4	ECL 16.33	0–.21	<.5	0–0.14	<.3
H	Total	0–0.21	<.5	0–0.17	<.4

species, *P. cepacia* and *P. cichorii*, differed from fluorescent species in 13 of the 23 acids, notably 12:0, 14:0, 18:0, and 19:0, and in the total for class G. However, *P. cepacia* could be differentiated from *P. cichorii* only by differences in one minor component, 30H-13:0, and in aggregated minor differences in ranges for each of the other acids and class totals.

The fluorescent species, as with the *Erwinia* species, could not be differentiated readily without the expert system. Two features of their profiles, however, were discernable. Percentages of 12:0, 16:0, and the class A total in *P. fluorescens* differed significantly from *P. viridiflava*, and 18:1 isomers were higher in *P. aeruginosa* (37.0%) than in other species (less than 20%).

Differentiation of species by the expert system. Species of *Erwinia* and *Pseudomonas* were identified correctly by the computer expert system using rules derived from ranges characteristic of each species. The rules and computer display for identification of *P. viridiflava* (ATCC 43557) are shown in Figures 1 and 2. Only two of the 141 *Erwinia* and *Pseudomonas* strains tested were identified ambiguously. Strain F8 of *P. viridiflava* also matched the profile of *P. syringae*, and strain ATCC 29284 of *E. rhapontici* also matched the profile of *E. herbicola.*

Statistical variation within a strain. Generally, there was no significant variability in fatty acid profiles resulting from the instrumentation. Methylated samples yielded reproducible fatty acid profiles when repeatedly chromatographed. However, there was variability within strains when, on different occasions, a bacterial sample was recultured and methylated, all other conditions remaining the same. This variability, expressed as a coefficient (of variation), ranged between 0.02 and 0.05, values generally less than the variation between species.

TABLE 3. Mean percentages of saturated, straight-chain (classes A and B) and unsaturated (class C) fatty acids in bacteria associated with postharvest diseases of fruits and vegetables

Fatty acid[w]		Genus[x]						
						Pseudomonas[y]		
Class	ECL	*Bacillus*	*Clostridium*	*Cytophaga*	*Erwinia*	nonfluorescent	fluorescent	*Xanthomonas*
A								
8:0	8.00	0.09	0.22	0.03	0.06	0.09	0.29	0.05
10:0	10.00	0.03	2.39	0	0.09	0.07	0.08	0.64
12:0	12.00	0.25	20.77	0.07	4.36	0.51	4.82	0.10
14:0	14.00	2.43	18.16	1.12	4.48	3.73	0.80	1.79
16:0	16.00	4.14	17.20	7.98	31.24	29.34	27.09	4.93
18:0	18.00	0.46	3.86	3.47	0.54	2.00	0.43	0.06
20:0	20.00	0.03	1.32	0.10	0.08	0.20	0.06	0.05
Total (mean)		7.43 a	63.90 c	12.77 a	40.89 bc	35.94 b	33.57 b	7.62 a
Range: low		3.82	31.02	8.09	28.45	27.23	24.16	5.31
high		18.68	78.63	18.22	53.25	42.08	41.13	15.90
B								
9:0	9.0	0.02	0.12	0.01	0.02	0.01	0.04	0.01
11:0	11.0	0	0.10	0	0.01	0	0.01	0.11
13:0	13.0	0.02	0.24	0.07	0.06	0.01	0.02	0.06
15:0	15.0	0.56	0.25	8.57	0.35	0.08	0.11	1.73
17:0	17.0	0.10	0.29	0.61	0.22	0.26	0.11	0.24
19:0	19.0	0.01	0.22	0.19	0.25	0.99	0.13	0
Total (mean)		0.71 a	1.23 a	9.44 b	0.90 a	1.33 a	0.42 a	2.14 a
Range: low		0.21	0.46	7.32	0.14	0.74	0.06	0.83
high		1.81	2.01	11.03	3.37	2.88	1.12	5.17
C								
11:1 *cis* 5	10.83	0	0.91	0	0	0	0	0.01
12:1 *cis* 5	11.84	0.61	0.25	0.03	0.19	0.30	0.09	0.15
12:1 *cis* 11	11.92	0	0.64	0	0	0	0	0
13:1	12.85	0.24	1.73	0	0	0	0	0
14:1 *cis* 7	13.84	0.25	0.09	0.07	0.23	0.02	0	0.07
14:1 *cis* 9	13.91	0	0.11	0	0.14	0.02	0	0
Unknown[z]	14.55	0.07	0.29	0	0	0	0	0
15:1	14.85	0.08	1.39	2.97	0.01	0.06	0.01	0.61
Unknown[z]	15.33	1.84	0	0	0	0	0	0
16:1 *cis* 9	15.74	3.31	4.11	13.67	26.65	16.56	33.95	2.31
16:1 *trans* 9	15.82	0.05	2.58	0.76	0.11	0.10	0.51	18.70
Unknown[z]	16.33	2.13	0.02	0	0.02	0.01	0.02	0
17:1 *cis* 5	16.81	0	0.01	1.74	0.08	0.06	0.03	0.96
17:1 *cis* 12	16.92	0	0.17	0	0	0.38	0	0
18:1 *cis* 9	17.73	0.16	0.81	0.18	5.79	0.01	0.04	0.38
18:1 *trans* 9	17.81	0.07	1.72	0.16	8.67	17.37	18.40	0.30
20:4 *cis* 5	19.20	0.31	0.21	0.13	0.02	0.08	0.06	0.02
20:4 *cis* 8	19.40	1.05	0.08	0	0	0	0	0
20:2	19.72	0	0.61	0.09	0.06	0.39	0.01	0.01
Total (mean)		8.91 a	16.31 a	19.98 a	42.11 b	35.33 b	53.10 b	23.53 ab
Range: low		2.32	7.45	16.68	10.12	24.96	31.56	20.81
high		18.60	33.58	24.74	63.33	43.69	66.07	29.15

[w] Fatty acids by chemical class: class A = saturated, even-carbon, straight-chain fatty acids; class B = saturated, odd-carbon, straight-chain fatty acids; class C = unsaturated, straight-chain fatty acids. ECL = equivalent (carbon) chain length: a mean based on at least three determinations for bacteria of each genera. Means of each class total not followed by the same letter are significantly different at the 99% level of probability ($P = 0.01$).

[x] Means based on strains described in Table 1, grown for 24 h on King's medium B agar at 25 C.

[y] Fluorescent pseudomonads: *P. aeruginosa, P. cichorii, P. fluorescens, P. putida, P. syringae* pv. *syringae,* and *P. viridiflava*; nonfluorescent pseudomonads: *P. cepacia* and *P. gladioli.*

[z] Unknown fatty acid with unsaturated sites, as determined by confirmatory chemical tests described in the text.

TABLE 4. Mean percentages of hydroxy-substituted (class D), saturated and unsaturated branch-chained (classes E and F), and cyclopropane (class G) fatty acids in bacteria associated with postharvest diseases of fruits and vegetables

Fatty acid[w]		Genus[x]						
						Pseudomonas[y]		
Class	ECL	*Bacillus*	*Clostridium*	*Cytophaga*	*Erwinia*	nonfluorescent	fluorescent	*Xanthomonas*
D								
20H-10:0	11.22	0.04	0.11	0	0.18	0.06	0.05	0.10
30H-10:0	11.46	0.35	0.19	0.40	0.09	0.27	2.96	0.89
i30H-11:0	12.10	0	0	0.01	0	0	0	1.29
30H-11:0	12.44	0	0.31	0.04	0.03	0	0.22	0.08
20H-12:0	13.16	0	0.06	0.02	0.04	0.01	3.07	0.07
30H-12:0	13.46	0.04	0.13	1.30	0.11	0.02	1.33	0.60
i30H-13:0	14.12	0	0	0	0	0	0	1.37
20H-13:0	14.10	0	0.45	0	0	0	0.06	0.13
30H-13:0	14.47	0	0.14	0	0	0.34	0.03	0
20H-14:0	15.17	0.17	0.11	0.17	0.22	0.08	0.05	0
30H-14:0	15.49	0	0.03	0.51	5.72	6.33	0.04	0
i30H-15:0	16.12	0.34	0	3.31	0.20	0.88	0.17	0
20H-15:0	16.18	0.10	0.10	0	0.03	0	0	0.04
30H-15:0	16.49	0	0	0	0	0	0	0.01
20H-16:0	17.19	0.01	0	0.26	0.03	0.35	0.03	0.02
30H-16:0	17.52	0	0	1.99	0.03	3.59	0.01	0.11
i30H-17:0	18.16	0	0.34	2.81	0.11	0.24	0.31	0.05
20H-17:0	18.23	0	0	0	0	0	0.02	0
30H-17:0	18.34	0	0.30	0.20	0.72	1.84	0.45	0.16
Total (mean)		1.06 a	2.28 a	11.00 b	7.51 ab	13.97 b	8.87 ab	4.92 ab
Range: low		0	0.36	4.44	3.78	10.33	5.43	3.48
high		3.69	11.72	18.38	12.26	16.79	21.88	7.12
E								
i11:0	10.57	0	0.04	0.03	0.06	0.02	0.34	3.61
a11:0	10.66	0	0	0	0	0	0	0.19
i13:0	12.62	3.60	1.68	0.33	0.03	0	0.18	0.27
a13:0	12.71	0.81	0.07	0	0	0	0	0.13
i14:0	13.64	5.00	1.01	0.16	0.04	0.02	0.08	0.58
a14:0	13.74	0.10	1.40	0	0.17	0	0	0.05
i15:0	14.63	25.55	3.20	32.52	0.03	0.02	0.01	27.13
a15:0	14.72	27.82	0.18	1.06	0.02	0.04	0.01	14.89
i16:0	15.65	3.41	0.57	0.37	0.01	0	0	1.62
a16:0	15.73	1.27	0.03	0	0	0	0	0
i17:0	16.64	5.55	2.01	0.77	0.10	0.13	0.04	5.28
a17:0	16.73	4.42	0.07	1.31	0.10	0.12	0.09	1.23
a18:0	17.72	0	0	0	0.12	0	0	0
i19:0	18.55	0.04	0.64	0	0.48	1.09	0.25	0
a19:0	18.71	0.02	0.20	0	0.02	0.06	0.10	0
Total (mean)		77.58 d	11.08 b	36.53 c	1.07 a	1.43 a	1.10 a	54.98 d
Range: low		56.72	5.86	30.29	0.06	0.75	0.16	48.36
high		91.04	20.97	39.71	3.80	2.27	3.30	60.38
F								
i15:1	14.43	0.21	0.23	3.10	1.54	0	0	0.76
a15:1	14.53	0	0.01	0	0	0	0	0.11
i16:1	15.42	0.99	0.01	0	0	0	0	0
a16:1	15.52	0.88	0	0	0	0	0	0
i17:1	16.42	1.38	0.70	3.52	0.12	0.12	0.03	5.01
a17:1	16.52	0.57	0.48	0.82	0	0.09	0.01	0.30
i18:1	17.42	0.02	0.23	0	0.01	0	0	0
Total (mean)		4.04 bc	1.65 b	7.43 c	1.68 b	0.20 a	0.04 a	6.19 bc
Range: low		0.35	0.17	4.18	0.52	0.01	0	2.67
high		9.95	4.90	10.84	2.65	0.39	0.25	10.05
G								
cyclo 17:0	16.85	0	0.24	0.27	4.57	7.89	1.90	0.23
cyclo 19:0	18.86	0	0.49	0.05	0.44	2.55	0.23	0
Total: mean		0 a	0.73 a	0.32 a	5.01 ab	10.45 ab	2.12 ab	0.23 a
Range: low		0	0	0.17	0	3.30	0	0.05
high		0	1.90	0.48	21.68	18.97	5.48	0.45
Unknown[z]		0.25	2.82	2.53	0.83	1.35	0.87	0.39

[w] Fatty acids by chemical class: class D = hydroxy-substituted fatty acids; class E = saturated, branched-chain fatty acids; class F = unsaturated, branched-chain fatty acids; class G = cyclopropane fatty acids. ECL = equivalent (carbon) chain length, a mean based on at least three determinations for bacteria of each genera. Nomenclature of fatty acids: i = *iso*, and a = *anteiso*. Means of each class total not followed by the same letter are significantly different at the 99% level of probability ($P = 0.01$).

[x] Means based on strains described in Table 1, grown for 24 h on King's medium B agar at 25 C.

[y] Fluorescent pseudomonads: *P. aeruginosa, P. cichorii, P. fluorescens, P. putida, P. syringae* pv. *syringae*, and *P. viridiflava*; nonfluorescent pseudomonads: *P. cepacia* and *P. gladioli.*

[z] Unknown fatty acid with unsaturated sites, as determined by confirmatory chemical tests described in text.

TABLE 5. Simplified taxonomic key based on class analysis of cellular fatty acids for genera of bacteria associated with postharvest diseases of fruits and vegetables

Step	Factor[w] (class)	Value (%)	Interpretation[x]	Probability[y] (%)
1	B	>7	*Cytophaga*	100
		<7	all other genera (go to step 2)	
2	A	<23	*Bacillus, Xanthomonas* (go to step 3)	100
		>23	*Erwinia, Pseudomonas, Clostridium* (go to step 4)	100
3	C + D	<23	*Bacillus*	100
		>23	*Xanthomonas*	100
4	E	>4	*Clostridium*	100
		<4	*Erwinia, Pseudomonas* (go to step 5)	100
5	F	>0.4	*Erwinia*	100
		<0.4	*Pseudomonas* (go to step 6)	100
6	C/D	<3.5[z]	fluorescent pseudomonads	92
		>3.5[z]	nonfluorescent pseudomonads	100

[w] Class A = saturated, even-carbon, straight-chain fatty acids; class B = saturated, odd-carbon, straight-chain fatty acids; class C = unsaturated, straight-chain fatty acids; class D = hydroxy-substituted fatty acids; class E = saturated, branched-chain fatty acids; class F = unsaturated, branched-chain fatty acids.
[x] Based on strains described in Table 1.
[y] Probability = percentage of strains tested that fit the indicated values.
[z] Ratio of fatty acids.

DISCUSSION

Fatty acid analysis can serve as a primary taxonomic tool or, more conservatively, as a confirmatory technique for previous identifications of bacteria. Genera that postharvest pathologists have encountered were included in this study, except for *Enterobacter cloacae* (22), which will be studied in the future, as strains become available. *P. pseudoalcaligenes* also was not included, but an abbreviated fatty acid profile for the species has been published (28) and fits the pattern described for the nonfluorescent pseudomonads.

Genus identification by class analysis of fatty acids was relatively uncomplicated. Once class totals are calculated and statistically analyzed, a dichotomous key can be used to identify genera. Although the key is based on data for the 190 strains listed in Table 1, it has been used successfully in identifying the genus of over 500 additional strains in our database.

Fatty acid profiles can be affected by culture conditions, physiological age of cells, and experimental factors in the laboratory (4). Our data was developed from cells grown on KB agar, in contrast to the data of others who have used cells grown in Trypticase soy agar (TSA) (6,18,26,30). We selected KB agar because all bacteria grew abundantly on KB in 24 h at 25 C, because of its diagnostic value in differentiating fluorescent and nonfluorescent pseudomonads, and because of the greater diversity of fatty acids detected in cells grown on KB than in cells grown on TSA (4). *E. amylovora* cells grown on KB have higher proportions of unsaturated fatty acids (class C) and lower saturated, odd-carbon, straight-chain and cyclopropane (classes B and G) fatty acids (4,33). Other genera are affected similarly

TABLE 6. Mean percentages of fatty acids and class totals significantly different in *Erwinia* species associated with postharvest diseases

Fatty acid and class totals[v]	*Erwinia* species (%)[w]					
	amylovora (14)[x]	*ananas* (12)	*herbicola* (11)	*carotovora* (20)	*chrysanthemi* (7)	*rhapontici* (6)
12:0	4.64 b	3.79 ab	3.66 ab	6.43 b	2.32 a	3.92 ab
14:0	5.37 b	4.33 b	4.95 b	1.76 a	3.62 ab	4.41 b
16:0	31.61 b	35.17 b	33.01 b	27.96 ab	24.41 a	36.08 b
Class A	42.30 b	44.65 b	42.36 b	36.91 a	31.70 a	45.01 b
SD	±2.27	±2.58	±5.46	±3.48	±2.91	±3.49
19:0	0.28 ab	0.43 ab	0.50 b	0.01 a	0.04 a	0.27 ab
Class B[y]	1.10 a	0.91 a	0.81 a	1.06 a	0.68 a	0.88 a
SD	±0.50	±0.28	±0.51	±0.52	±0.51	±0.57
13:1	0 a	0 a	0.03 a	0.74 b	0 a	0 a
16:1 *cis* 9	27.04 ab	17.31 a	19.97 ab	33.92 c	35.97 c	28.59 b
Class C	39.59 a	35.19 a	34.14 a	54.19 b	57.64 b	40.31 a
SD	±5.68	±2.35	±12.46	±2.79	±6.23	±5.68
30H-17:0	0.84 ab	0.92 ab	1.46 b	0.02 a	0.11 a	0.79 ab
Class D[y]	7.71 a	8.57 a	8.86 a	5.30 a	6.32 a	8.17 a
SD	±1.42	±1.81	±3.46	±1.03	±1.76	±1.68
i19:0[z]	0.51 ab	0.78 b	1.01 b	0.03 a	0.11 ab	0.62 ab
Class E	1.04 ab	1.50 ab	1.88 b	0.60 a	0.75 ab	1.00 ab
SD	±0.35	±0.48	±1.38	±0.68	±0.59	±0.41
cyclo 17:0	5.15 b	4.70 b	7.55 b	0.07 a	0.74 ab	1.97 ab
Class G	5.88 bc	5.23 b	8.19 c	0.09 a	0.91 ab	2.11 ab
SD	±4.30	±0.87	±5.39	±0.11	±1.26	±1.73

[v] Class A = saturated, even-carbon, straight-chain fatty acids; class B = saturated, odd-carbon, straight-chain fatty acids; class C = unsaturated, straight-chain fatty acids; class D = hydroxy-substituted fatty acids; class E = branched-chain fatty acids; class F = unsaturated, branched-chain fatty acids; class G = cyclopropane fatty acids. Means within the same row with no letter in common are significantly different ($P = 0.01$). SD = standard deviation of the population (calculated for class means only).
[w] Strains grown 24 h at 25 C on King's medium B agar. Means based on duplicate or triplicate analyses of each type strain and a single analysis for all others.
[x] Numbers in parentheses refer to the number of strains tested per species.
[y] Differences for classes B and D not significant.
[z] i = *iso*-branched.

(J. Wells, *unpublished data*). Nevertheless, these differences did not significantly affect the value of the dichotomous key, presented in Table 4, when data from cells grown on TSA were used, provided the chromatograms were sufficiently detailed.

The detail of the chromatogram from which percentages were generated, was an important factor in the quality of the data. In the final step of sample methylation, the hexane ethyl ether extract was concentrated to at least 20–30 μl, of which 2 μl was injected into the gas chromatograph. This yielded chromatograms with at least 30 identifiable components located between the 8 and 20 carbons. Most chromatograms contained 40–60 component peaks. Those with less than 30 were not used in the database. As a result, fatty acids averaging less than 1% of the total made important contributions to class totals, particularly in classes B and F.

In the differentiation of the *Erwinia* and *Pseudomonas* genera, *Erwinia* strains of all species grown on KB agar had an unsaturated, branched-chain fatty acid (class F) with an ECL of 14.43, identified as *iso*-15:1, ranging from 0.44 to 2.26% of the total fatty acid content. This component was absent in all *Pseudomonas* strains tested. The percentage of class F, therefore, was important for a one-step separation of *Erwinia* from *Pseudomonas* in the dichotomous key.

Species within *Erwinia* and *Pseudomonas* could not be separated with simple parameters, such as one or a combination of a few class totals or of individual fatty acids. Successful differentiation of these species involved consideration of multiple factors, requiring the assistance of a PC microcomputer.

The expert system described in this report was designed for use in any laboratory capable of analyzing bacterial fatty acids by GLC. The data are keyed into a program on a PC computer containing at least 256 K of internal memory. Data input and expert-system operation are uncomplicated and are accomplished by responding to commands and menus. The domain expert can be from the laboratory's own fatty acid analyses, from published information (if experimental conditions and chromatogram interpretations are the same), or from analyses done by other laboratories. The knowledge base founded on the domain expert, by necessity, must be built within the parameters of the RDMS. The selection of a RDMS is critical to the success of the expert system. The system may be identical to the one described here, or a system may be customized using the same general approaches.

TABLE 7. Mean percentages of fatty acids and class totals significantly different in *Pseudomonas* species associated with postharvest diseases

	Nonfluorescent pseudomonads (%)[y]		Fluorescent pseudomonads (%)					
Fatty acid and class totals[x]	*cepacia* (6)	*gladioli* (5)	*aeruginosa* (6)	*cichorii* (5)	*fluorescens* (18)	*putida* (6)	*syringae* (10)	*viridiflava* (15)
12:0	0.50 a	0.53 a	3.13 b	4.60 cd	3.83 bc	4.87 cd	6.51 d	6.18 d
14:0	3.76 a	3.69 a	0.80 b	0.44 b	1.29 b	0.95 b	0.56 b	0.45 b
16:0	29.33 a	29.36 a	24.88 ab	28.10 ab	29.76 a	28.53 ab	28.03 ab	22.48 b
18:0	2.04 a	1.96 a	0.47 b	0.39 b	0.38 b	0.54 b	0.50 b	0.39 b
Class A	35.93 a	35.96 a	29.61 b	33.97 ab	35.99 a	35.16 a	35.77 a	29.78 b
SD	±5.79	±2.47	±3.02	±3.22	±3.73	±4.19	±3.51	±3.01
19:0	1.18 a	0.79 a	0.04 b	0.02 b	0.20 b	0.28 b	0.11 b	0.08 b
Class B	1.64 a	1.03 a	0.46 ab	0.20 b	0.62 ab	0.54 ab	0.35 b	0.26 b
SD	±0.82	±0.30	±0.17	±0.05	±0.29	±0.18	±0.12	±0.09
16:1 *cis* 9	17.41 a	15.70 a	21.54 ab	34.95 ab	34.82 ab	30.02 ab	35.57 ab	40.70 b
17:1 *cis* 12	0.51 a	0.25 a	0 b	0 b	0 b	0 b	0 b	0 b
18:1 *cis* 9 + *trans* 9	18.58 a	16.19 a	36.98 b	17.76 a	12.36 a	13.14 a	17.80 a	19.94 a
20:2	0.36 a	0.42 a	0 b	0 b	0.03 b	0 b	0 b	0 b
Class C	37.52 a	33.14 a	59.62 c	52.94 bc	47.43 bc	46.32 bc	53.70 bc	60.86 c
SD	±7.10	±7.78	±3.34	±4.12	±6.78	±4.66	±2.24	±3.94
30H-10:0	0 a	0.54 a	3.11 b	3.25 b	2.96 b	3.46 b	2.60 b	2.62 b
20H-12:0	0.02 a	0 a	3.76 b	3.09 b	3.23 b	3.10 b	3.30 b	2.25 b
30H-12:0	0.01 a	0.02 a	1.27 b	1.53 b	1.53 b	1.16 b	1.21 b	1.12 b
30H-13:0	0.01 a	0.66 b	0.01 a	0.09 a	0.04 a	0.06 a	0 a	0.01 a
30H-14:0	5.52 a	7.14 a	0.01 b	0.12 b	0.05 b	0.08 b	0.01 b	0.01 b
i30H-15:0[z]	0.83 a	0.92 a	0.04 b	0.01 b	0.29 ab	0.53 ab	0 b	0.08 b
20H-16:0	0.44 a	0.27 a	0.02 b	0.04 b	0 b	0.01 b	0.09 b	0.05 b
30H-16:0	3.66 a	3.59 a	0.01 b	0.02 b	0 b	0 b	0.05 b	0.01 b
30H-17:0	1.67 a	2.00 a	0.13 b	0.07 b	0.82 ab	1.17 ab	0.14 b	0.15 b
Class D	12.44 a	15.51 a	8.52 b	9.68 b	9.40 b	10.49 ab	8.01 b	6.93 b
SD	±1.70	±0.78	±1.09	±0.78	±1.65	±1.65	±1.03	±0.75
i11:0	0.01 a	0.02 a	0.17 a	0.57 b	0.41 ab	0.71 b	0.08 a	0.12 a
i13:0	0 a	0 a	0.07 a	0.20 b	0.25 b	0.37 b	0.07 a	0.09 a
i19:0	0.93 a	1.09 a	0.07 b	0.12 b	0.39 b	0.53 ab	0.22 b	0.12 b
Class E	1.42 b	1.45 b	0.70 ab	1.09 ab	1.33 ab	1.98 b	0.84 ab	0.66 a
SD	±0.67	±0.29	±0.18	±0.56	±0.52	±0.28	±0.56	±0.18
cyclo 17:0	7.05 a	8.74 a	0.27 c	0.41 c	3.91 b	3.80 b	0.66 bc	0.82 bc
cyclo 19:0	2.30 a	2.81 a	0.22 b	0.14 b	0.35 b	0.41 b	0.10 b	0.10 b
Class G	9.35 a	11.55 a	0.48 c	0.55 c	4.26 b	4.21 b	0.76 c	0.92 c
SD	±1.25	±1.98	±0.18	±0.05	±1.00	±0.97	±0.52	±0.54

[x] Class A = saturated, even-carbon, straight-chain fatty acids; class B = saturated, odd-carbon, straight-chain fatty acids; class C = unsaturated, straight-chain fatty acids; class D = hydroxy-substituted fatty acids; class E = branched-chain fatty acids; class G = cyclopropane fatty acids. Means within the same row with no letter in common are significantly different ($P = 0.01$). SD = standard deviation of the population (calculated for class totals only).

[y] Strains, listed in Table 1, grown 24 h at 25 C on King's medium B agar. Means based on duplicate or triplicate analyses of each type strain and a single analysis for all others. Numbers in parentheses refer to the number of strains tested per species.

[z] i = *iso*-branched.

The system described here is particularly useful for laboratories with an interest in a specialized bacterial group. In addition, the database and expert system can be continuously edited, modified, and expanded as required.

The data for the expert system were percent composition values for a total of 78 fatty acids, 59 of them applying to *Erwinia* and 56 to *Pseudomonas*. Nine of the *Erwinia* and 13 of the *Pseudomonas* components were major fatty acids in at least one of the species; the balance was provided by minor components, each averaging less than 1% of the total. Some of the minor components, primarily unsaturated and hydroxy-substituted fatty acids, have not been documented previously for these genera. Some were major components (over 1% of the total) in individual strains: unsaturated 12:1 *cis* 5, 13:1, 14:1 *cis* 7, and 17:1 *cis* 12 and hydroxy 30H-11:0, 30H-13:0, *iso*-30H-15:0, 20H-15:0, *iso*-30H-17:0, and 30H-17:0. Their identification was provisional, based on chemical tests for unsaturated and hydroxy-substituted fatty acids and on comparisons with published values for their ECLs. Confirmation of these minor components by our mass spectrometer was inconclusive because of the low cellular concentrations.

Two of the 190 strains in the database were inconclusively identified. Strain F8, acquired as *P. viridiflava*, matched the fatty acid profiles of *P. viridiflava* and *P. syringae*, two species with relatively high-similarity coefficients of 0.971, based on a comparison of their mean fatty acid profiles. Strain ATCC 29284 of *E. rhapontici* matched the profiles of *E. rhapontici* and *E. herbicola*, species with similarity coefficients of 0.954. These strains, therefore, could be considered phenotypically transitional based on their fatty acid composition.

One feature of the expert system is its ability to handle special problems, such as bacterial samples that cannot be initially identified or multiple identifications for one sample. Control rules determine the cause of the problem in terms of which fatty acids are involved, and then the fatty acids in question are linked to display rules that activate appropriate statements. Another useful feature is the system's ability to determine how closely the fatty acid composition of a particular bacterial strain agrees with profiles in the database. Because true/false statements are displayed for each field of rules (Fig. 2), a rapid determination can be made of which rules are true (=1) and which are false (=0) for a particular sample. Successful identification means all statements are true for one genus and species. If there are any false statements, data can then be matched with unsatisfied rules to determine which rules fell outside established rules for that fatty acid.

The special features of the expert system, not all present in proprietary systems, include: 1) utilization of existing laboratory equipment for analysis of fatty acids and off-the-shelf hardware and software; 2) a database containing sample profiles with specific percent values for each fatty acid, which is built, expanded, and modified as desired by the investigator; 3) the ability to determine which specific fatty acids (and their values) may fall beyond the normal, established range for a particular taxonomic group; and 4) statistical subprograms that analyze the basis of group differentiation and similarity coefficients, enabling comparative studies.

Field c1 (GENUS=1)*[5]

Field c2 (Display genus name)

Field c3 [#2=5)]

Field 1 (#2=5) & (A1>0) & (A1<1.6) & (A2>0) & (A2<.3) & (A3>4.8) & (A3<8.1) & (A4>0)

Field 2 (#2=5) &(A4<1.3) &(A5>15) &(A5<28) & (A6>0) &(A6<1.2) &(A7<.6) & (A>23)

Field 3 (#2=5) & (A<37) & (B1<.3) &(B2<.1) &(B3<.1) &(B4>0) &(B4<.4) & (B5>0)

Field 4 (#2=5) &(B5<.9) &(B6>0) & (B6<.7) & (B>0) & (B<1.3) & (C1<.1) & (C2>0)

Field 5 (#2=5) & (C2<.6) & (C3<.1) & (C4<.1) & (C5<.1) & (C6<.1) & (C7<.1) & (C1C>34)

Field 6 (#2=5) & (C1C<46) & (C10<.2) & (C11<.1) & (C12<.1) & (C2C>11) & (C2C<30)

Field 7 (#2=5) & (C15<.7) & (C16<.1) & (C17<.1) & (C>52) & (C<69) & (D1<.2) & (D2>1.9)

Field 8 (#2=5) & (D2<3.6) & (D3<.1) & (D4<.6) & (D5>1.3) & (D5<3.7) & (D6>.4)

Field 9 (#2=5) & (D6<2.1) & (D7<.1) & (D8<.3) & (D9<.1) & (D10<.3) & (D11<.1)

Field 10 (#2=5) & (D12<.6) & (D13<.1) & (D14<.1) & (D15<.4) & (D16<.2) & (D17>0)

Field 11 (#2=5) & (D17,1.4) & (D18<.1) & (D19>0) & (D19<.6) & (D>4.8) & (D<11) & (E1>0)

Field 12 (#2=5) & (E1<.7) & (E2<.1) & (E3>0) & (E3<.5) & (E4<.1) & (E5>0) & (E5<.2)

Field 13 (#2=5) & (E6<.1) & (E7<.1) & (E8<.1) & (E9<.1) & (E10<.1) & (E11<.2) & (E12<.8)

Field 14 (#2=5) & (E13<.1) & (E14<.1) & (E15>0) & (E15<.6) & (E16<.7) & (E>.1) & (E<1.3)

Field 15 (#2=5) & (F1<.1) & (F2<.1) & (F3<.1) & (F4<.1) & (F5<.1) & (F6<.2) & (F7<.1)

Field 16 (#2=5) & (F<.2) & (G1>.1) & (F1<3) & (G2>0) & (G2<.7) & (G>.1) & (G<3.2)

Field 17 (#2=5) & (H1<.1) & (H2<.1) & (H3<.1) & (H4<.1) & (H<.1)

Field c4 + c5 (1+2+3+4+5+6+7+8+9+10+11+12+13+14+15+16+17)=17

Field c6 (SPECIES=1)*[8]

Field c7 (Display species name)

Fig. 1. Computer documentation of process and control rules by field position for the species *Pseudomonas viridiflava*. Fields c1–c7 contain the control program. Fields 1–17 contain the process data. Processing fields contain rules (as shown in Table 2) for fatty acids in each genus and species. Processing data from a sample yields true (=1) or false (=0) statements for each rule. If all statements are true for a genus or species, links are made to control rules. Field c3, displaying a [5], indicates that the analyzed sample passed all rules and matches the profile of genus 5 (*Pseudomonas*). Field c3 links genus to species fields (shown only for *P. viridiflava*). Fields c4 and c5 add true statements from each preceeding field of rules. Because all fields contain true statements, field c6 identifies the sample as species 8, and field c7 displays *viridiflava*.

GENUS	PROCESSING FIELDS 12345678901234567		
Bacillus	00000001000010O	=	{0}[0][c]
Clostridium	00000000000001	=	{0}[0]
Cytophaga	00000000001100001	=	{0}[0]
Erwinia	11111001111111	=	{0}[0]
Pseudomonas	1111111111111	=	{1}[5]
Xanthomonas	0000000010000001	=	{0}[0]
	Genus is *Pseudomonas*		
SPECIES	1234567890123456 78		
aeruginosa	011011001111001011	=	{0}[0]
cepacia	001110000000001001	=	{0}[0]
cichorii	0010111101111111	=	{0}[0]
fluorescens	11111111101111001	=	{0}[0]
gladioli	001100000000000000	=	{0}[0]
putida	011101010101110001	=	{0}[0]
syringae	1111111101111111	=	{0}[0]
viridiflava	11111111111111111	=	{1}[8]
	Species is *viridiflava*		

Fig. 2. Computer screen display for analysis of *Pseudomonas viridiflava*, strain ATCC 43557. Braced and bracketed values trigger program control fields. When all processing fields are true, a 1 appears in the braced field, generating the number of the genus or species in the bracketed field.

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