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**Differentiation of the Soft-Rotting Erwinias  
(the *Carotovora* Group)  
by Fatty Acid Composition**

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**Abstract**

About 90% of total cellular fatty acids in *E. carotovora*, grown on KB medium for 1 day at 28°C, were the saturated, even-carbon straight chains 12:0 (5.9%), 14:0 (1.7%) and 16:0 (30.1%), and the unsaturated 16:1 (36.6%) and 18:1 (15.0%) fatty acids. Other components were the hydroxy-substituted 3-OH 14:0 (5.3%) and 21 minor fatty acids each occurring less than 0.1% of the total — 14 of them reported herein for the first time in *Erwinia*. The ratio of 16:1/18:1 in KB-grown cells was as useful in differentiating subspecies of *E. carotovora* as previously reported by other workers for TSA-grown cells. A comparison of fatty acid profiles of *E. carotovora* on 4 different media, KB, TSA, NA and PDA, indicated that on KB there was the greatest proportion of Class A and C fatty acids, and the highest number of detectable components. Significant differences were noted in the 5 major fatty acids and in cyclic fatty acids among the 4 species of the *carotovora* group — *E. carotovora*, *E. chrysanthemi*, *E. rhapontici* and *E. cypripedii*. These differences could be expressed as algorithms that, when used in sequential dichotomous steps, could differentiate the 4 species.

**Zusammenfassung**

**Differenzierung der Weichfäule Erwinias (*carotovora*-Gruppe)  
durch die Fettsäurezusammensetzung**

Nach eintägiger Kultivierung bei 28°C auf KB-Medium machten die saturierten, mit geradzahigen Kohlenstoffen, in gerade Ketten 12:0 (5,9%), 14:0 (1,7%) und 16:0 (30,1%), und die ungesättigten 16:1 (36,6%) und 18:1 (15,0%) Fettsäuren circa 90% der gesamten zellularen

Fettsäuren in *E. carotovora* aus. Andere Bestandteile waren die Hydroxy-substituierte 3-OH 14 : 0 (5,3%) und 21 untergeordneten Fettsäuren, von denen alle weniger als 0,1% zur Gesamtmenge beitragen — 14 dieser untergeordneten Fettsäuren sind zum ersten Mal in *Erwinia* beobachtet worden. Das Verhältnis 16 : 1/18 : 1 in KB-gezüchteten Zellen war bei der Differenzierung von *E. carotovora*-Subspezies genauso brauchbar wie das von anderen Autoren für TSA-gezüchtete Zellen Publiziert. Ein Vergleich der Fettsäureprofile von *E. carotovora* nach der Kultivierung auf vier verschiedenen Medien, KB, TSA, NA und PDA, zeigte, daß der höchste Anteil an Klasse A und C Fettsäuren, und die höchste Anzahl an feststellbaren Bestandteilen auf dem KB-Medium zu ermitteln war. Signifikante Unterschiede wurden bei den fünf Hauptfettsäuren und bei den zyklischen Fettsäuren bei vier Spezies der *carotovora*-Gruppe — *E. carotovora*, *E. chrysanthemi*, *E. rhapontici* und *E. cypripedii* — festgestellt. Diese Unterschiede konnten als Algorithmen dargestellt werden, die die vier Spezies differenzieren konnten, wenn sie in aufeinanderfolgenden zweispaltigen Schritten angewandt wurden.

The “soft-rotting” *erwinias* are gram-negative, non-sporeforming, facultatively anaerobic bacteria that induce necrosis in plant tissues and are referred to as the *carotovora* group by DYE (1969). They include the species *E. carotovora* (Jones) Bergey *et al.*, *E. chrysanthemi* Burkholder *et al.*, *E. cypripedii* (Hori) Bergey *et al.*, and *E. rhapontici* (Millard) Burkholder (LELLIOT and DICKEY 1984, SELLWOOD and LELLIOT 1978). In addition, *E. carotovora* contains the subspecies *carotovora* and *atroseptica* (van Hall) Dye. The current edition of Bergey’s Manual of Systematic Bacteriology does not consider these organisms as a separate group, but they resemble each other phenotypically with the exception of *E. cypripedii* which is transitory to the group typified by *E. herbicola* (LELLIOT and DICKEY 1984, VERDONCK *et al.* 1987). *E. cypripedii* and *E. rhapontici* are not pectolytic nor cause soft rot *per se*, although they do induce necrosis in their respective hosts.

As decay-causing bacteria, *E. carotovora* and *chrysanthemi*, and also *rhapontici*, occur on rotting plant tissues and since they are not always host specific, can be isolated from the same field samples (PEROMBELON and KELMAN 1980, TOWNER and BERAHA 1976). Although definitive physiological and biological tests can be used for identification of these isolates, more rapid analytical techniques such as fatty acid analysis are being developed (MOSS 1981).

The fatty acid composition of *E. carotovora* is the subject of two previous studies (DE BOER and SASSER 1986, MATYSHEVSKAYA 1981). In addition, the soft-rotting *erwinias*, among other bacteria, can be identified by pattern recognition algorithms of gas-chromatographic (GC) analyses of cellular fatty acids. One recent study on the subspecies of *E. carotovora* (DE BOER and SASSER 1986) identifies major fatty acids and some minor components. The full quantitative capabilities of GC analysis have not always been used in these reports, and some closely-eluted components are not completely identified. Detailed studies are therefore useful in defining the basis of differentiation by fatty acid analysis within specialized groups of bacteria. This report presents the fatty acid profiles of the soft-rotting group of *erwinias* as defined by Dye including *E. cypripedii* and *E. rhapontici* (but not *E. carotovora* subspp. *wasabiae* and *betavasculorum*, and pathovars of *E. chrysanthemi*) and describes a procedure to identify members of the group through differences in their fatty acid profiles.

## Materials and Methods

### Bacterial strains

A total of 13 strains of *E. carotovora* subsp. *atroseptica*, 14 of subsp. *carotovora*, 6 of *E. chrysanthemi*, and 5 strains each of *E. cypripedii* and *E. rhapontici* were used in this study (Table 1). All cultures were maintained on trypticase soy agar (TSA) at 4°C and transferred every 3–4 weeks. Fatty acid composition of bacteria grown on agar of 4 different media, King's B (KB), nutrient agar (NA), potato-dextrose agar (PDA), and TSA was analyzed. KB was selected as the medium for this study because, among other reasons described below, cells on this medium produced the highest proportions of the fatty acid classes used by previous workers to differentiate the subspecies (DE BOER and SASSER 1986).

Table 1  
Identity and sources of *Erwinia* strains used in this study

Species	Strain designation	Source*
<i>E. carotovora</i> subsp. <i>atroseptica</i>	33260	ATCC
	C2, E11, E15, E18, E25s, E26s, E27s1, E27s2, E3, E4, E6, E8	(BURKHOLDER and SMITH 1949)
subsp. <i>carotovora</i>	15713	ATCC
	C14, C3s, C3s2, C6, C7s, C9, E21, E22, E31, E32, E37, E40, E60	(BURKHOLDER and SMITH 1949)
<i>E. chrysanthemi</i>	11663, 27385, 27550, 29261	ATCC
	635	F. LUKAZIC
	PD 581	J. JANSE
<i>E. cypripedii</i>	29267, 29268, 29269, 29270	ATCC
	636	F. LUKAZIC
<i>E. rhapontici</i>	23376, 29284, 29289	ATCC
	1025, 1026	NCPPB

\* ATCC = American Type Culture Collection, Rockville, MD 20852; NCPPB = National Collection of Plant Pathogenic Bacteria, Harpenden, U.K.; Dr. F. LUKAZIC, Dept. of Plant Pathology, Penn. State Univ. College Station, PA; Dr. J. D. JANSE, Dept. Bacteriol., Plantenziertenkundige Dienst, Wageningen, The Netherlands. Literature citations in parenthesis.

### Saponification and methyl esterification

A loopful of bacteria (approximately 250 mg wet weight) grown on agar medium for 1, 3 or 6 days at 28°C, according to the experimental design, was saponified and esterified by an adaption of the method described by DE BOER and SASSER (1986). Cells were added to 1.0 ml of 1.2 N NaOH in 50% aqueous methanol and heated for 30 min in a capped tube in a boiling water bath. The preparation was then acidified and esterified by adding 0.5 ml of 6 N HCl and 1.0 ml of 12% BCl<sub>3</sub>-methanol (Supelco, Inc., Bellefonte, PA 16823), and heated for 5 min at 85°C. Fatty acyl methyl esters were then extracted with 1.0 ml of a 1:1 mixture of hexane-diethylether, washed with 3.0 ml of 0.3 N NaOH, and concentrated under a stream of filtered, high-purity N<sub>2</sub> to a volume of 50 µl.

### Gas-liquid chromatography and mass spectrometry

Two µl of the concentrated sample were injected into a Varian Model 3700 Gas Chromatograph (Varian Associates, Sunnyvale, CA 94809) equipped with a flame-ionization detector. The column was of capillary glass, 15 m × 0.25 mm I.D., coated with a 0.25 µ film of SPB-1 (dimethyl-

*Table 2*  
Average percentage of cellular fatty acids in 12 strains of each subspecies of *Erwinia carotovora*  
grown on KB agar for 1 day at 28°C

Fatty acids by class	Subspecies <i>atroseptica</i>			Subspecies <i>carotovora</i>		
	Average	Range	SD*	Average	Range	SD
<b>A. Saturated straight chains, even-carbon:</b>						
8 : 0	0.06	0.01— 0.12	±0.03	0.7	0.05— 0.10	±0.02
10 : 0	0.17	0.01— 0.56	±0.13	0.16	0.01— 0.23	±0.06
12 : 0	5.90	4.61— 6.91	±0.91	6.61	5.96— 7.06	±0.42
14 : 0	1.72	1.18— 3.72	±0.66	1.66	1.26— 2.08	±0.26
16 : 0	30.12	26.33—34.45	±2.43	29.30	27.13—30.85	±1.40
18 : 0	0.21	0.04— 0.54	±0.15	0.64	0.05— 0.98	±0.26
20 : 0	0.02	0— 0.06	±0.02	0.03	0— 0.06	±0.02
Total Class A	38.19	34.02—44.51	±3.29	38.28	34.82—39.93	±1.56
<b>B. Saturated straight chains, odd-carbon:</b>						
9 : 0	0.02	0— 0.04	±0.02	0.02	0— 0.07	±0.02
11 : 0	0.03	0— 0.06	±0.02	0.01	0— 0.02	±0.01
13 : 0	0.09	0— 0.21	±0.06	0.03	0— 0.09	±0.03
15 : 0	0.61	0.11— 1.26	±0.40	0.18	0.6 — 0.36	±0.09
17 : 0	0.30	0.02— 0.69	±0.22	0.13	0.05— 0.22	±0.05
19 : 0	0.20	0— 0.14	±0.04	0	—	—
Total Class B	1.06	0.21— 2.11	±0.69	0.36	0.11— 0.66	±0.17
<b>C. Unsaturated acids:</b>						
16 : 1	36.56	33.08—39.55	±2.16	33.91	32.16—35.35	±1.74
18 : 2	0.03	0— 0.07	±0.02	0.01	0— 0.03	±0.01
18 : 1	14.96	10.90—18.35	±2.23	18.41	17.19—20.16	±0.92
Total Class C	51.53	44.51—54.84	±2.96	52.33	49.54—56.14	±1.83
<b>D. Hydroxy acids:</b>						
3-OH 12 : 0	0.03	0— 0.06	±0.02	0.01	0— 0.03	±0.01
2-OH 14 : 0	0.16	0.13— 0.19	±0.03	0.16	0.14— 0.18	±0.01
3-OH 14 : 0	5.28	3.97— 6.28	±0.79	5.23	4.05— 5.61	±0.49
2-OH 16 : 0	0.01	0— 0.06	±0.01	0.01	0— 0.02	±0.01
Total Class D	5.48	4.14— 6.49	±0.79	5.39	4.22— 6.05	±0.50
<b>E. Branched-chains:</b>						
i-15 : 0	0.02	0— 0.08	±0.02	0	—	—
a-15 : 0	0.02	0— 0.09	±0.02	0	—	—
i-17 : 1	0.01	0— 0.03	±0.01	0	—	—
i-17 : 0	0.11	0— 0.35	±0.10	0.02	0— 0.13	±0.02
a-17 : 0	0.08	0— 0.21	±0.08	0.05	0— 0.17	±0.05
Total Class E	0.24	0.02— 0.61	±0.17	0.08	0— 0.22	±0.07
<b>F. Cyclic acids:</b>						
D-17 : 0	0.06	0— 0.17	±0.06	0.01	0— 0.04	±0.01
D-19 : 0	0.01	0— 0.04	±0.01	0.01	0— 0.04	±0.01
Total Class F	0.07	0— 0.20	±0.07	0.02	0— 0.08	±0.02
<b>G. Unidentified:</b>						
Total percentage	3.08	2.19— 4.01	±0.54	3.33	2.26— 4.81	±1.02
	99.66			99.80		
Ratio of 16 : 1 / 18 : 1	2.5	2.2 — 2.7		1.8	1.6 — 2.0	
Sum of Classes B + E + F	1.4	0.3 — 2.7		0.4	0.1 — 1.0	

\* Standard deviation from the mean.

*Table 3*  
Effect of growth medium on fatty acid composition of *Erwinia carotovora* grown for 3 days at 28°C

Fatty acid or class	Average percentage on each medium*			
	KB	NA	PDA	TSA
<b>A. Saturated straight chains, even-carbon:</b>				
8 : 0	0.10	0	0	0.03
10 : 0	0.29	0.02	0	0.09
12 : 0	6.68	6.77	7.51	6.34
14 : 0	1.93 ab	1.36 a	3.20 b	1.30 a
16 : 0	30.80 a	21.06 b	37.33 a	20.61 b
18 : 0	0.40	0.34	0.38	0.22
20 : 0	0.09 a	0.55 b	0.61 b	0.20 a
Total Class A	40.29 a	30.10 b	49.30 a	28.27 b
<b>B. Saturated straight chains, odd-carbons:</b>				
9 : 0	0.01	0	0	0
11 : 0	0.01	0.10	0	0.31
13 : 0	0.04	0.63	0.04	1.20
15 : 0	0.27	2.24	0.69	3.42
17 : 0	0.15	2.65	0.87	2.00
19 : 0	0.05	0.07	0	0.03
Total Class B	0.47 a	5.58 b	1.60 ab	6.95 b
<b>C. Unsaturated acids:</b>				
16 : 1	34.73	31.60	34.89	34.85
18 : 2	0.02	0.22	0	0.06
18 : 1	16.82	20.65	6.11	19.37
Total Class C	51.55 a	52.44 a	41.00 b	54.28 a
<b>D. Hydroxy acids:</b>				
3-OH 12 : 0	0.01	0	0	0.05
2-OH 14 : 0	0.21	0.49	0.47	0.22
3-OH 14 : 0	3.74	2.49	2.82	2.39
2-OH 16 : 0	0.01	0.60	0.93	0.13
Total Class D	3.97	3.59	4.22	2.83
<b>E. Branched-chains:</b>				
i-15 : 0	0.01	0	0	0
a-15 : 0	0.01	0.07	0.02	0.41
i-17 : 0	0.11	0	0	0
a-17 : 0	0.25	3.93	0.88	2.11
Total Class E	0.38 a	4.00 b	0.90 a	2.53 b
<b>F. Cyclic acids:</b>				
D-17 : 0	0.03	0.37	0	0.21
D-19 : 0	0.08	0.44	0.34	0.18
Total Class F	0.11 a	0.81 b	0.34 ab	0.39 ab
Sum of Class A + C	91.8	72.5	90.3	82.5
Major components	6	9	6	10
Minor components	20	12	10	13

\* Data based on duplicate analyses of 6 strains per medium. Averages on each line not followed by the same letter are significantly different (P = 0.05).

unsaturated 16 : 1 divided by 18 : 1. This ratio was above 2.2 for all *atroseptica* strains, and below 2.0 for *carotovora* subspecies (Table 2). The sum of the fatty acid classes B, E and F were of supportive diagnostic value — averaging 1.38 for subsp. *atroseptica* and 0.47 for subsp. *carotovora*. However, two of the 13 strains of pv. *atroseptica*, C-2 and E-11, had unusually low values (0.34 and 0.21, respectively) which placed them in the range typical of subsp. *carotovora*.

#### Effect of growth medium

Growth medium had effects on selected groups of fatty acids of *E. carotovora*. On TSA and NA there were a greater number of major fatty acids (each exceeding 1% of the total) than on KB or PDA. On KB and PDA, the saturated odd-carbon straight chains 13 : 0, 15 : 0 and 17 : 0, were present only at percentages of less than 1% of the total, and there was a corresponding increase in Class A, the even-carbon saturated straight chains (Table 3). Similarly, the

Table 4  
Classes of cellular fatty acids affected by physiological age in 4 species of *Erwinia* grown on KB agar for 1, 3 or 6 days at 28°C

Fatty acid or class	Percentage of total fatty acids*		
	Day 1	Day 3	Day 6
Saturated straight chain, odd-carbons (Class B):			
<i>E. carotovora</i>	0.38 a	0.60 a	0.71 a
<i>E. chrysanthemi</i>	0.46 a	1.41 a	1.44 a
<i>E. cypripedii</i>	1.12 a	2.41 a	1.95 a
<i>E. rhapontici</i>	0.78 a	1.76 a	1.77 a
Branched-chain acids (Class E):			
<i>E. carotovora</i>	0.10 a	0.11 a	0.14 a
<i>E. chrysanthemi</i>	0.01 a	0.86 b	0.59 b
<i>E. cypripedii</i>	0.56 a	1.09 a	0.73 a
<i>E. rhapontici</i>	0.79 a	1.08 a	1.39 a
Cyclic acids (Class F):			
<i>E. carotovora</i>	0.02 a	0.11 a	0.14 a
<i>E. chrysanthemi</i>	1.46 a	3.34 b	3.88 b
<i>E. cypripedii</i>	1.72 a	10.96 b	9.05 b
<i>E. rhapontici</i>	3.20 a	10.50 b	10.09 b
Total of Classes B + E + F:			
<i>E. carotovora</i>	0.50 a	1.18 a	1.36 a
<i>E. chrysanthemi</i>	1.93 a	5.61 b	5.91 b
<i>E. cypripedii</i>	3.40 a	14.46 b	11.73 b
<i>E. rhapontici</i>	4.77 a	13.34 b	13.25 b

\* Based on duplicate analyses of 5 to 6 strains per species. Averages on each line followed by the same letter are significantly different (P = 0.05).

branched-chain a-17 : 0 was a significant component on TSA and NA (2.11 and 3.93 %, respectively) but a minor component on KB and PDA. Percentages of unsaturated acids, one of the classes useful in the differentiation of *E. carotovora* subspecies, were equivalent on TSA, NA and KB, but the level of 18 : 1 was significantly reduced on PDA. In general, growth on KB medium yielded a fatty acid profile enriched in the two classes useful for subspecies differentiation. Class A and Class C accounted for 91.8% of the total fatty acids on KB compared with 82.5% on TSA. Also noteworthy, was that the greatest number of components occurred in bacteria grown on KB medium. The fatty acids 9 : 0, i-15 : 0 and i-17 : 0 were detected only on KB-grown cells.

#### Effect of physiological age

In general, and in comparison to other groups of bacteria, the fatty acid profiles of the soft-rotting *erwinias* were affected relatively little by the physiological age of the cells. Although the profile of 1, 3 and 6 day-old cells of

Table 5  
Class totals and fatty acids variable in 4 species of *Erwinia* grown on KB agar for 3 days at 28°C

Fatty acid	Average percentage of total fatty acids*			
	<i>E. carotovora</i>	<i>E. chrysanthemi</i>	<i>E. cyripedii</i>	<i>E. rhapontici</i>
12 : 0	5.99 a	0.29 b	4.13 a	4.51 a
14 : 0	2.07 a	6.37 b	6.55 b	4.94 a
16 : 0	31.40 a	27.27 a	35.84 a	36.19 a
Class A total	40.30	34.59	47.70	46.51
Class B total	0.56	1.20	1.86	1.77
16 : 1	34.50 a	30.10 a	10.44 b	18.12 b
18 : 1	16.45 a	16.27 a	14.20 a	8.95 b
Class C total	50.95	46.37	24.87	27.16
Class D total	4.56	5.40	5.24	5.47
Class E total	0.34	0.59	0.78	1.05
Class F totals:				
Day 1	0.09 a	1.46 b	1.72 b	2.24 b
Day 3	0.10 a	3.15 b	10.01 b	8.02 b
Ratio Class A + B/C	0.8 a	0.8 a	2.2 b	2.0 b
Range	0.6—1.0	0.6—1.1	1.2—3.5	1.0—3.3
Ratio 16 : 1/18 : 1	2.2 a	1.9 a	0.7 b	2.0 a
Range	1.8—3.1	1.5—2.5	0.4—1.5	1.4—3.1
Ratio 16 : 0/12 : 0	5.4 a	104.0 b	8.9 a	8.4 a
Range	3.7—7.1	61.9—150.9	7.4—10.5	6.4—11.7
Ratio 12 : 0/14 : 0	2.9 a	0.1 b	0.6 b	1.0 ab
Range	2.0—5.1	0—0.01	0.5—0.7	0.7—2.9

\* Data based on duplicate analyses of 5 to 6 strains of each species. Strains for *E. carotovora* include 3 of each of the subspecies. Averages on each line not followed by the same letter are significantly different ( $P = 0.05$ ).

*E. carotovora* (both subspecies) were identical, significant changes occurred with age in the other species examined (Table 4). There were increases in Class E (hydroxy-substituted acids) and Class F (cyclopropane fatty acids) in *E. chrysanthemi*, while in *E. cypripedii* and *E. rhapontici* there were increases in Class F only. The stability of the fatty acid profile of *E. carotovora* and the variability in the other species could be statistically demonstrated by an analysis of the sums of percentages of Classes B, E and F which were unchanged in *E. carotovora*, but increased with age in the other species.

#### Species differences

The 4 species of *erwinia* had basically similar fatty acid profiles but with important variations. Fatty acids that varied quantitatively were some of those used in the differentiation of *E. carotovora* subspecies — the 12 : 0, 14 : 0, 16 : 0, 16 : 1, 18 : 1 and the cyclic fatty acids. Particularly noticeable was the relatively low percentage of cyclic fatty acids in *E. carotovora*, of 12 : 0 in *E. chrysanthemi*, and of 16 : 1 in *E. cypripedii* (Table 5). No one component or ratio of components could be used for the differentiation of all 4 species. The ratio of Class A and B divided by C was statistically lower for *E. carotovora* and *E. chrysanthemi*, but their range of 0.6 to 1.1 was overlapped by one strain of *E. rhapontici* with a ratio of 1.0. All other strains of *E. rhapontici* and *E. cypripedii* had ratios of over 1.2. Similarly, ratios of other variable fatty acids had ranges unique to one or two species but not to all four. However, utilizing a series of these ratios, a sequence of dichotomous steps could be arranged, such as in a taxonomic key, that successfully differentiated the 4 species on the basis of Class or individual fatty acid percentages (Table 6).

Table 6  
Tentative scheme for differentiation of strains of the *carotovora* group by fatty acid composition of cells grown on KB agar 3 days at 28°C\*

Step	Fatty acid data	Value	Identification
1.	Class A + B divided by C	less than 1.5 . . . . .	go to step 2
		more than 1.5 . . . . .	go to step 3
2.	Class F (on day 3)	less than 0.5 . . . . .	go to step 4
		more than 0.5 . . . . .	<i>E. chrysanthemi</i>
3.	Ratio 16 : 1/18 : 1	less than 1 . . . . .	<i>E. cypripedii</i>
		more than 1 . . . . .	<i>E. rhapontici</i>
4.	Ratio 16 : 1/18 : 1	below 2.2 . . . . .	<i>E. carotovora</i> ssp. <i>carotovora</i>
		over 2.2 . . . . .	<i>E. carotovora</i> ssp. <i>atroseptica</i>

\* Key valid for *erwinias*: Gram-negative, rod-shaped, facultatively anaerobic phyto-bacteria capable (except for *E. cypripedii*) of maceration of potato tissue.

#### Conclusions

The soft-rotting *erwinias*, particularly *E. carotovora* and *E. chrysanthemi*, are ecologically diverse and represent strains with considerable physiological and serological variation (JANSE and RUISSEN 1988, MOLINE 1985). The fatty acid

profiles developed in this report were based on a limited number of sample strains. Reliable data, however, can be obtained from a relatively small sampling of representative strains. In the case of *E. carotovora*, differentiation of subspecies was as good with the 12 strains of this study as with the 24 or more in the report of DE BOER and SASSER (1986). Although the profiles of the other *Erwinia* species were based on only 5 to 6 strains, those selected were fully characterized and usually included type strains. Nevertheless, the taxonomic scheme proposed in this report should be regarded as tentative until the algorithms can be verified or modified in tests with larger groups of field isolates.

Gas-liquid chromatography has become a more useful analytical tool with the advent of fused-silica capillary columns (MOSS *et al.* 1980). In general, capillary column can resolve and detect fatty acid components comprising as little as 0.01% of total fatty acids. This capability has not been fully used in previous studies of bacterial fatty acids. In this report, 14 of these minor fatty acids are shown for the first time to occur in *Erwinia*. Some of them could be of taxonomic significance in future studies.

KB was the preferred medium for several reasons in addition to that discussed above: all strains of the bacteria grew abundantly on KB in 24 h, and KB could be used as a standard medium for direct comparisons of fatty acid profiles of other pectolytic bacteria such as fluorescent strains of *Pseudomonas*, *Xanthomonas* and *Cytophaga* (LIAO and WELLS 1986). Current work is being directed to a specialized taxonomic scheme to differentiate all species of soft-rotting bacteria by fatty acid analysis.

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### Literature

- BRIAN, L. B., and E. W. GARDNER, 1968: A simple procedure for detecting the presence of cyclopropane fatty acids in bacterial lipids. *Appl. Microbiol.* 16, 549—552.
- DE BOER, S. H., and M. SASSER, 1986: Differentiation of *Erwinia carotovora* ssp. *carotovora* and *E. carotovora* ssp. *atroseptica* on the basis of cellular fatty acid composition. *Can. J. Microbiol.* 32, 796—800.
- BURKHOLDER, W. H., and W. L. SMITH, Jr., 1949: *Erwinia atroseptica* (Van Hall) Jennison and *Erwinia carotovora* (Jones) Holland. *Phytopathology* 39, 887—897.
- DYE, D. W., 1969: A taxonomic study of the genus *Erwinia*. II. The "carotovora" group. *N. Z. J. Sci.* 12, 81—97.
- JANSE, J. D., and M. A. RUISSEN, 1988: Characterization and classification of *Erwinia chrysanthemi* strains from several hosts in The Netherlands. *Phytopathology* 78, 800—808.
- LELLIOT, R. A., and R. S. DICKEY, 1984: Genus VII. *Erwinia*, pp. 469—476. In: KRIEG, N. R., and J. G. HOLT (eds), *Bergey's Manual of Systemic Bacteriology*. Vol. 1. Williams and Wilkins, Baltimore/London.
- LIAO, C. H., and J. M. WELLS, 1986: Properties of *Cytophaga johnsonae* strains causing spoilage of fresh produce at food markets. *Appl. Environ. Microbiol.* 52, 1261—1265.
- MATYSHEVSKAYA, L. A., 1981: Composition of fatty acids of the Genus *Xanthomonas* bacteria. *Mikrobiol. Z.* 43, 448—453.

- MOLINE, H. E., 1985: Differentiation of postharvest soft rotting bacteria with two-dimensional polyacrylamide gel electrophoresis. *Phytopathology* 75, 549—553.
- MOSS, C. W., S. B. DEES, and G. O. GUERRANT, 1980: Gas-liquid chromatography of bacterial fatty acids with a fused silica capillary column. *J. clinical Microbiol.* 12, 127—130.
- , 1981: Gas-liquid chromatography as an analytical tool in microbiology. *J. Chromatogr.* 203, 337—347.
- PEROMBELON, M. C. M., and A. KELMAN, 1980: Ecology of the soft rot *erwinias*. *Annu. Rev. Phytopathol.* 18, 361—387.
- SELLWOOD, J., and R. A. LELLIOT, 1978: Internal browning of hyacinth caused by *Erwinia rhapontici*. *Plant Path.* 27, 120—124.
- TOWNER, D. B., and L. BERAHA, 1976: Core-rot: A bacterial disease of carrots. *Plant Dis. Reprtr* 60, 357—359.
- VERDONCK, L., J. MERGAERT, C. RIJCKAERT, J. SWINGS, K. KESTERS, and J. DE LEY, 1987: Genus *Erwinia*: Numerical analysis of phenotypic features. *Internat. J. system. Bacteriol.* 37, 4—18.