

U. S. Department of Agriculture, ARS, Philadelphia, PA, USA

Differentiation of *Erwinia* Species in the "Amylovora" Group by Class Analysis of Cellular Fatty Acids

J. M. WELLS, T. VAN DER ZWET and C. N. HALE

Authors' addresses: J. M. WELLS, Eastern Regional Research Center, U.S. Department of Agriculture, 600 E. Mermaid Lane, Philadelphia, PA 19118 (USA). T. VAN DER ZWET, Appalachian Fruit Research Station, U.S. Department of Agriculture, Kearneysville, WV (USA). C. N. HALE, Department of Scientific and Industrial Research, Plant Protection, Auckland, New Zealand.

Received April 1, 1991; accepted April 16, 1993

Abstract

Cellular fatty acids of *Erwinia* species in the Amylovora group (*E. amylovora*, *E. nigrifluens*, *E. quercina*, *E. rubrifaciens*, *E. salicis* and *E. tracheiphila*), grown on King's medium B agar for 1, 3, and 6 days at 28 °C, were analyzed by gas-liquid chromatography, grouped by chemical class, and compared in order to differentiate the species. For the group in general, the average percentage in 1-day-old cells ranged 36.4—41.5 % for the saturated straight-chain even-carbon fatty acids (Class A), 0.3—0.7 % for saturated straight-chain odd-carbons (Class B), 43.4—53.3 % for unsaturated acids (Class C), 4.5—7.1 % for hydroxy-substituted acids (Class D), 0.2—2.0 % for branched-chain acids (Class E), and 0—7.5 % for cyclopropane fatty acids (Class F). Species could be differentiated in 4 sequential steps based on: 1) percentage of the 12 : 0 fatty acid (*E. amylovora* > 3.6 %, *E. quercina* < 1 %); 2) sum of Classes E + F (*E. tracheiphila* < 0.3 %); 3) ratio Class A/(E + F) (*E. salicis* < 8); and percentage of 18 : 1 (*E. rubrifaciens* > 14 %, *E. nigrifluens* < 14 %). Physiological age significantly affected relative percentages of Classes B, C and F in *E. amylovora* and *E. salicis*; of Classes A, C and F in *E. quercina*; of Class A in *E. tracheiphila*; and of Class C in *E. nigrifluens*.

Zusammenfassung

Differenzierung von *Erwinia*-Spezies der „Amylovora“-Gruppe durch eine Klassenanalyse der zellulären Fettsäuren

Die zellulären Fettsäuren von *Erwinia*-Spezies der Amylovora-Gruppe (*E. amylovora*, *E. nigrifluens*, *E. quercina*, *E. rubrifaciens*, *E. salicis* und *E. tracheiphila*), die für 1, 3 bzw. 6 Tage bei 28 °C auf King's Medium B kultiviert worden waren, wurden durch GLC analysiert, nach chemischen Klassen gruppiert und miteinander verglichen, um die Spezies differenzieren zu können. Für die Gruppe im allgemeinen gilt: in 1 Tag alten Zellen lag der durchschnittliche Prozentsatz für die saturierten, geradkettigen Fettsäuren mit geraden Kohlenstoffmolekülen bei 36,4—41,5 % (Klasse A), bei 0,3—0,7 % für die saturierten, geradkettigen Fettsäuren mit ungeraden Kohlenstoffmolekülen (Klasse B), bei 43,4—53,3 % für die ungesättigten Fettsäuren (Klasse C), bei 4,5—7,1 % für die hydroxy-substituierten Fettsäuren (Klasse D), bei 0,2—2,0 % für die verzweigt-kettigen Fettsäuren (Klasse E), und bei 0—7,5 % für die Cyclopropanfettsäuren (Klasse F). Differenziert wurden die Spezies in 4 aufeinanderfolgenden Gruppen anhand von den folgenden Merkmalen: 1) dem Prozent-

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

satz der 12 : 0 Fettsäure (*E. amylovora* > 3,6 %, *E. quercina* < 1 %); 2) der Summe der Klassen E + F (*E. tracheiphila* < 0,3 %); 3) dem Verhältnis Klasse A/(E + F) (*E. salicis* < 8); 4) dem Prozentsatz der 18 : 1 Fettsäure (*E. rubrifaciens* > 14 %, *E. nigrifluens* < 14 %). Das physiologische Alter der Zellen beeinflusste die relativen Prozentsätze der Klassen B, C sowie F in *E. amylovora* und *E. salicis*; der Klassen A, C sowie F in *E. quercina*; der Klasse A in *E. tracheiphila*; und der Klasse C in *E. nigrifluens*.

WINSLOW *et al.* (1920) established the genus *Erwinia* to combine all peritrichous plant pathogenic bacteria known at the time into one group, *Erwinia amylovora* being the type species. Detailed taxonomic studies of the genus *Erwinia* were completed by DYE (1968) in New Zealand. He recognized 4 groups within the genus, one of which was the Amylovora group. Based on the lack of differences in biochemical characteristics or in carbohydrate utilization patterns, DYE proposed the Amylovora group include *E. amylovora*, and 5 subspecies: *E. amylovora* spp. *nigrifluens*, *quercina*, *rubrifaciens*, *salicis*, and *tracheiphila*. In the recent issue of Bergey's Manual (LELLIOT and DICKEY 1984), all are listed as species of *Erwinia*, each with their characteristic pathogenicity: *E. amylovora* causing fire blight of apple (*Malus sylvestris* Mill), pear (*Pyrus communis* L.) and quince (*Cydonia oblonga* Mill); *E. nigrifluens* causing bark necrosis of Persian walnut (*Juglans regia* L.); *E. quercina* causing shoot blight of *Quercus* spp., *E. rubrifaciens* causing phloem necrosis of Persian walnut; *E. salicis* causing vascular wilt of *Salix* spp.; and *E. tracheiphila* causing vascular wilt of *Cucurbita* spp.

Analysis of cellular fatty acid composition has been of chemotaxonomic value for bacteria. The fatty acid composition of 143 strains of *E. amylovora* was described by VAN DER ZWET and SASSER (1985) and by VAN DER ZWET *et al.* (1987). The most abundant fatty acids were the straight-chain 12 : 0, 14 : 0 and 16 : 0, the unsaturated 16 : 1 and 18 : 1, 3-hydroxy 14 : 0, and cyclopropane 17 : 0. Minor fatty acids included the straight chains 15 : 0, 17 : 0 and 18 : 0.

The present study was undertaken to analyze the cellular fatty acid profiles of the *Erwinia* species in the Amylovora group, and to understand which specific fatty acids or groups of fatty acids could be important in their differentiation. A commercially-available, minicomputer-based, analytical system is available for identification of bacteria by fatty acid composition (MIDI, Microbial Identification System, Newark, DE, USA). The data presented in this study can be used in conjunction with such a system to assist in interpretation, or in independent analyses.

Materials and Methods

This study was based on a collection of 25 strains of *E. amylovora*, and 6—7 each of the other species in the group (Table 1). Also included was one strain of *E. mallotivora* (ATCC 29575), a species closely related to *E. amylovora* (GOTO 1976) but not included as such in Bergey's Manual. Bacteria were cultured (and maintained) on King's medium B agar (KB, Difco). Cells for fatty acid analyses were grown for 1, 3, and 6 days at 28 °C. Only 6 of the 21 strains of *E. amylovora* (595, WA 212, RIF-NY, WV 55, WV 260, and ATCC 15580) were used for the 3- and 6-day analyses.

Fatty acid analyses were conducted by the method described in a previous report (CASANO *et al.* 1988). Cells were saponified and fatty acids esterified by mixing one loopful into 1.0 ml of 1.2 N NaOH in 50 % aqueous methanol and heating for 30 min in capped tubes in a boiling water bath. One-half ml of 6 N HCl and 1.0 ml of 12 % BCl₃-methanol was then added and heated for 5 min at 85 °C. Methylated acids were then extracted with 1.0 ml of hexane-diethylether (1 : 1), washed with 3.0 ml of 0.3 N NaOH, and concentrated under a stream of high-purity nitrogen. Two μ l were injected into a Varian Model 3700 Gas Chromatograph (Varian Associates, Sunnyvale, CA 94809).

Table 1
Strains of species in the *Amylovora* group of *Erwinia* used in this study

Bacterium	Strains	Source
<i>E. amylovora</i>	15580	ATCC ^x
	WV 55, 260, 404, 428, 480	original isolations ^y , West Virginia, USA
	WV 1112, 1228, 1473, 2028	original isolations ^y , West Virginia, USA
	MO-30, 31, 55, 65	original isolations ^y , Missouri, USA
	1004, 1005, 1006, 1455	W. G. BONN ^z , Canada
	RIF-NY	S. V. BEER ^z , New York, USA
	UT 111, 112	S. V. THOMSON ^z , Utah, USA
	WA 210, 212, 214	R. P. COVEY ^z , Washington, USA
	595	NCPPB ^a
<i>E. nigrifluens</i>	13028, 29275	ATCC
	1391, 1575, 4789	ICMP ^b
	637	F. LUKEZIC ^z , Pennsylvania, USA
<i>E. quercina</i>	27595, 29281, 29282	ATCC
	1845, 1846, 1919	ICMP
<i>E. rubrifaciens</i>	29291	ATCC
	1915, 1917, 4792, 5948, 5950	ICMP
<i>E. salicis</i>	29294	ATCC
	1587, 1588, 5988, 9136, 9137, 9308	ICMP
<i>E. tracheiphila</i>	11417, 27003, 33245	ATCC
	1396, 1586, 5845	ICMP
<i>E. mallotivora</i>	29575	ATCC

^x ATCC = American Type Culture Collection, 12301 Parklawn Dr., Rockville, MD, USA.

^y Original isolations by T. van der Zwet.

^z W. G. Bonn, Canada Agr., Harrow, Ont.; S. V. Beer, Cornell Univ., Ithaca, NY; S. V. Thomson, Utah State Univ., Logan, UT; R. P. Covey (deceased), Washington State Univ., Tree Fruit Res. Ctr., Wenatchee, WA; F. Lukezic, Penn. State Univ., State College, PA, USA.

^a NCPPB = National Collection of Plant Pathogenic Bacteria, Harpenden, UK.

^b ICMP = International Collection of Microorganisms from Plants, DSIR, Auckland, NZ.

equipped with a flame ionization detector and a 15 m × 0.25 mm I.D. capillary glass column coated with SPB-1 as a non-polar stationary phase (Supelco Inc., Bellefonte, PA 16823). Flow rate of helium carrier gas was 30 cc/min, and operating conditions were: injector temperature, 230 °C; detector temperature, 250 °C; and temperature program rate of 4 °C/min. Chromatograms with a minimum of 25 to 30 individual components between 8 and 20 carbons in length were obtained for each sample. Eluted fractions were integrated and quantified as percent of total peak area with a Model 4270 Chromatography System (Varian Associates). Data was then entered into an Apple III micro-computer programmed with an Omnis 3 database manager (Blythe Software Inc., San Mateo, CA 94403).

Fatty acids were tentatively identified by co-chromatography with reference standards (Supelco Inc.). Major fatty acids (comprising over 1 % of the total) were confirmed by mass spectroscopy, and unsaturated, hydroxy-substituted, and cyclopropane fatty acids were confirmed chemically, all by methods previously described (WELLS and MOLINE 1991).

Fatty acids were organized into chemical classes, and class totals were generally used for comparative analysis of the different species. Class A, the saturated, straight-chain even-carbon fatty acids, included the 8 : 0, 10 : 0, 12 : 0, 14 : 0, 16 : 0, 18 : 0 and 20 : 0 fatty acids. Class B, the

saturated straight-chain odd-carbon fatty acids included 9 : 0, 13 : 0, 15 : 0, 17 : 0 and 19 : 0. Class C, the unsaturated acids, included *cis* 9—14 : 1, and *cis* and *trans*-16 : 1 and 18 : 1. Class D, hydroxy-substituted fatty acids, included 3-OH 10 : 0, 3-OH 12 : 0, 2-OH 14 : 0, 3-OH 14 : 0, 2-OH 16 : 0

Table 2
Average percentage (of total) and range of cellular fatty acids in species of the *Amylovora* group cultured for 1 day at 28 °C on KB agar

Fatty acids	<i>Erwinia</i> species*					
	<i>amylovora</i>	<i>nigrifluens</i>	<i>quercina</i>	<i>rubrifaciens</i>	<i>salicis</i>	<i>tracheiphila</i>
FATTY ACID CLASS:						
A. saturated, straight-chain, even-carbons						
Average	40.9	38.9	41.4	37.5	36.4	41.5
Range ^y	33—45	36—40	35—46	34—40	32—42	39—43
B. Saturated, straight-chain, odd-carbons						
Average	0.7	0.3	0.4	0.3	0.5	0.3
Range	0.3—2.1	0.1—0.4	0.1—0.8	0.1—0.9	0.2—0.7	0.1—0.5
C. Unsaturated acids						
Average	43.4	48.9	45.3	53.3	45.9	48.7
Range	38—52	38—55	33—49	50—54	37—52	47—51
D. Hydroxy-substituted						
Average	7.1	4.5	5.9	5.7	4.9	5.7
Range	5.7—8.7	2.7—7.0	4.9—6.5	4.8—8.4	3.2—5.8	4.6—6.9
E. Branched-chain acids						
Average	1.2	1.0	1.9	0.4	2.0	0.2
Range	0.3—2.7	0.5—3.2	1.0—5.2	0.2—4.9	1.0—4.4	0.2—0.3
F. Cyclopropane acids						
Average	2.8	2.5	3.9	0.6	7.5	0
Range	0.4—8.8	0.2—4.4	2.0—5.2	0.2—0.8	4.3—12.0	0
G. Unknown components						
	3.5	3.8	2.7	2.2	2.5	2.3
SELECTED FATTY ACIDS: ^z						
12 : 0 Average	5.2	2.6	0.5	2.6	3.7	2.6
Range	3.6—6.2	1.9—3.1	0.4—0.8	2.1—3.2	3.0—4.3	2.4—3.5
18 : 1 Average ^a	10.8	12.2	9.0	15.4	15.1	11.7
Range	7—18	11—14	6—12	14—17	14—17	9—16
SELECTED RATIOS:						
(a) Class E + F Average	4.1	3.5	5.8	1.0	9.5	0.2
Range	1.4—10.7	2.2—4.4	2.1—10.3	0.4—1.6	6.0—14.6	0.2—0.3
(b) Class A/E + F Average	13.5	12.5	8.7	44.6	4.5	347
Range	5.0—30.6	8.9—17.4	4.4—18.3	22.3—91.6	2.4—7.1	145—748

* Data based on 25 strains of *E. amylovora* and 6 to 7 each of the other species.

^y Ranges for Classes A and B, and 18 : 1 rounded to lowest and highest integer; ranges for Classes C, D, E and F, and 12 : 0 rounded to lowest and highest tenth.

^z Selected by usefulness in differentiating species of the *Amylovora* group.

^a Totals of *cis*- and *trans*-18 : 1.

and a partly-identified hydroxy-17 : 0 (equivalent chain length = 18.35). Class E, branched-chain acids, included *iso*-14 : 0, *iso*-17 : 1, *iso*-17 : 0, *anteiso*-17 : 0, *iso*-19 : 0 and *anteiso*-19 : 0. Class F, the cyclopropane fatty acids, included cyclo-17 : 0 and cyclo-19 : 0. The significance of changes in fatty acid composition due to physiological age of cells was tested by linear regression analysis. Each strain was tested once, and the data incorporated into the species averages.

Results

Class analysis of fatty acids by species

The class distribution of cellular fatty acids of *E. amylovora* grown for 1 day at 28 °C on KB agar was similar to that previously published (CASANO *et al.* 1988), and was 40.9 % saturated even-carbons, 0.7 % saturated odd-carbons, 43.4 % unsaturated acids, 7.1 % hydroxy-substituted acids, 1.2 % branched-chains, 2.8 % cyclopropane acids, and 3.5 % unidentified components (Table 2). The distribution for the other species in the *Amylovora* group was generally similar, but with consistent differences in some features, depending on the species.

Totals for Class A and B fatty acids in strains of all species ranged from 32 to 46 % with no consistent differences due to species. Class C values ranged from 33 to 55 % in all species, with values for *E. amylovora* tending to be higher — all strains averaging above 50 %. Values for Class D, hydroxy acids, ranged from 2.7 to 8.7 % with those of *E. amylovora* tending to be higher (all strains 5.7 % or above). Class E averages ranged from 0.2 to 4.9 % with strains of *E. salicis* and *E. quercina* tending to be higher (all strains averaging 1 % or above). Class F, cyclopropane acids, with averages ranging from 0 to 12.0 % were not detected in *E. tracheiphila* and were consistently low for *E. rubrifaciens* (all strains 0.8 % or below).

The individual fatty acids of greatest taxonomic value, in terms of differentiating species, were the 12 : 0 and 18 : 1 (sum of *cis*- and *trans*-18 : 1). Values for 12 : 0 in *E. amylovora* were exclusively above 3.6 %, *E. quercina* exclusively below 1 %, and the other species inclusively within the 1 to 3.5 % range (Table 2). Percentages of the 18 : 1 at or below 14 were characteristic of *E. amylovora* (24 of 25 strains), *E. nigrifluens* and *E. quercina*; percentages above 14 were characteristic of *E. rubrifaciens* and *E. salicis*; and the *E. tracheiphila* range overlapped from 9 to 16 %.

E. mallotivora, originally described as a distinct species (GOTO 1976), had a fatty acid composition closely resembling but distinct from that of *E. amylovora* (data not shown). The percentage of 12 : 0 was 5.8 %, satisfying the criterion for *E. amylovora*, but percentages of 14 : 0 (2.9 %) and Class B (0.14 %) fell outside the typical range. In addition, *E. mallotivora* contained 0.17 % of 3OH-16 : 0 (average of 3 determinations), a fatty acid undetected in any other species of *Erwinia*.

Effects of physiological age on fatty acid composition

As previously reported (CASANO *et al.* 1988), physiological age of cells affected relative percentages of Class B, C and F fatty acids in *E. amylovora* (Table 3). Classes affected by age differed in species of the *Amylovora* group. In *E. nigrifluens*, only Class C was affected — percentages tended to decrease from 49 % on day 1 to 44 % on day 6. Because of strain variability, however, the regression could not be confirmed statistically at the 95 % level of probability ($P = 0.05$). In *E. quercina*, Classes A, C and F were significantly affected by age, but only Class A in *E. tracheiphila*. The composition of *E. rubrifaciens* was unaffected by age, and *E. salicis* was similar to *E. amylovora* in that classes B, C and F were affected.

Table 3
Classes of fatty acids in species of the *Amylovora* group affected by physiological age of cells, and percentage of total fatty acids of cells grown for 1, 3 and 6 days on KB agar at 28 °C

Fatty acids	Days in culture	<i>Erwinia</i> species ^x					
		<i>amylovora</i>	<i>nigrifluens</i>	<i>quercina</i>	<i>rubrifaciens</i>	<i>salicis</i>	<i>tracheiphila</i>
Class A	1	40.9*	39.0	41.4	37.5	36.4	41.6
	3	40.9	43.1	44.6	38.9	43.7	44.0
	6	41.5	43.0	48.9	38.9	42.9	46.0
	variance ratio ^y	n.s.	n.s.	*	n.s.	n.s.	*
Class B	1	0.7	0.3	0.4	0.3	0.4	0.3
	3	2.3	0.3	0.6	0.2	1.3	0.7
	6	2.8	0.5	1.0	0.1	4.4	0.6
	variance ratio	*	n.s.	n.s.	n.s.	*	n.s.
Class C	1	43.4	48.9	43.5	53.3	45.9	49.6
	3	41.6	45.0	34.1	52.4	22.3	48.2
	6	35.6	44.0	30.0	53.2	19.1	48.0
	variance ratio	*	n.s.	**	n.s.	*	n.s.
Class F	1	2.8	2.5	3.9	0.6	7.5	0
	3	2.7	3.7	8.7	0.9	22.6	0
	6	4.7	2.0	11.9	1.1	19.1	0
	variance ratio	*	n.s.	*	n.s.	*	n.s.

^x Data based on 25 strains of *E. amylovora* and 6 to 7 each of the other species.

^y Variance ratio, F (at 5 degrees of freedom for greater mean square), for sample regression coefficient, b, of average percent on physiological age. Values of F at the 1 % and 5 % points of distribution designated * and **, respectively; n.s. = not significant.

Table 4
Dichotomous key for differentiation of species in the *Amylovora* group based on differences in cellular fatty acid composition^c

Step	Fatty acid or factor	Value ^y	Interpretation
1.	12 : 0	more than 3.6 %	= <i>E. amylovora</i>
	12 : 0	less than 1 %	= <i>E. quercina</i>
	12 : 0	between 1 and 3.5 %	[go to step 2]
2.	Sum of Classes E + F	less than or equal to 0.3 %	= <i>E. tracheiphila</i>
	Sum of Classes E + F	more than 0.3 %	[go to step 3]
3.	Ratio of Class A/(E + F)	less than 8	= <i>E. salicis</i>
	Ratio of Class A/(E + F)	more than 8	[go to step 4]
4.	18 : 1	more than 14 %	= <i>E. rubrifaciens</i>
	18 : 1	less than 14 %	= <i>E. nigrifluens</i>

^c Cells grown on KB agar for 1 day at 28 °C.

^y Percentages are based on total cellular fatty acids.

The one strain of *E. mallotivora* analyzed resembled *E. quercina* in that the percentages of Class A increased (38—49 %), Class C decreased (52—38 %) and Class F increased (1.8—5.1 %) with age. However, variability in *E. mallotivora* could not be evaluated in this study because of unavailability of strains.

Dichotomous key for differentiation of species

Based on the differences in fatty acid composition described above, the following factors were selected for rapid differentiation of the species in the *Amylovora* group: (1) percentage of 12 : 0; (2) total percentage of Classes E and F; (3) ratio of Class A divided by the sum of Classes E + F; and (4) percentage of 18 : 1. Factor 1 differentiated all strains of *E. amylovora*, *E. quercina* from the balance of the group; factor 2 differentiated *E. tracheiphila*; factor 3 *E. salicis*; and factor 4 distinguished *E. rubrifaciens* from *E. nigrifluens* (Table 4). The order in which we differentiated the species was arbitrary.

Discussion

The data presented in this study was based on 25 strains of one species, *E. amylovora*, and 6 to 7 of each of the others. Greater certainty can thus be placed on the data for *E. amylovora*; and that for the other species could be considered tentative until more detailed studies with a larger collection of strains become available. Nevertheless, reproducibility of the fatty acid profiles by the analytical technique was high, and characteristics of the species were consistent enough to permit statistical analysis in most cases with the limited number of strains.

Fatty acid profiles of *E. amylovora* are affected by the composition of the medium in which the cells are grown (CASANO *et al.* 1988). The medium of our choice was KB agar because of our interest in drawing comparisons with the Pseudomonads, another major group of plant pathogenic bacteria for which the medium is especially useful. Several previous studies (as well as the database of the commercially-available MIDI identification system) were based on cells grown on trypticase-soy agar (TSA, Difco), a good general purpose bacteriological medium. KB-grown cells have less Class B (saturated, odd-carbons) and Class F (cyclic acids) fatty acids, and more of Class C (unsaturated acids) than TSA-grown cells (CASANO *et al.* 1988).

The *Amylovora* group was originally separated from species in the *Herbicola* and *Carotovora* groups on the basis of pathogenicity and physiological characteristics (DYE 1968). Furthermore, sufficient differences exist among members of the group to merit species designations (BRENNER 1984). Similarly, fatty acid composition was significantly different for each species based on a limited sampling of representative strains. Once databases are established with larger collections of strains, fatty acid composition can be used as a reliable taxonomic tool.

E. mallotivora is a recently described species of which we were able to obtain only one specimen. The results of the fatty acid analysis confirm the conclusion of GOTO (1976), that *E. mallotivora* closely resembles *E. amylovora* but is sufficiently distinct to merit species designation. Of interest was the detection of 3-OH 16 : 0, a hydroxy-substituted fatty acid not previously found by us in any *Erwinia* sp. This characteristic, if confirmed by analysis of other strains, could be used to differentiate *E. mallotivora* from *E. amylovora*.

Regarding other fatty acids described herein for the *Erwinia*, all have been previously reported as being cellular components. Qualification is appropriate, however, since the possibility of some being artifacts of extraction and acid hydrolysis has not been eliminated — particularly the *trans* 9 isomer of 18 : 1, a form not generally found in nature. Nevertheless, these components are directly or indirectly involved in the chemotaxonomic characterization of the *Erwinia*.

Literature

- BRENNER, D. J., 1984: Family I. *Enterobacteriaceae*. In: KRIEG, N. R., and J. G. HOLT (eds), *Bergey's Manual of Systematic Bacteriology*, Vol. 1, pp. 408—420. The Williams & Wilkins Co., Baltimore, MD.
- CASANO, F., J. WELLS, and T. VAN DER ZWET, 1988: Fatty acid profiles of *Erwinia amylovora* as influenced by growth medium, physiological age and experimental conditions. *J. Phytopathology* **121**, 267—274.
- DYE, D. W., 1968: A taxonomic study of the genus *Erwinia*. I. The "Amylovora" group. *N.Z. J. Sci.* **11**, 590—607.
- GOTO, M., 1976: *Erwinia mallotivora* sp. nov., the causal organism of bacterial leaf spot of *Mallotus japonicus* Muell. *Arg. Internat. J. of System. Bact.* **26**, 467—473.
- LELLIOT, R. A., and R. S. DICKEY, 1984: GENUS VII. *Erwinia*. In: KRIEG, N. R., and J. G. HOLT (eds), *Bergey's Manual of Systematic Bacteriology*, Vol. 1, pp. 469—476. The Williams & Wilkins Co., Baltimore, MD.
- VAN DER ZWET, T., and M. SASSER, 1985: Characterization of *Erwinia amylovora* through fatty acid profiles. *Phytopathology* **75**, 1281 (Abstr.).
- —, 1986: Identification, symptomatology, and epidemiology of fire blight on Le Conte pear in the Nile Delta of Egypt. *Plant Dis.* **70**, 230—234.
- —, M. SASSER, and J. M. WELLS, 1987: Determination of fatty acid profiles relevant to the characterization of *Erwinia amylovora*. *Proc. 6th Internat. Conf. Plant Path. Bact.* 821—829.
- WELLS, J. M., and H. MOLINE, 1991: Differentiation of soft-rotting *Erwinia* of the Carotovora group by fatty acid analysis. *J. Phytopathology* **131**, 22—32.
- WINSLOW, C. E. A., J. BROADHURST, and R. E. BUCHANAN, 1920: The families and genera of the bacteria: *Erwineae*. *J. Bact.* **5**, 209.