Numerical Taxonomy of *Erwinia* Species Using API Systems

By J. MERGAERT,[1] L. VERDONCK,[1] K. KERSTERS,[1] J. SWINGS,[1]* J.-M. BOEUFGRAS[2] AND J. DE LEY[1]

[1] *Laboratorium voor Microbiologie en microbiële Genetica, Rijksuniversiteit, K. L. Ledeganckstraat 35, B-9000 Gent, Belgium*

[2] *Laboratoire de Recherche API, La Balme-les-Grottes, F-38390, Montalieu-Vercieu, France*

(*Received 9 February 1984; revised 19 April 1984*)

API 20E, API ZYM and eight other enzymic API systems were tested on 123 strains belonging to 18 *Erwinia* species, six *Enterobacter agglomerans* strains and 22 reference strains belonging to other phytopathogenic genera and other enterobacterial species. The data obtained, from a total of 130 tests, were subjected to numerical analysis. Test reproducibility within the API 20E system varied from 88 to 100%. The numerical analysis revealed 12 phenons; in six of these phenons two or three subphenons could be differentiated. Several of these (sub)phenons corresponded to established *Erwinia* species and could be differentiated from each other by 25 characters. No clearcut distinction could be made between the 'amylovora', 'carotovora' and 'herbicola' groups. Seven phenons were further analysed with the API 50CHE system. The results provided evidence for the retention of *Er. quercina*, *Er. nigrifluens*, *Er. salicis*, *Er. amylovora*, *Er. rubrifaciens*, *Er. mallotivora*, *Er. stewartii*, *Er. cypripedii* and *Er. chrysanthemi* as separate taxa and supported the synonymy within the pairs *Er. ananas* and *Er. uredovora*, *Er. dissolvens* and *Enterobacter cloacae*, *Er. carotovora* subsp. *atroseptica* and *Er. carotovora* subsp. *carotovora*, *Er. milletiae* and one of the *Er. herbicola* clusters. The inadequacy of the present classification of several *Erwinia* species, such as *Er. herbicola* and *Er. rhapontici*, is highlighted. The results show that API systems are a useful and rapid alternative to conventional phenotypical testing for the classification and identification of *Erwinia* species.

INTRODUCTION

During the last decade miniaturized biochemical procedures have been developed for the rapid identification of clinical *Enterobacteriaceae* and are now commercially available. One of these, API 20E (API Systems SA, Montalieu-Vercieu, France) contains 23 standardized biochemical tests and has been successfully tested in many clinical bacteriological laboratories (Butler *et al.*, 1975; Blazevic *et al.*, 1976; Rutherford *et al.*, 1977; Holmes *et al.*, 1977, 1978; Aldridge *et al.*, 1978; Murray, 1978; Blackall, 1980; Devenish & Barnum, 1980; Freeman *et al.*, 1981). Non-clinical isolates of *Erwinia herbicola* have already been characterized using the API 20E system (Neilson & Sparell, 1976; van Vuuren *et al.*, 1978; De Smedt & De Ley, 1979; De Vos *et al.*, 1980; Ingledew *et al.*, 1980; Haahtela *et al.*, 1981). A standardized microsystem for the semiquantitative assay of 19 different enzymes (API ZYM) has been described by Monget (1978) and applied to the identification of many bacteria (Humble *et al.*, 1977; Tharagonnet *et al.*, 1977; Kilian, 1978; Frank & Gerber, 1981; Mutimer & Woolcock, 1982) and has also been used in taxonomic studies (Gauthier, 1976; Guillermet, 1980; O'Donnell *et al.*, 1980; Holmes *et al.*, 1981, 1982). Another commercially available system, API 50CHE (i.e. the API 50CH gallery used with the API 50CHE medium), is suitable for the detection of acid production from 49 carbohydrates. A similar system, API 50E, has been used for the classification and identification of various bacteria (Imbs, 1974; Ljungh *et al.*, 1977; De Smedt & De Ley, 1979; Logan *et al.*, 1979; Schwan *et al.*, 1979; De Vos *et al.*, 1980; O'Donnell *et al.*, 1980).

0022-1287/84/0001-1774 $02.00

The genus *Erwinia* includes organisms causing important diseases of plants; others constitute a significant fraction of the nonphytopathogenic epiphytic flora of plant surfaces and some strains are opportunistic pathogens of man and animals. Dye (1968, 1969*a*, *b*, *c*) subdivided the genus into four so-called 'natural groups'. Strains of the 'amylovora' group (Dye, 1968), cause dry necrotic or wilt diseases on plants and include *Er. amylovora*, *Er. salicis*, *Er. rubrifaciens*, *Er. tracheiphila*, *Er. nigrifluens*, *Er. quercina*, and *Er. mallotivora* [assigned to the 'amylovora' group by Goto (1976)]. The 'carotovora' group (Dye, 1969*a*), consists of *Erwinia* species with strong pectolytic capacities, causing soft rots in a wide variety of plants. This group includes *Er. carotovora*, *Er. chrysanthemi*, *Er. rhapontici* and *Er. cypripedii*. Some authors assign the 'carotovora' group to the genus *Pectobacterium* (Waldee, 1945; Brenner *et al.*, 1973). The 'herbicola' group (Dye, 1969*b*) consists of erwinias that (usually) produce a yellow pigment; it includes epiphytes (*Er. herbicola*), some phytopathogens (*Er. ananas*, *Er. uredovora*, *Er. milletiae* and *Er. stewartii*) and yellow-pigmented and related nonpigmented clinical isolates, often named *Enterobacter agglomerans* (Ewing & Fife, 1972). Dye's fourth group (Dye, 1969*c*) contains 'atypical' erwinias, such as *Er. dissolvens*.

Many rearrangements have been proposed for the genus *Erwinia* (Brenner *et al.*, 1972; Gardner & Kado, 1972; Starr & Chatterjee, 1972; Murata & Starr, 1974; Sakazaki *et al.*, 1976; Young *et al.*, 1978; Dye, 1981) but a consensus has not yet been reached. The taxonomic uncertainty is illustrated by the inclusion of 21 *Erwinia* species as well as their synonyms (i.e. *Pectobacterium*, *Enterobacter agglomerans*) in the Approved Lists of Bacterial Names (Skerman *et al.*, 1980). The assignment of an *Erwinia* culture to one of Dye's major groups (Dye, 1968, 1969*a*, *b*) is largely made on the basis of pectolytic capacity (soft-rot erwinias), yellow pigmentation ('herbicola' group) and the lack of these traits in the white, non-pectolytic, wilt-causing 'amylovora' group (Starr, 1981). However, *Er. rhapontici* and *Er. cypripedii* both belong to the 'carotovora' group but do not degrade pectate (Lelliott, 1974) and only 70% of the strains from the '*Er. herbicola–En. agglomerans* complex' are yellow pigmented (Ewing & Fife, 1972). Identification at the species level is problematical, unless it is based on a known phytopathogenic capacity (Starr, 1981).

This paper presents the results of an extensive biochemical and enzymic characterization of 123 *Erwinia* strains, 6 *En. agglomerans* strains and 22 reference strains using API 20E and enzymic API systems (API ZYM, OSIDASES, ESTERASES, AMINOPEPTIDASES). These results were submitted to a numerical analysis. The production of acid from carbohydrates was investigated with the API 50CHE system for 89 of these strains. Our aims were (i) to study the physiological and biochemical diversity within the genus *Erwinia*, (ii) to compare the groupings obtained from the numerical analysis with the current classification of *Erwinia* species and (iii) to investigate the applicability of various API systems for the rapid identification of members of the genus *Erwinia* and allied species.

METHODS

Strains investigated. Strains used in this study (Table 1) included the type strains of all *Erwinia* species, 22 reference strains belonging to other phytopathogenic genera and other enterobacterial species and 6 *Enterobacter agglomerans* strains.

API systems. The commercially available API 20E and API 50CHE systems and nine enzymic API strips were used. The commercially available API ZYM and the experimental API galleries OSIDASES, ESTERASES, AMINOPEPTIDASES AP1, AP2, AP3, AP4, AP5 and AP6 encompass 107 enzymic test substrates (Table 2), and enable the assay of 61 aminopeptidases (arylamidases), 28 glycosidases, 13 esterases, 2 phosphatases, 2 proteinases and 1 phosphoamidase.

For API 20E tests, strains were grown on nutrient agar (Oxoid) slants at 28 °C for 24 h. Harvested cells were suspended in 5 ml sterile distilled water at a density of approximately 10^8 cells ml^{-1} (McFarland no. 1) as recommended by Murray (1978). The API 20E test strips were then handled as described by the manufacturer. The galleries were incubated at 30 °C, as most *Erwinia* strains are not able to grow at 37 °C.

For API 50CHE tests, strains were grown on nutrient agar slants at 28 °C for 24 h. Preparations of suspensions and inoculation were carried out as described by the manufacturer. Positive reactions after 3, 6, 24 and 48 h incubation at 30 °C were coded as 5, 4, 3 and 2, respectively. No acid production after 48 h incubation was coded as zero.

Table 1. *Bacterial strains investigated and their clustering, using the Euclidian distance coefficient*

Phenon and subphenon	Strain no.	Species name according to Skerman *et al.* (1980)	Received from*	Isolated from	Place and/or year of isolation	API 20E 7-digit code
—	M2/1	*Agrobacterium radiobacter*	M. Bernaerts	Ditch water	Belgium, 1952	1015046
—	B6†	*Agrobacterium tumefaciens*	P. Manigault	*Malus* sp.	USA, 1934	1010007
—	Kerr 38	*A. tumefaciens*	A. Kerr	*Prunus* sp.	Australia	1010006
—	NCPPB 796	*Xanthomonas campestris* pv. *vasculorum*‡	NCPPB	*Saccharum officinarum*	Mauritius	0001000§
—	NCPPB 457†	*Xanthomonas axonopodis*	NCPPB	*Axonopus scoparius*	Columbia, 1949	0001000§
—	NCPPB 528†	*X. campestris* pv. *campestris*‡	NCPPB	*Brassica oleracea* var. *gemmifera*	UK, 1957	1203000§
—	NCPPB 2969†	*Xanthomonas albilineans*	NCPPB	*Saccharum officinarum*	Fiji, 1961	1001000§
—	VT139	*Corynebacterium fascians*	R. Vantomme	*Lilium* sp.	Belgium, 1981	1010000§
—	D188	*C. fascians*		*Chrysanthemum morifolium*	1946	0011000§
—	NCPPB 1468†	*Corynebacterium michiganense*	NCPPB	*Lycopersicon esculentum*	UK, 1962	1005002
—	ATCC 13880†	*Serratia marcescens*	ATCC	Pond water		5306563
—	NCPPB 281†	*Pseudomonas syringae*	NCPPB	*Syringa vulgaris*	UK, 1950	0006022
—	NCPPB 2192†	*Pseudomonas tolaasii*	NCPPB	*Agaricus bisporus*	UK, 1965	2206006
A	NCPPB 1852†	*Erwinia quercina*	NCPPB	*Quercus* sp.	USA, 1964	0205121
A	NCPPB 1853	*Er. quercina*	NCPPB	*Quercus* sp.	USA, 1963	0205121
B	NCPPB 564†	*Erwinia nigrifluens*	NCPPB	*Juglans regia*	USA, 1955	0005773
B	NCPPB 565	*Er. nigrifluens*	NCPPB	*Juglans regia*	USA, 1955	0005773
B	NCPPB 566	*Er. nigrifluens*	NCPPB	*Juglans regia*	USA, 1955	0005773
—	NCPPB 2531	*Erwinia salicis*	NCPPB	*Salix caprea*	UK, 1972	0204731
—	NCTC 9001†	*Escherichia coli*	NCTC	Human, urine		5144553
C	NCPPB 447†	*Er. salicis*	NCPPB	*Salix alba* var. *caerulea*	UK, 1957	0005361
C	NCPPB 2534	*Er. salicis*	NCPPB	*Salic alba* var. *vitellina*	UK, 1972	0005361
C	NCPPB 2522	*Er. salicis*	NCPPB	*Salix alba* var. *caerulea*	UK, 1971	0005361
C	NCPPB 1466	*Er. salicis*	NCPPB	*Salix alba* var. *caerulea*	UK, 1957	0005161
C	NCPPB 2310	*Er. salicis*	NCPPB	*Salix* sp.	UK, 1970	0005361
C	HIM 588-6	*Er. salicis*	HIM	*Salix alba*	Netherlands, 1975	0005121
—	NCPPB 2291	*Erwinia amylovora*	NCPPB	*Rubus idaeus*	USA, 1949	0005020
D1	VT74	*Er. amylovora*	R. Vantomme	*Crataegus* sp.	Belgium, 1980	0005522
D1	VT82	*Er. amylovora*	R. Vantomme	*Cotoneaster* sp.	Belgium, 1980	0005522
D1	VT75	*Er. amylovora*	R. Vantomme	*Cotoneaster salicifolius* var. *floccosus*	Belgium, 1980	0005522
D1	VT71	*Er. amylovora*	R. Vantomme	*Cotoneaster repens*	Belgium, 1980	0005522
D1	VT72	*Er. amylovora*	R. Vantomme	*Pyrus communis*	Belgium, 1980	0005522
D1	VT56	*Er. amylovora*	R. Vantomme	*Crataegus* sp.	Belgium, 1980	0005522
D1	VT70	*Er. amylovora*	R. Vantomme	*Cotoneaster salicifolius* var. *floccosus*	Belgium, 1980	0007522

Table 1 (*continued*)

Phenon and subphenon	Strain no.	Species name according to Skerman *et al.* (1980)	Received from*	Isolated from	Place and/or year of isolation	API 20E 7-digit code
D1	VT87	*Er. amylovora*	R. Vantomme	*Crataegus* sp.	France, 1980	0005122
D1	VT1	*Er. amylovora*	R. Vantomme	*Pyrus communis*	Belgium, 1979	0007522
D1	NCPPB 683†	*Er. amylovora*	NCPPB	*Pyrus communis*	UK, 1959	0005122
D1	100-4	*Er. amylovora*	J. Hockenhull	*Cydonia oblonga*	Denmark, 1972	0005522
D1	VT57	*Er. amylovora*	R. Vantomme	*Crataegus* sp.	Belgium, 1980	0005522
D1	CNBP 2001	*Er. amylovora*	CNBP	*Pyrus communis*	Canada, 1972	0005522
D1	HIM 616-4	*Er. amylovora*	HIM	*Sorbus aria*	UK, 1959	0005522
D1	NCPPB 1272	*Er. amylovora*	NCPPB	*Malus sylvestris*	UK, 1962	0007522
D1	VT3	*Er. amylovora*	R. Vantomme	*Pyrus communis*	Belgium, 1977	0007522
D1	NCPPB 2791	*Er. amylovora*	NCPPB	*Pyrus communis*	USA, 1975	0005522
D2	NCPPB 2021†	*Erwinia rubrifaciens*	NCPPB	*Juglans regia*	USA, 1965	0004022
D2	NCPPB 2022	*Er. rubrifaciens*	NCPPB	*Juglans regia*	USA, 1966	0004122
D2	NCPPB 2020	*Er. rubrifaciens*	NCPPB	*Juglans regia*	USA, 1963	0004122
E1	NCPPB 2852	*Erwinia mallotivora*	NCPPB	*Mallotus japonicus*	Japan, 1975	1005522
E1	NCPPB 2853	*Er. mallotivora*	NCPPB	*Mallotus japonicus*	Japan, 1975	1005522
E1	NCPPB 2851†	*Er. mallotivora*	NCPPB	*Mallotus japonicus*	Japan, 1975	1005562
E2	NCPPB 2294	*Erwinia stewartii*	NCPPB	*Zea mays*	USA, 1932	1005162
E2	NCPPB 449	*Er. stewartii*	NCPPB	*Zea mays* var. *rugosa*	USA	1004162
E2	NCPPB 1553	*Er. stewartii*	NCPPB	*Zea mays*	USA, 1963	1004162
E2	NCPPB 2295†	*Er. stewartii*	NCPPB	*Zea mays* var. *rugosa*	USA	1004162
F1	Gilardi 698	*Enterobacter agglomerans*	F. Gavini	Human, urine	USA	3307173
F1	NCPPB 656	*Erwinia herbicola*	NCPPB	*Malus sylvestris*	UK, 1958	1005173
F2	JM1	*Erwinia milletiae*	D. C. Graham	*Wisteria floribunda*	Japan, 1956	1205173
F2	CNBP 1189	*Er. herbicola*	F. Gavini	*Malus sylvestris*	USA	1005173
F2	G155	*Er. herbicola*	D. C. Graham	Human, tonsils	USA	1005173
F2	NCPPB 2971†	*Er. herbicola*	NCPPB	Cereals	Canada	1005173
F2	G139	*Er. herbicola*	D. C. Graham	*Avena sativa*	UK	1005133
F2	NCTC 9381†	*En. agglomerans*	NCTC	Human, knee	Zimbabwe, 1956	1005173
F2	NCPPB 2600	*Er. milletiae*	NCPPB	*Wisteria floribunda*	Japan, 1970	1205133
F2	NCPPB 955	*Er. milletiae*	NCPPB	*Wisteria floribunda*	Japan	1207133
F2	NCPPB 2601	*Er. milletiae*	NCPPB	*Wisteria floribunda*	Japan, 1970	1207133
F2	NCPPB 2519†	*Er. milletiae*	NCPPB	*Wisteria floribunda*	Japan, 1970	1205133
F2	G140	*Er. herbicola*	D. C. Graham	*Avena sativa*	UK	1205133
F3	G146	*Er. herbicola*	D. C. Graham	*Vigna sinensis*	Tanzania	1205173
F3	ATCC 14589	*Er. herbicola*	F. Gavini	Soil	Japan	1205162
F3	NCIB 9746	*Er. herbicola*	NCIB	*Allium cepa*	Netherlands, 1965	1205153
–	PeH_220	*Er. herbicola*	M. E. Rhodes-Roberts	*Psychotria emetica*		1204353

Phenon and subphenon	Strain no.	Species name according to Skerman *et al.* (1980)	Received from*	Isolated from	Place and/or year of isolation	API 20E 7-digit code
G1	ICPB XU102†	*Erwinia uredovora*	ICPB	*Puccinia graminis* f.sp. *tritici*	USA, 1954	1247573
G1	NCPPB 1419	*Er. uredovora*	NCPPB	*Puccinia graminis* f.sp. *tritici*	Hungary, 1956	1244773
G1	NCPPB 1044	*Er. uredovora*	NCPPB	*Puccinia graminis*	Zimbabwe, 1961	1246773
G1	NCPPB 1846†	*Erwinia ananas*	NCPPB	*Ananas comosus*	Brazil, 1965	1245573
G1	NCPPB 1416	*Er. uredovora*	NCPPB	*Puccinia graminis* f.sp. *tritici*	Hungary, 1956	1245773
G1	NCPPB 391	*Er. uredovora*	NCPPB	*Puccinia graminis* f.sp. *tritici*	Hungary	1245773
G1	NCPPB 802	*Er. uredovora*	NCPPB	*Puccinia graminis* f.sp. *tritici*	Hungary, 1957	1245773
G1	BG2	*Er. ananas*	D. C. Graham	*Ananas comosus*	Hawaii, 1957	1245773
G1	BG1	*Er. ananas*	D. C. Graham	*Ananas comosus*	Hawaii, 1957	1245773
G2	Y1	*Er. ananas*	D. C. Graham	*Ananas comosus*	Hawaii, 1958	1245563
G2	MF28	*Er. ananas*	D. C. Graham	*Ananas comosus*	Hawaii, 1958	1245563
G2	NCPPB 2275	*Er. herbicola*	NCPPB	*Pennisetum glaucum*	India, 1961	1245163
H1	NCPPB 751	*Erwinia cypripedii*	NCPPB	*Cypripedium* sp.	USA, 1950	1204373
H1	PDDCC 1567	*Er. cypripedii*	PDDCC	*Cypripedium* sp.	USA	1204373
H1	HIM 579-1	*Er. cypripedii*	HIM	*Paphiopedilum* sp.		1204373
H1	NCPPB 3004†	*Er. cypripedii*	NCPPB	*Cypripedium* sp.	USA	1204373
H1	NCPPB 750	*Er. cypripedii*	NCPPB	*Cypripedium* sp.	USA, 1950	1204373
H1	NCPPB 752	*Er. cypripedii*	NCPPB	*Cypripedium* sp.	USA, 1950	1204373
H2	NCPPB 657	*Er. herbicola*	NCPPB	*Crataegus oxycantha*	UK, 1958	1005753
H2	NCPPB 655	*Er. herbicola*	NCPPB	*Pyrus communis*	UK, 1958	1004753
H3	NCPPB 2560	*Erwinia rhapontici*	NCPPB	*Triticum aestivum*	UK, 1973	1205773
H3	NCPPB 2559	*Er. rhapontici*	NCPPB	*Triticum aestivum*	UK, 1973	1205773
H3	NCPPB 2548	*Er. rhapontici*	NCPPB	*Triticum aestivum*	France	1205773
H3	NCPPB 2606	*Er. rhapontici*	NCPPB	*Triticum aestivum*	UK, 1973	1205773
H3	NCPPB 2549	*Er. rhapontici*	NCPPB	*Triticum aestivum*	France	1205773
–	Paulin 240-7	*Er. herbicola*	F. Gavini	*Crataegus* sp.		1005773
–	Paulin 238-3	*Er. herbicola*	F. Gavini	*Crataegus* sp.		1005733
I	NCPPB 2636	*Er. cypripedii*	NCPPB	*Paphiopedilum philippinense*	FRG, 1972	1205573
I	NCPPB 1850†	*Erwinia dissolvens*	NCPPB	*Zea mays*		3305573
I	NCTC 10005†	*Enterobacter cloacae*	NCTC	Human, spinal fluid		3305773
–	NCPPB 2176†	*Erwinia cancerogena*	NCPPB	*Populus canadensis*	1964	1305133
–	NCPPB 1625	*Erwinia carotovora* subsp. *carotovora*	NCPPB	*Solanum tuberosum*	Canada, 1918	1205573
–	ATCC 13883†	*Klebsiella pneumoniae*	ATCC			5214773
–	Gilardi 1081	*En. agglomerans*	F. Gavini	Human, hand wound	USA	1205353

Table 1 (*continued*)

Phenon and subphenon	Strain no.	Species name according to Skerman *et al.* (1980)	Received from*	Isolated from	Place and/or year of isolation	API 20E 7-digit code
J	ICPB 3423	*En. agglomerans*	F. Gavini	Human, knee fluid	USA	1044153
J	Leclerc 1783†	*Escherichia adecarboxylata*	F. Gavini	Drinking water		1004173
–	NCPPB 671	*Er. carotovora* subsp. *carotovora*	NCPPB	*Carnegiea gigantea*	USA, 1959	1205131
K1	UCPPB 1931	*Er. carotovora* subsp. *atroseptica*	UCPPB	*Beta vulgaris*	USA	1005133
K1	UCPPB 188	*Er. carotovora* subsp. *atroseptica*	UCPPB	*Beta vulgaris*	USA	1005133
K1	NCPPB 2792	*Er. carotovora* subsp. *atroseptica*	NCPPB	*Beta vulgaris*	USA, 1974	1005133
–	NCPPB 274	*Er. carotovora* subsp. *atroseptica*	NCPPB	*Solanum tuberosum*		1005573
K2	NCPPB 1640	*Er. carotovora* subsp. *carotovora*	NCPPB	*Saccharum officinarum*	Jamaica, 1963	1207173
K2	NCPPB 979	*Er. carotovora* subsp. *atroseptica*	NCPPB	*Delphinium ajacis*		1205173
K2	NCPPB 549†	*Er. carotovora* subsp. *atroseptica*	NCPPB	*Solanum tuberosum*	UK, 1957	1205173
K2	NCPPB 370	*Er. carotovora* subsp. *carotovora*	NCPPB	*Zantedeschia aethiopica*	Denmark, 1955	1207173
K2	NCPPB 552	*Er. carotovora* subsp. *carotovora*	NCPPB	*Zea mays*	Israel	1205173
K2	NCPPB 433	*Er. carotovora* subsp. *atroseptica*	NCPPB	*Solanum tuberosum*	Zimbabwe, 1955	1205173
K2	NCPPB 1277	*Er. carotovora* subsp. *atroseptica*	NCPPB	*Solanum tuberosum*	Northern Ireland, 1961	1205173
K2	NCPPB 1236	*Er. carotovora* subsp. *carotovora*	NCPPB	*Helianthus annuus*	Zambia, 1962	1205173
K2	NCPPB 352	*Er. carotovora* subsp. *carotovora*	NCPPB	*Schizanthus* sp.	Tanzania	1207173
K2	NCPPB 31	*Er. carotovora* subsp. *atroseptica*	NCPPB	*Apium graveolens* var. *dulce*	UK, 1939	1205173
K2	NCPPB 312†	*Er. carotovora* subsp. *carotovora*	NCPPB	*Solanum tuberosum*	Denmark	1207173
K2	NCPPB 1747	*Er. carotovora* subsp. *carotovora*	NCPPB	*Allium cepa*	Japan	1205173
K2	NCPPB 2418	*Er. carotovora* subsp. *carotovora*	NCPPB	*Chrysanthemum morifolium*	UK, 1971	1205173
–	NCPPB 2042	*Er. carotovora* subsp. *carotovora*	NCPPB	*Daucus carota* var. *sativa*	USA	1005173
–	van Vuuren 117	*En. agglomerans*	H. J. J. van Vuuren	Fermenting wort, brewery	South Africa	1205573
–	NCPPB 1578†	*Er. rhapontici*	NCPPB	*Rheum rhaponticum*	UK	1205373
–	NCPPB 518	*Erwinia chrysanthemi*	NCPPB	*Dianthus caryophyllus*	Denmark, 1957	1244173
L	NCPPB 453	*Er. chrysanthemi*	NCPPB	*Dianthus caryophyllus*	UK, 1956	1245173
L	NCPPB 2309	*Er. chrysanthemi*	NCPPB	*Chrysanthemum maximum*	Italy, 1969	1245173
L	NCPPB 402†	*Er. chrysanthemi*	NCPPB	*Chrysanthemum morifolium*	USA	1247173
L	NCPPB 2421	*Er. chrysanthemi*	NCPPB	*Begonia bertini*	Netherlands, 1969	1247173
L	NCPPB 1861	*Er. chrysanthemi*	NCPPB	*Parthenium argentatum*	USA, 1945	1247173
L	NCPPB 454	*Er. chrysanthemi*	NCPPB	*Philodendron* sp.	USA, 1957	1247373
L	NCPPB 516	*Er. chrysanthemi*	NCPPB	*Parthenium argentatum*	Denmark, 1956	1247373
L	NCPPB 1065	*Er. chrysanthemi*	NCPPB	*Zea mays*	Egypt, 1961	1247173
L	NCPPB 2538	*Er. chrysanthemi*	NCPPB	*Zea mays*	USA, 1970	1247173
L	NCPPB 2511†	*Er. paradisiaca*	NCPPB	*Musa paradisiaca*	Colombia, 1973	1245073
L	LV6	*Er. chrysanthemi*	L. Verdonck	*Dieffenbachia maculata*	Belgium, 1981	1245133
L	NCPPB 2308	*Er. chrysanthemi*	NCPPB	*Dieffenbachia picta* cv. *amoena*	Italy, 1969	1245133
L	NCPPB 569	*Er. chrysanthemi*	NCPPB	*Saccharum officinarum*	Australia, 1958	1245133

Phenon and subphenon	Strain no.	Species name according to Skerman *et al.* (1980)	Received from*	Isolated from	Place and/or year of isolation	API 20E 7-digit code
–	NCTC 10396†	*Edwardsiella tarda*	NCTC	Human faeces		4744000
–	NCPPB 1822	*Xanthomonas fragariae*	NCPPB	*Fragaria chiloensis* var. *ananassa*	USA, 1966	0003000§
–	NCPPB 325†	*Pseudomonas solanacearum*	NCPPB	*Lycopersicon esculentum*	USA	0201004§
–	NCPPB 359†	*Pseudomonas rubrilineans*	NCPPB	*Saccharum officinarum*	Mauritius, 1955	0211004§
–	Gilardi 664	*En. agglomerans*	F. Gavini	Hand brush	USA	3305173
–	NCPPB 956	*Er. carotovora* subsp. *carotovora*	NCPPB	*Brassica oleracea* var. *botrytis*	1902	1207123
–	NCPPB 2700	*Xanthomonas campestris* pv. *graminis*‡	NCPPB	*Dactylis glomerata*	Switzerland, 1973	0207026§
	PDDCC 1585¶	*Erwinia tracheiphila*	PDDCC	*Cucumis sativus*	USA, 1951	0004520
	PDDCC 1586¶	*Er. tracheiphila*	PDDCC			0004520
	PDDCC 5845†,¶	*Er. tracheiphila*	PDDCC	*Cucumis melo*	USA, 1972	0004520

* Sources: ATCC, American Type Culture Collection, Rockville, Maryland, USA; CNBP, Collection Nationale des Bactéries Phytopathogènes, Institut National de la Recherche Agronomique, Angers, France; HIM, Hygiene-Institut und Medizinal-Untersuchungsamt, Universität Marburg, FRG; ICPB, International Collection of Phytopathogenic Bacteria, Davis, California, USA; NCIB, National Collection of Industrial Bacteria, Aberdeen, UK; NCPPB, National Collection of Plant Pathogenic Bacteria, Harpenden, UK; NCTC, National Collection of Type Cultures, London, UK; PDDCC, Culture Collection of Plant Diseases Division, New Zealand Department of Scientific and Industrial Research, Auckland, New Zealand; UCPPB, University of California, Department of Plant Pathology, Berkeley, California, USA; Bernaerts, M., Centraal Laboratorium, Ministerie van Economische Zaken, Brussels, Belgium; Gavini, F., Institut National de la Santé et de la Recherche Médicale, Unité 146, Villeneuve-d'Ascq, France; Graham, D. C., Department of Agriculture and Fisheries for Scotland, Agricultural Scientific Services, Edinburgh, UK; Hockenhull, J., Department of Plant Pathology, The Royal Veterinary and Agricultural University, Copenhagen, Denmark; Kerr, A., Department of Plant Pathology, University of Adelaide, Glen Osmond, Australia; Manigault, P., Institut Pasteur, Paris, France; Rhodes-Roberts, M. E., Department of Botany and Microbiology, University of Aberystwyth, UK; Vantomme, R., Laboratorium voor Microbiologie en microbiële Genetica, Rijksuniversiteit, Gent, Belgium; van Vuuren, H. J. J., Department of Microbiology, University of the Orange Free State, Bloemfontein, Republic of South Africa.

† Type strains according to Skerman *et al.* (1980).

‡ Pathovar designation according to Young *et al.* (1976).

§ Seven-digit profile number obtained after 48 h incubation at 30 °C.

‖ Proposed type strain of *Er. carotovora* subsp. *betavasculorum* (Thomson *et al.*, 1981).

¶ Not included in the numerical analysis.

For the enzymic API tests, strains were grown in Roux flasks containing approximately 120 ml nutrient agar and incubated at 28 °C for 24 h. Cells were washed off the agar surface with sterile distilled water, harvested by centrifugation (15 min at 12000 r.p.m. and 4 °C in a Sorvall RC 2B centrifuge, type SS34 rotor), and washed with 20 ml sterile distilled water. The pellet was resuspended in approximately 1 ml sterile distilled water and from this dense suspension two dilutions, each containing approximately $1{\cdot}8 \times 10^9$ cells ml^{-1} (McFarland no. 6), were prepared: (i) in 2 ml of normal saline and (ii) in 10 ml of a 0·01 M-phosphate buffer pH 7. The test strips were placed in their moistened plastic incubation trays and inoculation was carried out as follows: two drops of suspension (i) were added to each cupule of the API ZYM strip, and two drops of suspension (ii) were added to the cupules of the OSIDASES, ESTERASES and AMINOPEPTIDASES AP1 to AP6 strips. All the enzymic galleries were incubated at 30 °C for 4 h. One drop of a 0·1 M-NaOH solution was then added to each cupule of the OSIDASES strips and positive reactions (denoted by the appearance of a yellow colour) were recorded immediately. One drop of reagent A [containing 10% (w/v) SDS in a 2 M-Tris/HCl buffer pH 7·6 to 7·8] and one drop of reagent B [containing 0·3% (w/v) Fast Blue B in 2-methoxyethanol] were added to each cupule of the other test strips, which were then exposed to a 600 W light source for 5 min to destroy excess reagent B. Positive reactions were denoted by a colour change. The results were graded 0 to 5 by comparison with the colour chart provided by the manufacturer. Codes 0, 1, 2, 3, 4 and 5 correspond to approximately 0, 5, 10, 20, 30 and over 40 nM, respectively, of hydrolysed substrate.

Data analysis. Numerical analysis was carried out on the data obtained from all strains (except for *Er. tracheiphila*; see below) listed in Table 1 with API 20E, API ZYM, OSIDASES, ESTERASES and AMINOPEPTIDASES AP1 to AP6 systems. The results obtained in the API ZYM, ESTERASES and AMINOPEPTIDASES AP1 to AP6 systems were coded as semiquantitative characters using the original record scores of 0 (negative), 1, 2, 3, 4 and 5 (weakly to strongly positive). The mean value of the positive reactions obtained in these systems was approximately 3 for this data set. Consequently, the results of the API 20E and OSIDASES tests were coded 3 (positive), 1 (weakly positive) and 0 (negative). The Euclidian distance and Canberra metric coefficients were used to compute dissimilarities and clustering was achieved by the unweighted average pair group method (UPGMA) (Sneath & Sokal, 1973) using the CLUSTAN program (Wishart, 1978) on the Siemens 7541 (BS 2000) computer of the Centraal Digitaal Rekencentrum of the Rijksuniversiteit Gent. The results of the API 20E tests were also recorded as seven-digit profile numbers as described by the manufacturer and the profiles obtained were identified using the API 20E Analytical Profile Index (1983 edition, no. 2019) and the API computer service.

RESULTS AND DISCUSSION

Test reproducibility

Seventeen strains were tested twice in the API 20E, API ZYM, OSIDASES, ESTERASES and AMINOPEPTIDASES strips. The Euclidian distance coefficients calculated between the replicates had a mean and standard deviation of $0{\cdot}48 \pm 0{\cdot}19$. Five tests, the hydrolysis of 2-naphthyl butyrate, L-leucyl-2-naphthylamide, 2-naphthyl-β-D-galactopyranoside, *p*-nitrophenyl-β-D-galactopyranoside-6-phosphate, and L-histidyl-L-phenylalanine-2-naphthylamide, showed considerable disagreement in more than 3 of 17 duplicated strains. Test reproducibility within the API 20E system varied from 88 to 100%. Highest reproducibility was obtained for the tests contained in the API 50CHE system, where only 3 tests showed a slight disagreement in one of the 7 duplicated strains.

Numerical analyses of the data obtained from the API 20E, API ZYM, OSIDASES, ESTERASES and AMINOPEPTIDASES AP1 to AP6 systems, applied to 151 strains

Numerical analysis, using the Euclidian distance coefficient, revealed 12 phenons at the distance level of $d = 0{\cdot}95$. In six of the phenons, definite subphenons could be distinguished (Fig. 1). Several of these (sub)phenons (A, B, C, D1, D2, E1, E2, H1, H3, K and L) corresponded to established *Erwinia* species (Table 1, Fig. 1). All *Erwinia* strains were separated from the non-enterobacterial strains at $d = 1{\cdot}83$, except for the two *Er. quercina* strains (phenon A) and *Er. carotovora* subsp. *carotovora* NCPPB 956 (unclustered). The numerical analysis using the Canberra metric coefficient gave similar results.

Biochemical and enzymic characteristics of Erwinia

The results of API 20E, API ZYM, OSIDASES, ESTERASES and AMINOPEPTIDASES AP1 to AP6 systems are listed in Table 2.

All strains classified in phenons F, G, H, I, J, K and L as well as the ungrouped strains classified between phenons F and L in the dendrogram (Fig. 1; Table 1) were submitted to further analysis with the API 50CHE system in order to examine the discriminative potential of this system, and to trace additional tests for the differentiation of phenons and subphenons F1, F2, F3, G1, G2, H1, H2, H3, I, J, K1, K2 and L. All the strains investigated with this system produced acid within 48 h from D-ribose, D-galactose, D-glucose, D-fructose, D-mannose, mannitol and *N*-acetylglucosamine, but not from L-xylose, β-methylxyloside or glycogen. None of the *Erwinia* or *En. agglomerans* strains studied with this system produced acid from L-sorbose, starch, D-tagatose and L-arabitol, and fewer than 5% produced acid from erythritol, adonitol, inulin, melezitose, xylitol, D-turanose, D-lyxose and D- and L-fucose. More than 95% of these *Erwinia* and *En. agglomerans* strains produced acid from L-arabinose, L-rhamnose, arbutin and salicin. The remaining tests were positive for at least 5% and at most 95% of these *Erwinia* and *En. agglomerans* strains. The API 50CHE system provided additional differential tests for separating the subphenons and phenons F1, F2, F3, G1, G2, H1, H2, H3, I, J, K1, K2 and L (Table 3).

Comments on the taxonomy of Erwinia *as revealed by API systems*

It is evident from the numerical analysis (Fig. 1) that no sharp distinction can be made between the 'amylovora', 'herbicola' and 'carotovora' groups. For example *Er. quercina* (phenon A) and *Er. nigrifluens* (phenon B) are remote from each other and from the other species of the 'amylovora' group (*Er. salicis, Er. amylovora, Er. rubrifaciens* and *Er. mallotivora*). *Erwinia stewartii* is phenotypically closer to the latter four species of the 'amylovora' group than to any species of the 'herbicola' group, and *Er. cypripedii*, considered as a member of the 'carotovora' group, is more related to some *Er. herbicola* strains than to *Er. carotovora* or *Er. chrysanthemi.* Both numerical analyses of phenotypic characteristics (Dye, 1981) and DNA:DNA hybridization studies (Murata & Starr, 1974; Azad & Kado, 1980) have failed to support these 'natural' groups. Dye (1981) compared four different clustering methods, but none provided a definite division into the three groups. Consequently these three groups have recently been abandoned (Lelliott & Dickey, 1984).

The six species of the 'amylovora' group, (*Er. quercina, Er. nigrifluens, Er. salicis, Er. amylovora, Er. rubrifaciens* and *Er. mallotivora*) were recovered as separate (sub)phenons A, B, C, D1, D2, and E1, respectively (Fig. 1). However, it should be noted that only very few strains of *Er. quercina, Er. nigrifluens, Er. rubrifaciens* and *Er. mallotivora* were available for inclusion in our study. *Erwinia tracheiphila* strains were not included in the numerical analyses because they grew poorly on nutrient agar and growth on this medium was a prerequisite for the standardized testing in the enzymic API systems. Indeed, it has been stressed that a strict control of medium and growth time is necessary in order to obtain comparable enzymic profiles (Westley *et al.*, 1967). In each of the four dendrograms presented by Dye (1981), the following species were recovered as well-separated clusters: *Er. nigrifluens, Er. salicis, Er. tracheiphila* and *Er. amylovora.* The strains of *Er. quercina* and *Er. rubrifaciens* consistently formed one phenon and segregated only at high similarity levels. Dye (1981) considered them as two pathovars of one species: *Er. quercina* pv. *quercina* and *Er. quercina* pv. *rubrifaciens.* However, our results and the data from DNA:DNA hybridizations (Brenner *et al.*, 1974; Murata & Starr, 1974; Azad & Kado, 1980) showed that *Er. amylovora, Er. nigrifluens, Er. salicis, Er. mallotivora, Er. tracheiphila, Er. quercina* and *Er. rubrifaciens* each form well-separated clusters, strongly suggesting that all should be retained as separate species. The API 20E system allows an easy differentiation between them. The specific and unique seven-digit profile numbers are given for each in Table 1. Within the genus *Erwinia*, the hydrolysis of L-hydroxyproline-2-naphthylamide is restricted to cultures of *Er. quercina.* Hydrolysis of this substrate seems to occur more frequently in other enterobacterial genera, e.g. *Serratia* (Godsey *et al.*, 1981).

The four strains of *Er. stewartii*, including the type strain NCPPB 2295, clustered together in a homogeneous subphenon E2 and showed higher similarity to members of the 'amylovora' group [*Er. mallotivora* (subphenon E1), *Er. rubrifaciens* (subphenon D2), *Er. amylovora* (subphenon D1) and *Er. salicis* (phenon C)] than to any species of the 'herbicola' group. In all four numerical

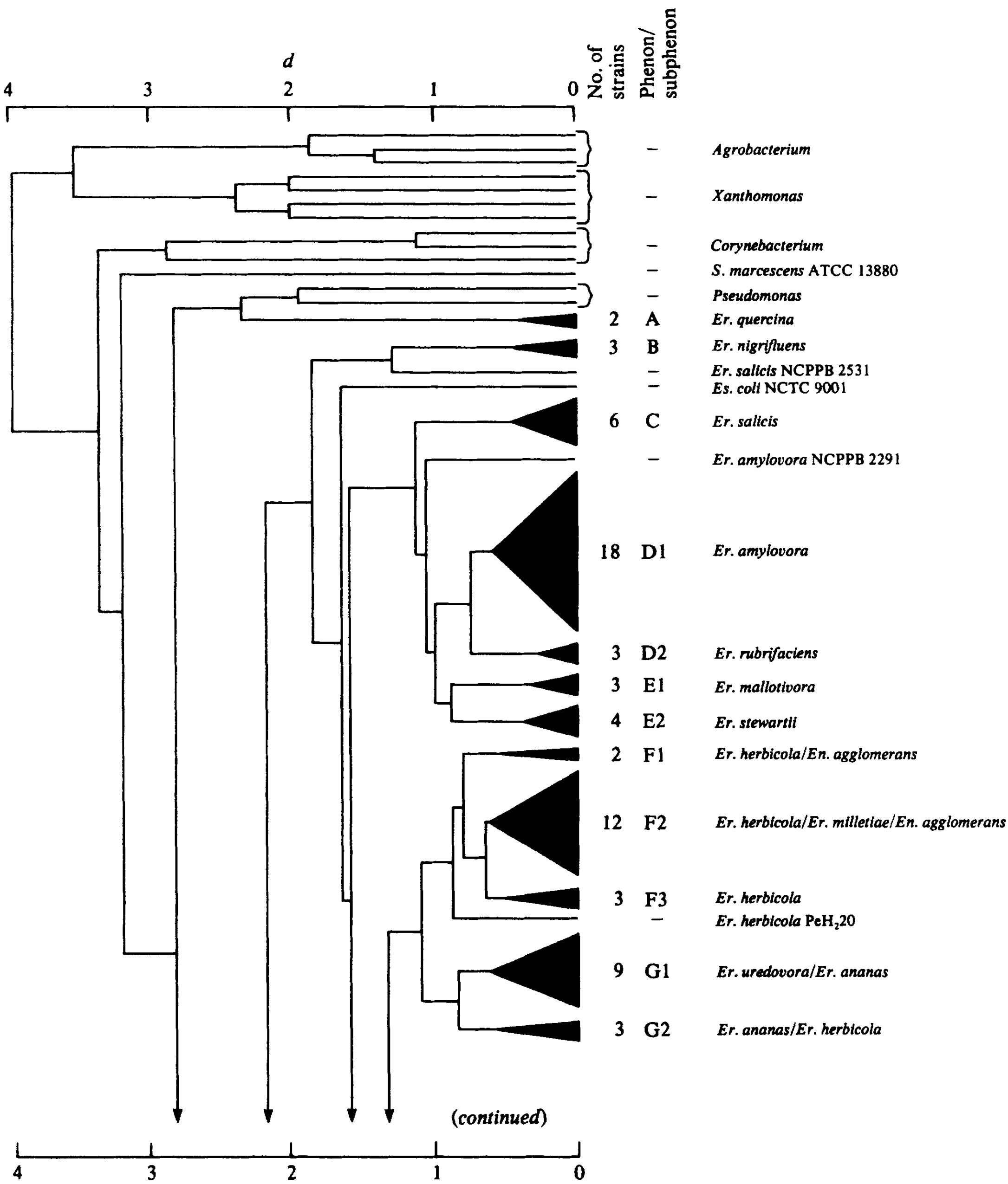

Fig. 1. Simplified dendrogram derived from the unweighted, average pair group clustering of Euclidian distance coefficients. For each of the 151 strains, 23 API 20E and 107 enzymic tests from the API ZYM, OSIDASES, ESTERASES and AMINOPEPTIDASES systems were determined. All strains are in the same sequence as in Table 1.

analyses prepared by Dye (1981), *Er. stewartii* constituted a homogeneous cluster close to *Er. amylovora*. It is clear that the current classification of *Er. stewartii* in the 'herbicola' group is no longer justified. *Er. stewartii* formed a distinct species that could be recognized by a specific API 20E profile (Table 1).

The strains labelled *Er. herbicola* were heterogeneous and were recovered in five (sub)phenons (F1, F2, F3, G2 and H2 in Fig. 1). Indeed some strains formed single-member clusters. This

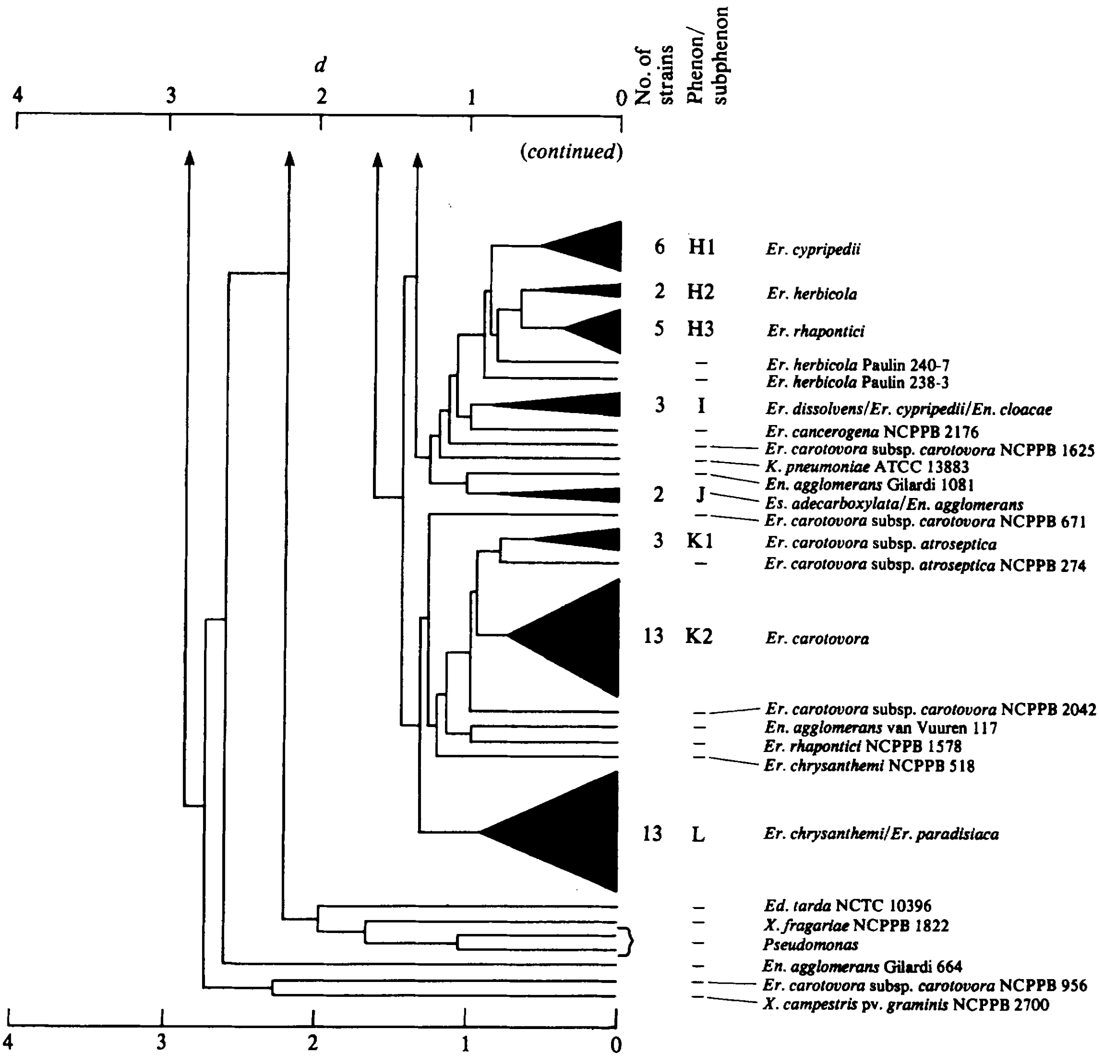

Fig. 1 (*continued*)

confirms earlier findings (Murata & Starr, 1974; Sakazaki *et al.*, 1976; Brenner, 1981; Gavini *et al.*, 1983; Mergaert *et al.*, 1983*a*; Brenner *et al.*, 1984). The type strains of *Er. herbicola* (NCPPB 2971) and *En. agglomerans* (NCTC 9381) and all five *Er. milletiae* strains investigated, including the type strain NCPPB 2519, were recovered together in subphenon F2. A partial overlap of *Er. herbicola*, *Er. milletiae* and *En. agglomerans* has been demonstrated by Murata & Starr (1974), Gavini *et al.* (1983) and Mergaert *et al.* (1983*b*). In the API 20E Analytical Profile Index, *Er. herbicola* has been named *En. agglomerans* and hundreds of different seven-digit profile numbers are used for this organism. Definite conclusions concerning the subdivisions within the '*Er. herbicola–En. agglomerans* complex', and their relationships to other *Enterobacteriaceae* are not yet possible but certainly a taxonomic problem arises when the type strains of *En. agglomerans* (NCTC 9381), *Er. herbicola* (NCPPB 2971) and *Er. milletiae* (NCPPB 2519) fall within the same subphenon F2. The high phenotypic and protein electrophoretic similarities of these type strains have already been stressed by Mergaert *et al.* (1983*b*) and in this study it was impossible to differentiate them from each other by the API systems used. This is further evidence for the synonymy of the three taxa as already proposed by Ewing & Fife (1972) and Lelliott (1974). As the phenotypic features of subphenon F2 fit *Er.*

Table 2. *Differential biochemical and enzymic tests for the separation of the phenons and subphenons*

Key: +, 90% or more of strains positive; –, 90% or more of strains negative; d, 11–89% of strains positive. Results for all the other tests are given in footnote‡.

Character* (Sub)phenon ... No. of strains ...	A 2	B 3	C 6	D1 18	D2 3	E1 3	E2 4	F1 2	F2 12	F3 3	G1 9	G2 3	H1 6	H2 2	H3 5	I 3	J 2	K1 3	K2 13	L 13
API 20E system:																				
β-D-Galactosidase	–	–	–	–	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Citrate utilization	+	–	–	–	–	–	–	d	d	+	+	+	+	–	+	+	–	–	+	+
Indole production	–	–	–	–	–	–	–	–	–	–	+	+	–	–	–	–	d	–	–	+
Acetoin production	+	+	+	+	–	+	d	+	+	+	d	+	+	d	+	+	–	+	+	+
Acid from:																				
Inositol	–	+	d	–	–	–	–	–	–	–	d	–	+	+	+	d	–	–	–	d
Sorbitol	–	+	–	d	–	+	–	–	–	–	+	d	–	+	+	+	–	–	–	–
L-Rhamnose	–	+	–	–	–	–	–	+	+	d	+	–	+	d	+	+	+	+	+	+
Melibiose	–	+	d	–	–	d	+	+	d	+	+	+	+	+	+	+	+	–	+	+
Amygdalin	+	+	+	–	–	–	–	+	+	d	+	+	+	+	+	+	+	+	+	+
L-Arabinose	–	+	–	+	+	d	+	+	+	+	+	+	+	d	+	+	+	+	+	+
Nitrate reduction to nitrite	–	–	–	–	–	–	–	+	+	–	d	–	d	+	+	+	+	+	+	d
OSIDASES system:																				
PNP-α-D-galactopyranoside	–	+	+	–	–	–	–	–	–	–	–	–	–	–	d	d	+	–	+	d
PNP-β-D-galactopyranoside-6-phosphate	–	–	–	–	–	d	–	–	d	–	+	+	–	d	d	d	–	–	–	d
PNP-α-L-arabinofuranoside	+	+	–	–	–	–	–	–	–	–	d	+	–	–	–	+	–	–	–	d
PNP-α-D-glucopyranoside	+	–	d	–	–	d	–	d	–	–	–	–	–	–	d	–	–	+	d	–
PNP-β-D-glucopyranoside	+	+	+	d	+	–	–	+	+	+	d	d	+	+	+	+	+	+	+	+
PNP-β-D-fucopyranoside	–	–	–	–	–	–	–	–	–	–	–	–	d	–	d	–	–	+	+	d
PNP-β-D-mannopyranoside	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	d	d	–	+	d
PNP-β-D-xylopyranoside	–	–	–	–	–	–	–	–	–	–	d	+	–	–	–	d	+	–	–	d
API ZYM system†:																				
2-Naphthyl-α-D-glucopyranoside	5	–	–	–	d	–	–	–	–	–	–	–	–	–	–	d	–	2	d	–
AMINOPEPTIDASES systems†:																				
L-Pyrrolidonyl-NA	–	–	–	–	–	1	2·5	2	2·8	1	3·1	3	d	3	2·4	2	4	1	1·4	–
L-Hydroxyprolyl-NA	5	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
γ-L-Glutamic acid-NA	–	–	d	2·5	–	–	–	4	4·8	2·7	4·7	4·7	4·7	5	4·8	4·3	5	2·3	3·8	d
L-Alanyl-L-phenylalanyl-L-proline-NA	2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	d
L-Valyl-L-tyrosyl-L-serine-NA	2·5	–	–	–	–	1	–	–	–	–	–	d	–	–	d	d	–	–	–	–

Footnotes to Table 2:

• Abbreviations: PNP-, *p*-nitrophenyl-; -NA, -2-naphthylamide.

† Positive reactions are expressed as mean values, calculated from the original codes obtained from all strains of each cluster.

‡ All the strains displayed no or very weak enzyme activities (scores ⩽ 1) with the following substrates in API ZYM: naphthol-AS-BI-β-D-glucuronate, 6-Br-2-naphthyl-α-D-mannopyranoside; in OSIDASES: PNP-β-L-fucopyranoside, PNP-*N*-acetyl-α-D-glucosaminide; in AMINOPEPTIDASES: *N*-benzoyl-L-leucine-NA and *N*-benzoyl-L-alanine-4-methoxy-NA.

All enterobacterial strains produced acid from D-glucose, were oxidase negative in API 20E and displayed no or very weak enzyme activities with the following substrates in API ZYM: 2-naphthyl-α-L-fucopyranoside, L-valyl-NA; in OSIDASES: PNP-α-L-fucopyranoside, PNP-β-D-galacturonide, PNP-α-D-xylopyranoside; in ESTERASES: 2-naphthyl myristate, 2-naphthyl palmitate; in AMINOPEPTIDASES: L-aspartyl-NA, L-tryptophyl-NA, *N*-carbobenzyloxy-L-arginine-4-methoxy-NA, α-L-glutamyl-α-L-glutamic acid-NA, α-L-glutamyl-L-histidine-NA, *N*-acetylglycyl-L-lysine-NA and L-histidyl-L-leucyl-L-histidine-NA.

Less than 5% of the *Erwinia* and *En. agglomerans* strains produced decarboxylases for lysine and ornithine, arginine dihydrolase, tryptophan deaminase, H_2S and urease in API20E and hydrolysed in API ZYM: L-cystyl-NA, *N*-glutarylphenylalanine-NA; in OSIDASES: PNP-α-maltoside, PNP-β-maltoside, PNP-α-D-mannopyranoside, PNP-β-D-glucuronide; in ESTERASES: 2-naphthyl caprate, 2-naphthyl laurate; in AMINOPEPTIDASES: L-prolyl-NA.HCl, L-hydroxyprolyl-NA, α-L-glutamyl-NA, L-isoleucyl-NA, L-threonyl-NA, β-alanyl-NA, glycyl-L-proline-NA, α-L-aspartyl-L-arginine-NA, glycyl-L-tryptophan-NA, L-phenylalanyl-L-proline-NA, L-alanyl-L-phenylalanyl-L-proline-NA, L-valyl-L-tyrosyl-L-serine-NA, L-leucyl-L-leucyl-L-valyl-L-tyrosyl-L-serine-NA.

Over 95% of the *Erwinia* and *En. agglomerans* strains produced acid from mannitol and sucrose in API 20E, produced acid phosphatase in API ZYM and hydrolysed L-lysyl-NA, L-alanyl-NA, L-glutamine-NA.HCl, L-leucyl-glycine-NA, glycyl-L-alanine-NA and L-leucyl-L-alanine-NA in AMINOPEPTIDASES. Hydrolysis of 2-naphthyl stearate in ESTERASES was omitted as this test already gave a positive reaction when inoculated with sterile distilled water alone.

All the other tests contained in the API 20E, API ZYM, OSIDASES, ESTERASES and AMINOPEPTIDASES strips were positive with at least 5% and at most 95% of the *Erwinia* and *En. agglomerans* strains. For the non-commercial systems these substrates are in OSIDASES: PNP-β-D-galactopyranoside, PNP-*N*-acetyl-β-D-glucosaminide, PNP-β-D-lactoside; in ESTERASES: 2-naphthyl butyrate, 2-naphthyl valerate, 2-naphthyl caproate, 2-naphthyl caprylate, 2-naphthyl nonanoate; in AMINOPEPTIDASES: L-tyrosyl-NA, L-phenylalanyl-NA, L-histidyl-NA, glycyl-NA, L-arginyl-NA, *S*-benzyl-L-cysteine-NA, DL-methionyl-NA, glycylglycine-NA.HBr, glycyl-L-phenylalanine-NA, L-seryl-L-tyrosine-NA, L-ornithyl-NA, L-seryl-NA, *N*-carbobenzyloxy-glycyl-glycyl-L-arginine-NA, L-alanyl-L-arginine-NA, L-alanyl-L-phenylalanyl-L-prolyl-L-alanine-NA, L-arginyl-L-arginine-NA, α-L-aspartyl-L-alanine-NA, glycyl-L-arginine-NA, L-histidyl-L-serine-NA, L-lysyl-L-alanine-NA, L-lysyl-L-lysine-NA, L-phenylalanyl-L-arginine-NA, L-phenylalanyl-L-prolyl-L-alanine-NA, L-prolyl-L-arginine-NA, L-seryl-L-methionine-NA, *N*-carbobenzyloxy-glycyl-glycyl-L-arginine-NA, L-histidyl-L-phenylalanine-NA, L-lysyl-L-serine-4-methoxy-NA.

Table 3. *Differential tests from the API 50CHE system for the separation of the phenons and subphenons F1, F2, F3, G1, G2, H1, H2, H3, I, J, K1, K2 and L*

Key: +, 90% or more of strains positive within 6 h; (+) 90% or more of strains positive within 48 h; –, 90% or more of strains negative after 48 h; d, 11–89% of strains positive within 48 h.

Character	(Sub)phenon ... No. of strains ...	F1 2	F2 12	F3 3	G1 9	G2 3	H1 6	H2 2	H3 5	I 3	J 2	K1 3	K2 13	L 13
Acid from:														
L-Rhamnose		(+)	(+)	(+)	(+)	–	(+)	(+)	(+)	+	(+)	(+)	(+)	(+)
α-Methylmannoside		–	–	–	(+)	d	–	–	–	–	–	–	–	–
α-Methylglucoside		–	–	–	–	–	–	–	–	(+)	–	(+)	d	–
Aesculin		+	(+)	d	d	d	(+)	(+)	+	d	+	+	+	+
D-Cellobiose		(+)	–	d	(+)	(+)	(+)	–	(+)	+	+	d	(+)	(+)
D-Maltose		(+)	(+)	d	(+)	(+)	d	(+)	(+)	+	+	d	–	–
Lactose		(+)	d	(+)	(+)	(+)	–	–	d	(+)	(+)	d	(+)	d
Melibiose		(+)	d	(+)	(+)	(+)	d	–	(+)	d	+	–	(+)	d
Sucrose		+	+	d	(+)	+	+	–	+	+	(+)	+	(+)	+
Inulin		–	–	–	–	–	–	–	–	–	–	(+)	–	–
Raffinose		–	–	d	(+)	(+)	–	–	(+)	d	d	d	(+)	d
D-Arabitol		(+)	d	d	(+)	(+)	–	(+)	–	–	+	–	–	–

herbicola (Lelliott, 1974) as well as *En. agglomerans* (Ewing & Fife, 1972) it is not clear which name should be retained for the strains of this subphenon. Different views concerning this subject have been developed by Dye (1964) and Ewing & Fife (1972), but the situation is still more obscured by the fact that within the '*Er. herbicola–En. agglomerans* complex' over 10 DNA homology groups (Brenner *et al.*, 1984), 14 phenons (Gavini *et al.*, 1983), 21 phenons (Murata & Starr, 1974) and 25 protein electrophoretic groups (Mergaert *et al.*, 1983*a*) have been revealed.

Erwina uredovora and *Er. ananas* clustered together in phenon G. Their close relationship was also revealed by Dye (1981) and Brenner *et al.* (1984), and both Young *et al.* (1978) and Dye (1981) proposed to unite them as separate pathovars in *Er. ananas* Serrano 1928, as *Er. ananas* pv. *ananas* and *Er. ananas* pv. *uredovora*. The probable synonymy of these two taxa is supported here as no differential features between *Er. ananas* and *Er. uredovora* were highlighted with the API systems. However, phenon G was slightly heterogeneous, comprising two subphenons G1 and G2 which could only be differentiated by acid production from L-rhamnose.

Six of the seven *Er. cypripedii* strains, including the type strain NCPPB 3004, constituted the homogeneous subphenon H1. *Erwinia cypripedii* NCPPB 2636 was a member of phenon I (see below). In each of Dye's (1981) numerical analyses, *Er. cypripedii* formed a well-delineated cluster. *Erwinia cypripedii* should be regarded as a species separate from other species of *Erwinia*. The API 20E seven-digit profile numbers were specific for this species, although they were identified as *En. agglomerans*, *Klebsiella ozaenae* or possible *Erwinia* species in the API 20E Analytical Profile Index.

Five strains labelled *Er. rhapontici*, isolated from *Triticum aestivum*, clustered in subphenon H3, whereas the type strain NCPPB 1578, which had been isolated from *Rheum rhaponticum*, was ungrouped. From Dye's (1981) dendrograms, it was apparent that the type strain NCPPB 1578 of *Er. rhapontici* is very similar to three other *Rheum* isolates of this species. From the present results it is questionable whether the *Triticum* isolates belong to the species *Er. rhapontici*. They were assigned to *Klebsiella pneumoniae* in the API 20E Analytical Profile Index, while the type strain of *Er. rhapontici* (NCPPB 1578) was assigned to *En. agglomerans* or possible *Erwinia* sp. In the API systems, the type strain differed in 15 out of 179 features from the *Triticum* isolates. Our data do not support Roberts' (1974) view that *Triticum* isolates are identical or very similar to *Rheum* isolates.

The type strains *Er. dissolvens* NCPPB 1850 and *En. cloacae* NCTC 10005 clustered in phenon I, together with strain NCPPB 2636, an apparently misnamed *Er. cypripedii* strain, although this strain was credited to be pathogenic in the NCPPB catalogue (Anonymous, 1977). Brenner *et al.* (1973) asserted that *Er. dissolvens* is more closely related to *En. cloacae* than to any *Erwinia* species.

Enterobacter agglomerans strain ICPB 3423 and the type strain Leclerc 1783 (= ATCC 23216) of *Escherichia adecarboxylata* formed phenon J. This agrees with the findings of Gavini *et al.* (1983) and conflicts with Ewing & Fife's (1972) proposal to incorporate *Es. adecarboxylata* in *En. agglomerans*.

Subphenon K2 contained strains labelled *Er. carotovora* subsp. *carotovora* and subsp. *atroseptica* together with their type strains, NCPPB 312 and NCPPB 549, respectively. These two subspecies could not be differentiated from each other by means of API systems. Others have also pointed to the high relatedness between *Er. carotovora* subsp. *carotovora* and subsp. *atroseptica* (Dye 1969*a*, 1981; Brenner *et al.*, 1973; Murata & Starr, 1974). We recommend that the use of both subspecies *carotovora* and *atroseptica* be discontinued. The three strains UCPPB 188, UCPPB 193 and NCPPB 2792 isolated from sugarbeets and labelled *Er. carotovora* subsp. *atroseptica* formed the separate subphenon K1. For these strains the name *Er. carotovora* subsp. *betavasculorum* has been proposed and UCPPB 193 (= NCPPB 2795) was designated as type strain (Thomson *et al.*, 1981). We adopt the view of Thomson *et al.* (1981) that the sugarbeet isolates should constitute a separate taxon.

Phenon L contained 12 *Er. chrysanthemi* strains, including the type strain NCPPB 402. *Er. chrysanthemi* has also been recognized as a separate taxon by DNA:DNA hybridization (Murata & Starr, 1974) and numerical taxonomy (Dye, 1981). The type strain of *Er. paradisiaca*, NCPPB 2511, was also included in phenon L, confirming the findings of Dickey & Victoria (1980).

Our numerical analyses give further support for the retention of the following species as separate taxa: *Er. quercina*, *Er. nigrifluens*, *Er. salicis*, *Er. amylovora*, *Er. rubrifaciens*, *Er. mallotivora*, *Er. stewartii*, *Er. cypripedii* and *Er. chrysanthemi*. On the other hand the results support the synonymy of the following pairs of taxa: *Er. ananas* and *Er. uredovora*, *Er. dissolvens* and *En. cloacae*, *Er. carotovora* subsp. *atroseptica* and *Er. carotovora* subsp. *carotovora*, *Er. milletiae* and one of the *Er. herbicola* clusters (subphenon F2). The heterogeneity of the '*Er. herbicola–En. agglomerans* complex' is confirmed.

Applicability of API systems for the classification and identification of Erwinia strains

The API systems constitute a fast alternative to conventional phenotypical testing procedures in classificatory surveys. The API 20E and API 50CHE systems were found to have the highest discriminatory potential and reproducibility within *Erwinia*. In the API 20E Analytical Profile Index, strains labelled *Er. cypripedii*, *Er. stewartii*, *Er. rhapontici*, *Er. herbicola*, *Er. milletiae*, *Er. uredovora*, *Er. ananas*, *Er. chrysanthemi*, *Er. carotovora* and *Es. adecarboxylata* were assigned on the basis of their API 20E seven-digit profile numbers to *En. agglomerans* or possible *Erwinia* species, thus reflecting the inadequacy of the present classification of the '*Er. herbicola–En. agglomerans* complex'. Other strains labelled *Er. ananas*, *Er. carotovora*, *Er. herbicola*, *Er. rhapontici* or *Er. uredovora* were assigned in the API 20E Analytical Profile Index to *Klebsiella* species or *Serratia* species. The seven-digit profile numbers of *Er. amylovora*, *Er. quercina*, *Er. nigrifluens*, *Er. salicis*, *Er. rubrifaciens*, *Er. mallotivora* and *Er. tracheiphila* are specific but were not listed in the API 20E Analytical Profile Index, which is mainly based on data obtained from clinical enterobacteria. They also remained unidentified after consultation of the API computer service. From the enzymic API systems, the 'OSIDASES' appeared to be the most promising for identification. Of the 58 substrates contained in the AMINOPEPTIDASES systems, 5 proved to be useful within *Erwinia* (see Table 2). Both the API ZYM and ESTERASES systems seemed to be of negligible discriminatory value within *Erwinia*. In general, our data agree well with those obtained by Godsey *et al.* (1981) and support the discriminative value of the following substrates for *Enterobacteriaceae*: L-prolyl-2-naphthylamide, L-hydroxyprolyl-2-naphthylamide, L-pyrrolidonyl-2-naphthylamide, *p*-nitrophenyl-β-D-xylopyranoside, *p*-nitrophenyl-β-D-glucopyranoside and *p*-nitrophenyl-α-L-arabinofuranoside.

J.D.L. is indebted to the Instituut tot Aanmoediging van het Wetenschappelijk Onderzoek in Nijverheid en Landbouw (IWONL, Belgium) and the Fonds voor Kollektief Fundamenteel Onderzoek (FKFO, Belgium) for research and personnel grants. J.M. and L.V. are indebted to the IWONL for scholarships, and J.S. and K.K. to the Nationaal Fonds voor Wetenschappelijk Onderzoek (NFWO, Belgium) for equipment grants.

REFERENCES

ALDRIDGE, K. E., GARDNER, B. B., CLARK, S. S. & MATSEN, J. M. (1978). Comparison of Micro-ID, API 20E, and conventional media systems in identification of Enterobacteriaceae. *Journal of Clinical Microbiology* 7, 507–513.

ANONYMOUS (1977). *Catalogue of Cultures in the National Collection of Plant Pathogenic Bacteria*, 1st edn. Harpenden: Ministry of Agriculture, Fisheries and Food.

AZAD, H. R. & KADO, C. I. (1980). Numerical and DNA:DNA reassociation analyses of *Erwinia rubrifaciens* and other members of the *Enterobacteriaceae*. *Journal of General Microbiology* **120**, 117–129.

BLACKALL, P. J. (1980). Evaluation of a multi-test microtube system for the identification of veterinary isolates of Enterobacteriaceae. *Veterinary Microbiology* **5**, 229–237.

BLAZEVIC, D. J., TROMBLEY, C. M. & LUND, M. E. (1976). Inoculation of API-20E from positive blood cultures. *Journal of Clinical Microbiology* **4**, 522–523.

BRENNER, D. J. (1981). The genus *Enterobacter*. In *The Procaryotes*, vol. II, pp. 1173–1180. Edited by M. P. Starr, H. Stolp, H. G. Trüper, A. Balows & H. G. Schlegel. Berlin, Heidelberg & New York: Springer-Verlag.

BRENNER, D. J., FANNING, G. R. & STEIGERWALT, A. G. (1972). Deoxyribonucleic acid relatedness among species of *Erwinia* and between *Erwinia* species and other enterobacteria. *Journal of Bacteriology* **110**, 12–17.

BRENNER, D. J., STEIGERWALT, A. G., MIKLOS, G. V. & FANNING, G. R. (1973). Deoxyribonucleic acid relatedness among erwiniae and other Enterobacteriaceae: the soft-rot organisms (genus *Pectobacterium* Waldee). *International Journal of Systematic Bacteriology* **23**, 205–216.

BRENNER, D. J., FANNING, G. R. & STEIGERWALT, A. G. (1974). Deoxyribonucleic acid relatedness among erwiniae and other Enterobacteriaceae: the gall, wilt, and dry-necrosis organisms (genus *Erwinia* Winslow *et al.*, *sensu stricto*). *International Journal of Systematic Bacteriology* **24**, 197–204.

BRENNER, D. J., FANNING, G. R., LEETE KNUTSON, J. K., STEIGERWALT, A. G. & KRICHEVSKY, M. I. (1984). Attempts to classify Herbicola group – *Enterobacter agglomerans* strains by deoxyribonucleic acid hybridization and phenotypic tests. *International Journal of Systematic Bacteriology* **34**, 45–55.

BUTLER, D. A., LOBREGAT, C. M. & GAVAN, T. L. (1975). Reproducibility of the Analytab (API 20E) system. *Journal of Clinical Microbiology* **2**, 322–326.

DE SMEDT, J. & DE LEY, J. (1979). Identification of Ruiter's strains, isolated from browned marinated herring, as members of *Erwinia herbicola*. *International Journal of Systematic Bacteriology* **29**, 183–187.

DEVENISH, J. A. & BARNUM, D. A. (1980). Evaluation of API 20E system and Encise Enterotube for the identification of Enterobacteriaceae of animal origin. *Canadian Journal of Comparative Medicine* **44**, 315–319.

DE VOS, P., KERSTERS, K. & DE LEY, J. (1980). Identification of the leaf nodule bacterial strain PeH_220 as *Erwinia herbicola* subsp. *herbicola*. *Zentralblatt für Bakteriologie, Mikrobiologie und Hygiene* (*Abteilung I, Originale C*) **1**, 237–242.

DICKEY, R. S. & VICTORIA, J. L. (1980). Taxonomy and emended description of strains of *Erwinia* isolated from *Musa paradisiaca* Linnaeus. *International Journal of Systematic Bacteriology* **30**, 129–134.

DYE, D. W. (1964). The taxonomic position of *Xanthomonas trifolii* (Huss, 1907) James, 1955. *New Zealand Journal of Science* **7**, 261–269.

DYE, D. W. (1968). A taxonomic study of the genus *Erwinia*. I. The '*amylovora*' group. *New Zealand Journal of Science* **11**, 590–607.

DYE, D. W. (1969*a*). A taxonomic study of the genus *Erwinia*. II. The '*carotovora*' group. *New Zealand Journal of Science* **12**, 81–97.

DYE, D. W. (1969*b*). A taxonomic study of the genus *Erwinia*. III. The '*herbicola*' group. *New Zealand Journal of Science* **12**, 223–236.

DYE, D. W. (1969*c*). A taxonomic study of the genus *Erwinia*. IV. 'Atypical' erwinias. *New Zealand Journal of Science* **12**, 833–839.

DYE, D. W. (1981). A numerical taxonomic study of the genus *Erwinia*. *New Zealand Journal of Agricultural Research* **24**, 223–229.

EWING, W. H. & FIFE, M. A. (1972). *Enterobacter agglomerans* (Beijerinck) comb. nov. (the herbicola-lathyri bacteria). *International Journal of Systematic Bacteriology* **22**, 4–11.

FRANK, S. K. & GERBER, J. D. (1981). Hydrolytic enzymes of *Moraxella bovis*. *Journal of Clinical Microbiology* **13**, 269–271.

FREEMAN, J. W., ROWLAND, R. W., OVERMAN, S. B. & GOODMAN, N. L. (1981). Laboratory evaluation of the AutoMicrobic system for identification of Enterobacteriaceae. *Journal of Clinical Microbiology* **13**, 895–898.

GARDNER, J. M. & KADO, C. I. (1972). Comparative base sequence homologies of the deoxyribonucleic acids of *Erwinia* species and other Enterobacteriaceae. *International Journal of Systematic Bacteriology* **22**, 201–209.

GAUTHIER, M. J. (1976). *Alteromonas rubra* sp. nov., a new marine antibiotic-producing bacterium. *International Journal of Systematic Bacteriology* **26**, 459–466.

GAVINI, F., LEFEBVRE, B. & LECLERC, H. (1983). Étude taxonomique de souches appartenant ou apparentées au genre *Erwinia*, groupe *herbicola*, et à l'espèce *Enterobacter agglomerans*. *Systematic and Applied Microbiology* **4**, 218–235.

GODSEY, J. M., MATTEO, M. R., SHEN, D., TOLMAN, G. & GOHLKE, J. R. (1981). Rapid identification of Enterobacteriaceae with microbial enzyme activity profiles. *Journal of Clinical Microbiology* **13**, 483–490.

GOTO, M. (1976). *Erwinia mallotivora* sp. nov., the causal organism of bacterial leaf spot of *Mallotus japonicus* Muell. Arg. *International Journal of Systematic Bacteriology* **26**, 467–473.

GUILLERMET, F. (1980). '*Fusobacterium fusiforme*' et '*F. nucleatum*'. *Annales de microbiologie* **131**, 95–96.

HAAHTELA, K., WARTIOVAARA, T., SUNDMAN, V. & SKUJINS, J. (1981). Root-associated N_2 fixation (acetylene reduction) by Enterobacteriaceae and *Azospirillum* strains in cold-climate spodosols. *Applied and Environmental Microbiology* **41**, 203–206.

HOLMES, B., WILLCOX, W. R., LAPAGE, S. P. & MALNICK, H. (1977). Test reproducibility of the API

(20E), Enterotube and Pathotec systems. *Journal of Clinical Pathology* **30**, 381–387.

HOLMES, B., WILLCOX, W. R. & LAPAGE, S. P. (1978). Identification of Enterobacteriaceae by the API 20E system. *Journal of Clinical Pathology* **31**, 22–30.

HOLMES, B., OWEN, R. J. & WEAVER, R. E. (1981). *Flavobacterium multivorum*, a new species isolated from human clinical specimens and previously known as group IIk, biotype 2. *International Journal of Systematic Bacteriology* **31**, 21–34.

HOLMES, B., OWEN, R. J. & HOLLIS, D. G. (1982). *Flavobaterium spiritivorum*, a new species isolated from human clinical specimens. *International Journal of Systematic Bacteriology* **32**, 157–165.

HUMBLE, M. W., KING, A. & PHILLIPS, I. (1977). API ZYM: a simple rapid system for the detection of bacterial enzymes. *Journal of Clinical Pathology* **30**, 275–277.

IMBS, M. A. (1974). *Contribution à l'étude de la taxonomie des bactéries du genre Erwinia*. PhD thesis, Nancy, France.

INGLEDEW, W. M., SIVASWAMY, G. & BURTON, J. D. (1980). The API 20E microtube system for rapid identification of Gram negative brewery bacteria. *Journal of the Institute of Brewing* **86**, 165–168.

KILIAN, M. (1978). Rapid identification of Actinomycetaceae and related bacteria. *Journal of Clinical Microbiology* **8**, 127–133.

LELLIOTT, R. A. (1974). Genus XII. *Erwinia*. In *Bergey's Manual of Determinative Bacteriology*, 8th edn., pp. 332–340. Edited by R. E. Buchanan & N. E. Gibbons. Baltimore: Williams & Wilkins.

LELLIOTT, R. A. & DICKEY, R. S. (1984). Genus VII. *Erwinia*. In *Bergey's Manual of Systematic Bacteriology*, pp. 469–476. Edited by N. R. Krieg. Baltimore: Williams & Wilkins.

LJUNGH, Å., POPOFF, M. & WADSTRÖM, T. (1977). *Aeromonas hydrophila* in acute diarrheal disease: detection of enterotoxin and biotyping of strains. *Journal of Clinical Microbiology* **6**, 96–100.

LOGAN, N. A., CAPEL, B. J., MELLING, J. & BERKELEY, R. C. W. (1979). Distinction between emetic and other strains of *Bacillus cereus* using the API system and numerical methods. *FEMS Microbiology Letters* **5**, 373–375.

MERGAERT, J., KERSTERS, K. & DE LEY, J. (1983*a*). Numerical comparison of protein electrophoregrams from strains of *Enterobacter agglomerans* (*Erwinia herbicola*) and allied species. In *Gram Negative Bacteria of Medical and Public Health Importance: Taxonomy–Identification–Applications*, p. 171. Edited by H. Leclerc. Paris: Éditions INSERM.

MERGAERT, J., GAVINI, F., KERSTERS, K., LECLERC, H. & DE LEY, J. (1983*b*). Phenotypic and protein electrophoretic similarities between strains of *Enterobacter agglomerans*, *Erwinia herbicola*, and *Erwinia milletiae* from clinical or plant origin. *Current Microbiology* **8**, 327–331.

MONGET, D. (1978). *Mise au point d'une microméthode de détection et de mesure d'activités enzymatiques (API ZYM). Résultats obtenus dans differents domaines d'application*. PhD thesis, Lyon, France.

MURATA, N. & STARR, M. P. (1974). Intrageneric clustering and divergence of *Erwinia* strains from plants and man in the light of deoxyribonucleic acid segmental homology. *Canadian Journal of Microbiology* **20**, 1545–1565.

MURRAY, P. R. (1978). Standardization of the Analytab Enteric (API 20E) system to increase accuracy and reproducibility of the test for biotype characterization of bacteria. *Journal of Clinical Microbiology* **8**, 46–49.

MUTIMER, M. D. & WOOLCOCK, J. B. (1982). API-ZYM for identification of *Corynebacterium equi*. *Zentralblatt für Bakteriologie, Mikrobiologie und Hygiene (Abteilung I., Originale C)* **3**, 410–415.

NEILSON, A. H. & SPARELL, L. (1976). Acetylene reduction (nitrogen fixation) by Enterobacteriaceae isolated from paper mill process waters. *Applied and Environmental Microbiology* **32**, 197–205.

O'DONNELL, A. G., NORRIS, J. R., BERKELEY, R. C. W., CLAUS, D., KANEKO, T., LOGAN, N. A. & NOZAKI, R. (1980). Characterization of *Bacillus subtilis*, *Bacillus pumilis*, *Bacillus licheniformis*, and *Bacillus amyloliquefaciens* by pyrolysis gas–liquid chromatography, deoxyribonucleic acid–deoxyribonucleic acid hybridization, biochemical tests, and API systems. *International Journal of Systematic Bacteriology* **30**, 448–459.

ROBERTS, P. (1974). *Erwinia rhapontici* (Millard) Burkholder associated with pink grain of wheat. *Journal of Applied Bacteriology* **37**, 353–358.

RUTHERFORD, I., MOODY, V., GAVAN, T. L., AYERS, L. W. & TAYLOR, D. L. (1977). Comparative study of three methods of identification of Enterobacteriaceae. *Journal of Clinical Microbiology* **5**, 458–464.

SAKAZAKI, R., TAMURA, K., JOHNSON, R. & COLWELL, R. R. (1976). Taxonomy of some recently described species in the family Enterobacteriaceae. *International Journal of Systematic Bacteriology* **26**, 158–179.

SCHWAN, O., NORD, C. E. & HOLMBERG, O. (1979). Biochemical characterization of unidentified microaerophilic cocci isolated from heifer and dry-cow mastitis. *Journal of Clinical Microbiology* **10**, 622–627.

SERRANO, F. B. (1928). Bacterial fruitlet brown-rot of pineapple in the Philippines. *Philippine Journal of Science* **36**, 271–305.

SKERMAN, V. B. D., MCGOWAN, V. & SNEATH, P. H. A. (1980). Approved lists of bacterial names. *International Journal of Systematic Bacteriology* **30**, 225–420.

SNEATH, P. H. A. & SOKAL, R. R. (1973). *Numerical Taxonomy. The Principles and Practice of Numerical Classification*. San Francisco: W. H. Freeman.

STARR, M. P. (1981). The genus *Erwinia*. In *The Procaryotes*, vol. II, pp. 1260–1271. Edited by M. P. Starr, H. Stolp, H. G. Trüper, A. Balows & H. G. Schlegel. Berlin, Heidelberg & New York: Springer Verlag.

STARR, M. P. & CHATTERJEE, A. K. (1972). The genus *Erwinia*: enterobacteria pathogenic to plants and animals. *Annual Review of Microbiology* **26**, 389–426.

THARAGONNET, D., SISSON, P. R., ROXBY, C. M., INGHAM, H. R. & SELKON, J. B. (1977). The API ZYM system in the identification of Gram-negative anaerobes. *Journal of Clinical Pathology* **30**, 505–509.

THOMSON, S. V., HILDEBRAND, D. C. & SCHROTH, M. N. (1981). Identification and nutritional differentiation of the *Erwinia* sugar beet pathogen from members of *Erwinia carotovora* and *Erwinia chrysanthemi*. *Phytopathology* **71**, 1037–1042.

VAN VUUREN, H. J. J., KERSTERS, K., DE LEY, J., TOERIEN, D. F. & MEISEL, R. (1978). *Enterobacter agglomerans* – a new bacterial contaminant isolated from lager beer breweries. *Journal of the Institute of Brewing* **84**, 315–317.

WALDEE, E. L. (1945). Comparative studies of some peritrichous phytopathogenic bacteria. *Iowa State College Journal of Science* **19**, 435–484.

WESTLEY, J. W., ANDERSON, P. J., CLOSE, V. A., HALPERN, B. & LEDERBERG, E. M. (1967). Aminopeptidase profiles of various bacteria. *Applied Microbiology* **15**, 822–825.

WISHART, D. (1978). *Clustan. User Manual*, 3rd edn. Program Library Unit. Edinburgh University, Edinburgh.

YOUNG, J. M., DYE, D. W., BRADBURY, J. F., PANAGOPOULOS, C. G. & ROBBS, C. F. (1978). A proposed nomenclature and classification for plant pathogenic bacteria. *New Zealand Journal of Agricultural Research* **21**, 153–177.