Taxonomy of the Genus *Serratia*

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SUMMARY

One hundred and fifty-six strains of *Serratia* and related bacteria including representatives of *Enterobacter liquefaciens*, *Enterobacter cloacae*, *Enterobacter aerogenes*, *Erwinia carotovora*, *Erwinia chrysanthemi*, *Erwinia herbicola* and *Erwinia nimipressuralis* were studied using 223 morphological, physiological, biochemical and carbon source utilization tests. The results were subjected to computer analysis. At the 80 % similarity level all strains, except two, grouped into eight phenons representing: (A) *Serratia marcescens* with the neotype CCM303 (ATCC13880); (B) *S. marinorubra* with the monotype NCTC10912 (ATCC27614); (C1) *S. liquefaciens* with the type ATCC14460; (C2) *S. plymuthica* with the monotype CCM640 (ATCC183); (D) *Erwinia herbicola* with the neotype of *Enterobacter agglomerans* NCTC9381; (E) *Enterobacter cloacae* with the neotype NCTC10005 and *Erwinia nimipressuralis*; (F) *Erwinia carotovora* with the type ATCC495, *Erwinia atrosepica* and *Erwinia chrysanthemi*; (G) *Klebsiella mobilis* with the neotype NCTC10006. At the 70 % similarity level the phenons formed two groups: (A, B, C1, C2) and (D, E, F, G).

The following conclusions were drawn. (1) There are three species of enterobacteria producing prodigiosin: *S. marcescens*, *S. plymuthica* and *S. marinorubra*. (2) There are four species of *Serratia*, one colourless (*S. liquefaciens*). (3) Subphenons (biovars) are described within the four species of *Serratia*. (4) Non-pigmented wild-type strains of *S. marcescens* can generally be differentiated from pigmented strains by characters other than pigmentation, because subphenons are homogeneous with respect to pigmentation.

This survey raised some problems of nomenclature because old descriptions could be found that could loosely fit the present phenons. Comparison with an authentic culture was considered to be the most objective way of identifying these phenons with earlier named species.

INTRODUCTION

The taxonomy of the genus *Serratia* is still confused. At least 42 species names have been associated with the generic name *Serratia*. Since the early work of Hefferan (1903) several taxonomists have tried to simplify the taxonomy of red-pigmented organisms. The greatest simplification was achieved by Ewing, Davis & Reavis (1959), Ewing, Davis & Johnson (1962) and Martinec & Kocur (1960, 1961 *a, b, c, d*). *Serratia* was then a monospecific genus. Besides *S. marcescens*, *S. marcescens* var. *kiliensis* was retained only because of its negative Voges Proskauer reaction. The only species accepted in the 8th edition of *Bergey's Manual of Determinative Bacteriology* (Buchanan & Gibbons, 1974) is *S. marcescens*. Evidence that there was more than one species of *Serratia* (Brisou & Cadeillan, 1959; Fulton, Forney &

Table 1. *Strains studied*

Strains from clinical material are from human sources unless otherwise noted.

Phenon	Strain no.	Source* and collection no.	Names as received and comments
A	4	Le Minor 18-65	Urine, Pontoise, 1965
	5	Le Minor 23-65	Sputum, Lyon, 1965
	12	Le Minor 19-66	Blood, Nantes, 1966
	16	Brisou	Pig
	57	Hôp. Pellegrin	Blood, urology ward, 1968
	60	Hôp. Pellegrin	Urine, urology ward, 1968
	204	Lab. Municipal	Effluent water from a distillery, Coutras, 1970
	207	Hôp. Militaire	Pus from wound, 1969
	208	Lab. Municipal	Well water, Saint-André-de-Cubzac, 1970
	222	Lab. Municipal	Spring water, Haux, 1971
	223	Lab. Municipal	Water from a 165 m boring
	225	Lab. Municipal	Water, 1971
	241	Le Minor 8-60	<i>S. marcescens</i> var. <i>kiliensis</i> , ATCC990
	242	Le Minor 25-60	<i>S. marcescens</i> var. <i>kiliensis</i> , CDC574-60
	243	Le Minor 15-61	Faeces, Strasbourg, 1961
	245	Le Minor 29-62	Pasteur Institute, Lyon, 1962
	246	Le Minor 43-62	Lepidopteran, Saint-Christol-les-Ales, 1962
	247	Le Minor 47-62	Insect, Saint-Christol-les-Ales, 1962
	248	Le Minor 62-62	Urine, Pasteur Institute, Lyon, 1962
	249	Le Minor 1-63	Blood, Nantes, 1963
	250	Le Minor 12-63	Urine, Paris, 1963
	251	Le Minor 27-65	Blood, Paris, 1965
	252	Le Minor 28-65	' <i>Serratia</i> intermédiaire', Chabbert strain CLB
	253	Le Minor 2-66	Urine, Pasteur Institute, Lyon, no. 65-083-4031
	254	Le Minor 17-66	Urine, Saint-Etienne, 1966
	255	Le Minor 20-66	Urine, Paris, 1966
	256	Le Minor 27-66	Urine, Nantes, 1966
	257	Le Minor 20-67	Running oedema, Saint-Etienne, 1967
	258	Le Minor 25-67	Pork sparerib, Bar-le-Duc, 1967
	259	Le Minor 20-68	Urine, Saint-Etienne, 1968
	260	Le Minor 26-68	Pasteur Hospital, Paris, 1968
	261	Le Minor 33-68	Blood, Suresnes, 1968
	263	Le Minor 27-69	Cerebrospinal fluid, Algiers, 1969
	265	Le Minor 24-70	Blood, Firminy, 1970
	266	Le Minor 28-70	Blood, Arras, 1970
	267	Le Minor 36-70	Faeces, Paris, 1970
	268	Le Minor 2-71	Lymphatic fluid, Dijon, 1971
	269	Le Minor 6-71	—
	284	CIP53-85	<i>S. marcescens</i> var. <i>kiliensis</i> , ATCC990, Breed BF
	285	CIP53-89	<i>S. indica</i> , ATCC4003, Breed CU37
	286	CIP53-100	<i>S. marcescens</i> , ATCC8195, Breed HY
	287	Sneath D106	Fulton 2690, <i>Serratia</i> pattern 1, pus, 1947
	290	Sneath D318	ATCC274, NCTC1377, <i>S. marcescens</i> working type (Sneath & Skerman, 1966)
	292	Brisou 4413	Sea water, Ré island
	293	Brisou 5645	River water (Vienne)
	295	Brisou 5568	River water (Vienne)
	296	Brisou 5751	River water, Anglin, DSM30126, centrotyp
	298	Brisou 6647	—
	323	Lab. Municipal	River water, Courpiac, 1971
	324	Lab. Municipal	Spring water, 1971
	326	Lab. Municipal	Sea water, Naujac, 1971
	327	Lab. Municipal	Water from a 30 m boring
	328	Lab. Municipal	River water, 1971
	354	Lab. Municipal	Water from a 640 m boring, Prayssas, 1971
	355	Lab. Municipal	Drinking water from a ship, 1971
	394	Le Minor 3-71	Blood, Lisieux, 1971
	395	Le Minor 14-71	Blood, Saint-Claude, 1971
	449	Veterinary lab.	Piglet, Dax, 1972
	481	Hôp. Pellegrin	' <i>Enterobacter liquefaciens</i> ', blood, 1972
	504	CCM303	ATCC13880, pond water, neotype of <i>S. marcescens</i> (Martinec & Kocur, 1961a)
	506	CCM377	Eltinge 12
A2	Hôp. Pellegrin		' <i>Enterobacter aerogenes</i> ', pus, surgery ward, 972

Table 1 (cont.)

Phenon	Strain no.	Source* and collection no.	Names as received and comments
B	20	Brisou 90	<i>S. plymuthicum</i> , pig
	22	Brisou 658	<i>S. kiliensis</i> , sea water, La Rochelle, NCTC10847
	36	Santé Navale	<i>Serratia</i> , cultivated mushroom, 1964
	37	Santé Navale	<i>Serratia</i> , cultivated mushroom, 1964, DSM30124, centrototype
	39	Santé Navale	<i>Serratia</i> , cultivated mushroom, 1966, NCTC10848
	224	Lab. Municipal	<i>Serratia</i> , well water, Camarsac, 1971
	230	I.P. Tunis 8376	<i>Serratia</i> , 1971
	231	I.P. Tunis	<i>Serratia</i> , 1971
	240	Le Minor 4-60	Atypical <i>Serratia</i> , ATCC268, Hefferan 'B. prodigiosus VIII'
	262	Le Minor 36-68	Atypical <i>Serratia</i> , animal faeces, Tangier, 1968
	288	Sneath D119	<i>S. marinorubra</i> , ZoBell S11, sea water, monotype, NCTC10912, ATCC27614
	291	Brisou 641	<i>Serratia</i> , water, La Rochelle
	294	Brisou 5648	<i>Serratia</i> , river water (Vienne)
	297	Brisou 6033	<i>Serratia</i> , river water in Savoy
	377	Lapage T37/66	<i>Serratia</i> sp. (biotype 2), pig faeces
	378	Lapage T40/66	<i>Serratia</i> sp. (biotype 2), sewer swab
	393	Le Minor 34-70	<i>Serratia</i> , throat swab, Creteil, 1970
	421	I.P. Tunis	<i>Serratia</i> , 1972
	511	CCM788	<i>S. marcescens</i> , Insect, Berkeley
	513	NCPPB1126	<i>S. marcescens</i> , Hayward B717, <i>Solanum tuberosum</i> , 1961
	530	Matsumoto	' <i>Serratia</i> resembling phenon B' (Grimont, 1969), sewage
	540	Le Minor 34-70	<i>S. rubidaea</i> (variant of no. 393)
C1	9	Le Minor 10-66	<i>Serratia</i> , French sausage, Lyon, 1966, NCTC10862, CCM2715
	221	Lab. Municipal	<i>Serratia</i> , school drinking water, 1971, CCM2716
	237	Lab. Municipal	<i>Serratia</i> , well water, 1971
	239	Lab. Municipal	<i>Serratia</i> , water, 1971
	271	Le Minor 3-61	<i>Enterobacter liquefaciens</i> , contaminated serum, 1961
	272	Le Minor 5-61	<i>E. liquefaciens</i> , blood, Rodez, 1961
	274	Le Minor 4-68	<i>E. liquefaciens</i> , CDC1105-51
	275	Le Minor 5-68	<i>E. liquefaciens</i> , CDC6136-66, CCM2717, DSM30125, centrototype
	276	Le Minor 6-68	<i>E. liquefaciens</i> , CDC6135-66
	277	Le Minor 7-68	<i>E. liquefaciens</i> , CDC6137-66
	278	Le Minor 8-68	<i>E. liquefaciens</i> , CDC2224-64
	279	Le Minor 9-68	<i>E. liquefaciens</i> , CDC5155-57
	280	Le Minor 10-68	<i>E. liquefaciens</i> , CDC1284-57, ATCC27592 proposed neotype of <i>Serratia liquefaciens</i> (Ewing <i>et al.</i> , 1973)
	281	Le Minor 11-68	<i>E. liquefaciens</i> , CDC2138-51
	282	Le Minor 12-68	<i>E. liquefaciens</i> , CDC4733-51
	283	Le Minor 13-70	<i>E. liquefaciens</i> , veal, 1970
	318	Le Minor 2-71	<i>E. liquefaciens</i> , blood, Paris, 1971
	319	Le Minor 4-71	<i>E. liquefaciens</i> , urine, Tangier, 1971
	390	Lab. Municipal	<i>Serratia</i> , water, Le Barp, 1972, CCM2718
	503	ATCC14460	<i>Aerobacter lipolyticus</i> , Grimes, cotype (Sneath & Skerman, 1966, and see Grimes, 1961; Grimes & Hennerty, 1931; American Type Culture Collection, 1964)
	507	CCM400	<i>S. marcescens</i> , CCEB213, Carpenter bee <i>Megachile</i> sp.
	508	CCM412	<i>S. marcescens</i> , CCEB309, Elm borer <i>Saperdia carcharias</i>
	509	CCM455	<i>S. marcescens</i> , CCEB308, Elm borer <i>Saperdia carcharias</i>
	514	CCEB006	<i>S. marcescens</i> var. <i>noctuarum</i> , from silkworm <i>Bombyx mori</i>
C2	33	Brisou 3617	<i>S. plymuthicum</i> , sea water, Oléron
	34	Brisou 3736	<i>S. plymuthicum</i>
	203	Lab. Municipal	<i>Serratia</i> , water, 1970
	235	Lab. Municipal	<i>Serratia</i> , water, 1971
	238	Lab. Municipal	<i>Serratia</i> , water, 1971
	273	Le Minor 22-61	'Atypical <i>E. liquefaciens</i> ', Pasteur Institute, Lille
	289	Sneath D317	<i>Serratia</i> , NCTC9493, Barrett REXXIVC, water, 1954
	299	Brisou 6912	<i>Serratia</i> , sea water, Oléron
	325	Lab. Municipal	<i>Serratia</i> , spring water, Creysse, 1971
	329	Lab. Municipal	<i>Serratia</i> , well water, Izon, 1971
	392	Lab. Municipal	<i>Serratia</i> , spring water, Bergerac, 1971, DSM30127, centrototype
	410	Lab. Municipal	<i>Serratia</i> , water supply, 1972
	500	ATCC992	<i>S. kiliensis</i> , ' <i>B. ruber</i> Kiel isolated by Lubinsky, Kiew'

Table 1 (cont.)

Phenon	Strain no.	Source* and collection no.	Names as received and comments
	501	ATCC4261	<i>S. kiliensis</i> , Kral collection through Novy, 'Probably an authentic strain of Breunig's original culture isolated from Kiel water, in 1888'
	502	ATCC7462	<i>S. marcescens</i> var. <i>kiliensis</i> proposed neotype (Martinec & Kocur, 1961d)
	510	CCM640	<i>S. plymuthica</i> , ATCC183, 'Original strain of Fischer, isolated by him from water supply of Plymouth, England, in 1887'
	512	NCIB8616	<i>S. kiliensis</i> , Breed K6 (same source as strain 500)
	515	NCIB4612	<i>Chromobacterium prodigiosum</i> , strain 'Norfolk Broads'
	516	NCIB8266	<i>Chromobacterium prodigiosum</i> , strain 82B, soil
	517	NCIB8285	<i>Chromobacterium prodigiosum</i> , canal water
D	E2	Hôp. Pellegrin	<i>Erwinia herbicola</i> , blood, 1971
	E15	Lab. Municipal	<i>Erwinia herbicola</i> , barley
	E16	Lab. Municipal	<i>Erwinia herbicola</i> , spring water
	E20	NCTC9381	<i>Chromobacterium typhiflavum</i> , laceration of knee, centrotypotype, neotype of <i>Enterobacter agglomerans</i> (Ewing & Fife, 1972)
	E23	Hôp. Pellegrin	<i>Erwinia herbicola</i> , pus, surgery ward, 1972
E	E62	NCPPB440	<i>Erwinia nimipressuralis</i> , Carter, <i>Ulmus</i> sp., 1946
	E63	NCPPB2045	<i>Erwinia nimipressuralis</i> , Carter, <i>Ulmus</i> sp., ATCC9912, cotype (Sneath & Skerman, 1966)
	C1	CIP6085	NCTC10005, ATCC13047, neotype of <i>Enterobacter cloacae</i> (Hormaeche & Edwards, 1960)
	C2	CIP57-33	NCTC8155, yellow pigmented <i>Enterobacter cloacae</i>
	C3	CIP68-1	<i>Enterobacter cloacae</i> , blood, Paris
	C4	Hôp. Pellegrin	<i>Enterobacter cloacae</i> , pus, surgery ward, 1972, centrotypotype
	C5	Hôp. Pellegrin	<i>Enterobacter cloacae</i> , urine, 1972
F	E25	ATCC495	<i>Erwinia carotovora</i> , soft rot of carrot, Jones, cotype (Sneath & Skerman, 1966)
	E26	NCPPB312	<i>Erwinia carotovora</i> , from <i>Solanum tuberosum</i>
	E27	NCPPB453	<i>Erwinia chrysanthemi</i> , from <i>Dianthus caryophyllus</i>
	E28	NCPPB547	<i>E. carotovora</i> var. <i>carotovora</i> , from <i>Persea americana</i> , centrotypotype
	E29	NCPPB549	<i>E. carotovora</i> var. <i>atroseptica</i> , from <i>Solanum tuberosum</i>
	E30	NCPPB929	<i>E. carotovora</i> var. <i>carotovora</i> , from <i>Zantedeschia aethiopica</i>
	E31	NCPPB1065	<i>Erwinia chrysanthemi</i> , from <i>Zea mays</i>
	E32	NCPPB1066	<i>Erwinia chrysanthemi</i> , from <i>Zea mays</i>
	E33	NCPPB1278	<i>E. carotovora</i> var. <i>atroseptica</i> , soil
	E34	NCPPB1590	<i>E. carotovora</i> var. <i>atroseptica</i> , soil
G	A1	CIP60-86	NCTC10006, ATCC13048. Neotype of <i>Enterobacter aerogenes</i> (Hormaeche & Edwards, 1960) and of <i>Klebsiella mobilis</i> (Bascomb <i>et al.</i> , 1971)
	A3	Hôp. Pellegrin	<i>Enterobacter aerogenes</i> , pus, dermatology ward, 1972
	A4	Hôp. Pellegrin	<i>Enterobacter aerogenes</i> , urine, urology ward, 1972
	A5	Hôp. Pellegrin	<i>Enterobacter aerogenes</i> , pus, haemodialysis unit, 1972, centrotypotype
Unclustered	38	Santé Navale	<i>Serratia</i> , cultivated mushrooms, 1965, as yet unidentified
	236	Lab. Municipal	Atypical <i>Serratia</i> , water, 1971, now identified as <i>Aeromonas hydrophila</i>

* NCIB, National Collection of Industrial Bacteria, Aberdeen, Scotland. ATCC, American Type Culture Collection, Rockville, Maryland, U.S.A. NCTC, National Collection of Type Cultures, London. NCPPB, National Collection of Plant Pathogenic Bacteria, Harpenden. CCM, Czechoslovak Collection of Microorganisms, Brno, Czechoslovakia. CCEB, Czechoslovak Collection of Entomogenous Bacteria, Prague, Czechoslovakia. CIP, Collection de l'Institut Pasteur, Paris, France. Le Minor, Prof. Le Minor, Institut Pasteur, Paris, France. Lapage, Dr S. P. Lapage, Central Public Health Laboratory, London. Brisou, Prof. J. Brisou, Poitiers University, France. Lab. Municipal, Laboratoire Municipal, Bordeaux, France (R. Serceau). Hôp. Pellegrin, Laboratoire de Bactériologie, Hôpital Pellegrin, Bordeaux, France. Santé Navale, Ecole de Santé des Armées, Bordeaux, France. Veterinary Lab., Centre Vétérinaire, Dax, France (Dr Vuillaume). I.P. Tunis, Institut Pasteur de Tunis, Tunis, Tunisia (R. Houissa). Matsumoto, Dr H. Matsumoto, Shinshu University, Matsumoto, Japan. CDC, Center for Disease Control, Atlanta, Georgia, U.S.A. DSM, Deutsche Sammlung von Mikroorganismen, Teilsammlung Weihenstephan, Freising, German Federal Republic.

Leifson, 1959) was not followed up until recently, when Grimont (1969), Bascomb *et al.* (1971) and Grimont & Dulong de Rosnay (1972) produced evidence that red-pigmented enterobacteria constituted more than one species. Although Edwards & Ewing (1972) had recognized only one species in the genus, Ewing, Davis & Fife (1972) and Ewing *et al.* (1973) recognized two species of red-pigmented *Serratia*, *S. marcescens* and *S. rubidaea*.

Relationships between *Serratia* and *Enterobacter liquefaciens* were not clear until Hamon, Le Minor & Peron (1970) showed that *S. marcescens* and *Ent. liquefaciens* were sensitive to the same bacteriocins. Close relationship between these two species was so evident in the work of Bascomb *et al.* (1971) that they transferred *Ent. liquefaciens* to the genus *Serratia*.

The present study was aimed at defining species within *Serratia*, circumscribing this genus and clarifying its nomenclature.

METHODS

Strains. These are listed in Table 1. Most strains showed colonial variation including pigment-less variants during preliminary culture. A colony of the dominant colonial type (a pigmented colony, if present) was selected for study. The strains were maintained on semi-solid nutrient agar containing: meat extract (Leibig), 3 g; yeast extract (Difco), 10 g; agar (BBL), 7.5 g; distilled water to 1 l (pH 7.4).

All tests were inoculated from 2-day-old cultures on nutrient agar (as above, but with agar at 15 g l⁻¹) and incubated at 30 °C unless otherwise stated. Divided Petri dishes ('replidishes'; Sneath & Stevens, 1967) were used wherever possible, with 3 ml medium per compartment unless otherwise stated. Inoculation of replidishes was as described by Stevens (1969).

Colonial and cell morphology. Bacteria were grown on nutrient agar at 30 °C for 2 days. Colonial morphology was examined under a magnifying glass ($\times 3$) and pigment production was noted. Gram-staining was performed at the same time. Odour was noted as being either similar to or different from that of strain 38 (strain 38 had a distinctive odour resembling vegetable matter, unlike the somewhat fishy or faecal odour of most *Serratia* strains).

Growth in nutrient broth. Nutrient broth (of the same formula as nutrient agar but with the agar omitted) was inoculated and incubated for 24 h. Growth was scored as moderate, dense or very dense.

Solubility and spectral properties of red pigments. Bacteria were grown on peptone/glycerol agar [Bacto-peptone (Difco), 5 g; glycerol, 10 ml; agar, 20 g; distilled water, 1 l], harvested and resuspended in 5 ml acetone. Pigment was extracted by the method of Williams, Green & Rappoport (1956). Solubility was noted in petroleum ether (b.p. 40 to 60 °C) and in water. The pigment which was soluble in petroleum ether was taken to dryness and then dissolved in 11 ml ethanol/1 M-HCl (10:1, v/v). Absorption spectra were determined for aqueous and ethanol/HCl solutions from 340 to 650 nm. Pigment of strain NIMA (much used in the study of pigment biosynthesis, kindly supplied by Professor R. P. Williams) was used as a control (maximum absorbance at 535 nm, shoulder at 510 nm in ethanol/HCl).

Growth at different temperatures. Bacteria were incubated in tubes of Trypticase Soy Broth (TSB; BBL) at 5, 10, 20, 37, 40, 42, 45 and 48 °C ± 0.5 °C. Turbidity was scored visually as slight, moderate, dense or very dense for up to 3 days, or up to 10 days, with a photometric reading at 550 nm on the 10th day in the case of growth at 5 °C. Absorbances of less than 0.03 corresponded to visually negative growth. Slight and moderate growth corresponded approximately to absorbances ranging from 0.03 to 0.30.

pH. Ability to grow on TSB at pH 4, 4.5, 5, 5.5 and 9 was recorded every other day for 10 days.

Motility. Tubes containing semi-solid nutrient agar (agar concentration reduced to 0.75 % w/v) were stab inoculated and growth was examined visually for motile swarms for up to 5 days. Motile cultures were stained by the method of Leifson (1960) and examined for flagella at $\times 1000$ magnification.

Growth in the presence of NaCl. Bacteria were incubated in TSB containing 4, 7, 8.5 and 10 % (w/v) NaCl in replidishes. Growth in 4 and 7 % NaCl was examined visually for up to 2 days. Growth in 8.5 and 10 % NaCl was examined visually for up to 10 days and photometrically on the 10th day.

Catalase and oxidase tests. One-day nutrient agar cultures were flooded with H_2O_2 (10 vols) and oxygen evolution was observed; oxidase was tested by the method of Kovacs (1956).

Growth in the presence of KCN. KCN medium (BBL) supplemented with KCN (75 mg l⁻¹) and triphenyltetrazolium chloride (50 mg l⁻¹) was inoculated, layered with paraffin oil and examined after 5 and 10 days for red insoluble formazan. A control medium without KCN was included.

Nitrate reduction. Bacteria were incubated in nitrate broth [Bacto-peptone (Difco), 5 g; glucose, 0.1 g; KNO_3 , 1 g; distilled water, 1 l (pH 7.4)] for 2 days and tested for nitrate by adding Griess-Ilosvay reagents. Negative results were checked by adding zinc powder (ZoBell, 1932).

Growth in the presence of $KClO_3$. VL agar [tryptone (Difco), 10 g; NaCl, 5 g; meat extract (Liebig), 2 g; yeast extract (Difco), 5 g; cysteine hydrochloride, 0.3 g; glucose, 2 g; Bacto-agar (Difco), 6 g; distilled water, 1 l (pH 7.4)] supplemented with 0.1 % (w/v) $KClO_3$ was dispensed into tubes (160 $\times$ 10 mm) to give a column 70 mm deep, and inoculated from an overnight culture in TSB diluted 1:20. A control tube without $KClO_3$ was also inoculated. Nitrate reductase A reduces chlorate anaerobically to hypochlorite which inhibits growth in the depths of the tube, whereas nitrate reductase B does not reduce chlorate, so that growth occurs throughout the tube as in the control (Pichinoty & Piéchaud, 1968). Strains unable to reduce nitrate (though they grew in the presence of $KClO_3$) were scored as 'no comparison'.

Tetrathionate reduction. The liquid medium of Le Minor *et al.* (1970) was sterilized by filtration, and then dispensed into tubes (120 $\times$ 12 mm), inoculated and incubated overnight. Unequivocal acidification was scored as positive. Negative tubes were read again at 2 days.

Voges Proskauer test. Clark and Lubs medium (BBL) was used. The test was carried out in three ways: (i) in test tubes (5 ml) tested for production of acetoin after 3 days incubation by the method of Barritt (1936); (ii) in replidishes (1.5 ml) tested after 3 days by the method of O'Meara (1931); and (iii) in replidishes (0.5 ml) tested after overnight incubation (Richard, 1972) with addition of 0.5 ml α -naphthol solution and 0.5 ml 4 M-NaOH. Acetoin is rapidly produced and detected by Richard's method. Some strains may quickly destroy acetoin and butanediol and thus give a negative reaction after the longer incubation which is necessary with the other two methods. The Richard and O'Meara tests may thus detect different characters of some strains, and so were scored separately.

Methyl red test and final pH measurements. Cultures were grown in Clark and Lubs medium (BBL) in test tubes for 3 days. pH was determined using 'Lyphan' pH indicator paper (G. Klotz, West Berlin). About 0.1 ml of each culture was placed in a well of a microtitre plate and a drop of methyl red solution was added. Positive, doubtful and negative results corresponded respectively to the following pH ranges: 5.2 or below, 5.5 to 5.8, and 6.1 or above.

Gas from glucose. Nutrient agar supplemented with 1 % (w/v) glucose was dispensed into tubes (160 $\times$ 10 mm), inoculated when molten and examined for bubbles of gas for up to 3 days.

Oxidative/fermentative utilization of glucose. The medium and method of Hugh & Leifson (1953) was used.

Production of acid from carbohydrates. To peptone water [Bacto-peptone (Difco), 10 g; NaCl, 5 g; distilled water, 1 l (pH 7.4)] containing bromothymol blue solution (12 ml l⁻¹: bromothymol blue, 0.2 g; 0.1 M-NaOH, 5 ml; distilled water, 95 ml), carbohydrates were added to give a final concentration of 0.5 % (w/v). Replidishes were examined for acidification for up to 5 days.

Gluconate test. Bacteria were incubated in the medium of Haynes (1951) for 2 days and the presence of reducing derivatives was tested by adding a Clinitest tablet (Ames Co., Slough). Colours ranging from light green to rusty yellow were scored as positive whereas a deep blue colour was scored as negative.

Iodoacetate test. The medium of Lysenko (1961) was sterilized by boiling for 30 min on three successive days, then supplemented with sodium iodoacetate (sterilized by filtration) at 208 mg l⁻¹. Cultures in this medium were examined for acid production for 5 days. Controls without iodoacetate were included.

Ammonia from peptone. Peptone water was dispensed into test tubes and inoculated. After 5 days incubation ammonia was detected by a spot test on a filter paper impregnated with Nessler reagent. Production of an orange to brown colour was scored as positive.

Hydrogen sulphide production. Peptone water supplemented with 0.01 % (w/v) L-cysteine hydrochloride (BDH) was inoculated. Lead acetate paper was placed in the lid of the dish to cover all compartments (Stevens, 1969) and was observed for blackening for 5 days. Doubtful results were repeated in tubes with strips of lead acetate paper inserted between the cotton plug and the tube.

Production of indole from peptone. Peptone water was dispensed in tubes and inoculated. Indole was detected with Kovacs' reagent after 1 and 6 days incubation. Positive results with pigmented strains were checked by using Difco indole test strips inserted between the cotton plug and the tube. Some precursors of prodigiosin (either volatile or not) may react with Ehrlich reagent (contained in Kovacs' reagent and on Difco indole test strips), so by 'indole' we mean an 'Ehrlich positive substance'.

Lysine, ornithine and arginine decarboxylases. Møller's medium (Møller, 1955) for decarboxylases (BBL) supplemented with either lysine, ornithine or arginine (1 %, w/v) was inoculated and covered by a layer of paraffin oil. Compartments were examined for alkalization for 4 days. Activity of arginine decarboxylase is not distinguished from that of arginine dihydrolase by this medium.

Deamination of phenylalanine. Medium [yeast extract (Difco), 3 g; DL-phenylalanine, 2 g; Na₂HPO₄, 1 g; NaCl, 5 g; Bacto-agar (Difco), 12 g; distilled water, 1 l] was inoculated and incubated for 2 days. Aqueous ferric chloride solution (13 %, w/v) was then added dropwise to the compartments and development of a green colour was read as positive.

Urease. The liquid medium of Stuart, Van Stratum & Rustigian (1945), sterilized by filtration, was inoculated and examined daily for alkali production for up to 10 days.

Malonate. Bacto-malonate broth (Difco) was inoculated and examined for alkalinity after 4 and 8 days. Cultures giving late positive results were retested in tubes.

Gelatin hydrolysis. Two methods were used. (1) Nutrient gelatin [Bacto-peptone (Difco), 5 g; meat extract (Liebig), 3 g; gelatin, 120 g; distilled water, 1 l] was dispensed in small tubes, inoculated and incubated at 20 °C; liquefaction was recorded after 15 days. (2) Nutrient agar containing 4 % (w/v) gelatin was poured into square Petri dishes (9.5 × 9.5 cm) and inoculated (25 strains per dish). Zones of hydrolysis were detected by flooding with

aqueous mercuric chloride (10 %, w/v) after incubation at 30 °C for 1 day. Negative strains were retested after 4 days incubation.

Chitin hydrolysis. Colloidal chitin was prepared by dissolving 2 g chitin (Fluka, Buchs, Switzerland) in 40 ml cold 11 M-HCl, allowing the solution to stand for 3 h and then filtering it through glass wool. The filtrate was diluted 1:10 in cold distilled water, and the precipitate was washed three times with distilled water, suspended in distilled water, neutralized with 0.1 M-NaOH, and again washed three times with distilled water. The chitin was then suspended in 40 ml distilled water, sterilized by heating at 80 °C for 30 min on three successive days, then added to molten nutrient agar to a final concentration of 4 % (w/v). After inoculation and incubation, plates were examined for up to 5 days for clearing around the growth.

Tributylin hydrolysis. Tributyrin was suspended in nutrient agar following the method of Hugo & Beveridge (1962). After inoculation and incubation, plates were observed for clearing for up to 15 days.

Acid from triacetin. The method of Hugo & Beveridge (1962) was used. Cultures were examined for acidification for up to 5 days.

Tween hydrolysis. Tween 40, 60 and 80 (Sigma), at final concentrations of 1 % (v/v), were added to medium [Bacto-peptone, 10 g; NaCl, 5 g; CaCl₂·H₂O, 0.1 g; agar (BBL), 15 g; distilled water, 1 l (pH 7.4)], and dispensed into square Petri dishes and multi-inoculated (25 strains per plate) with about 0.02 ml of an 18 h culture in TSB. Zones of precipitation were recorded for 5 days.

Lecithinase. A 2 % (w/v) sterile alcoholic solution of lecithin (Sigma) was diluted 1:3 in water, then sonicated briefly. The opalescent emulsion was added to nutrient agar (15 %, v/v) and dispensed as for Tween hydrolysis. Precipitation around colonies was recorded for up to 5 days.

Starch hydrolysis. Nutrient agar containing 0.5 % (w/v) soluble starch (Fluka) was used. Plates were flooded with Lugol's iodine and examined for clear zones after 4 days.

Deoxyribonuclease. Bacteria were grown on DNAase medium (BBL). After 3 days the plates were flooded with 1 M-HCl and inspected for areas of clearing. The test was repeated for negative strains after 5 days incubation.

Pectin hydrolysis. Minimal medium M70 (Véron, 1975) supplemented with 0.5 % (w/v) citrus pectin (Sigma) and 0.1 % (w/v) yeast extract (Difco) was used. After 5 days incubation, plates were flooded with a 1 % (w/v) aqueous solution of Cetrimide and examined for clear zones (Hankin, Zucker & Sands, 1971).

β-Galactosidase. Sterile microtitre plates were used. Overnight cultures on nutrient agar supplemented with 1 % (w/v) lactose were suspended in distilled water and dispensed into the wells in 0.05 ml portions followed by 0.05 ml 0.013 M-*o*-nitrophenyl-β-D-galactopyranoside (Sigma) in 0.25 M-phosphate buffer pH 7 (Le Minor & Ben Hamida, 1962). Cetrimide (0.025 ml; 0.001 %, w/v) was added in place of toluene (Reeves & Braithwaite, 1972). A control well with 0.05 ml distilled water instead of bacteria was included. Plates were incubated and examined for a yellow colour after 90 min. Negative strains were retested in tubes and examined for up to 24 h.

β-Xylosidase. The method of Brisou, Richard & Lenriot (1972) was adapted for use with sterile microtitre plates. Aqueous (1 %, w/v) *p*-nitrophenyl-β-xyloside was dispensed into the wells in 0.05 ml portions, followed by 0.05 ml of a fresh bacterial suspension in 0.25 M-phosphate buffer pH 7. Plates were examined for a yellow colour after 24 h.

Thallous acetate resistance. Mueller Hinton agar (Difco) supplemented with thallous

acetate was dispensed and inoculated as described for Tween hydrolysis. Growth was recorded daily for 7 days.

Cetrimide resistance. Mueller Hinton agar (Difco) supplemented with Cetrimide was dispensed, inoculated, and examined as for Tween hydrolysis.

Tetracycline resistance. Mueller Hinton agar (Difco) containing tetracycline, dispensed as described for Tween hydrolysis, was inoculated with diluted 18 h TSB cultures [one platinum loop (diam. 3 mm) of culture in 10 ml distilled water]. Growth was recorded after 18 h.

Colistin resistance. Mueller Hinton agar (Difco) supplemented with colistimethate (Sigma) was dispensed, inoculated and examined as for tetracycline resistance.

Carbon source utilization tests. Defined medium M70 (Véron, 1975) was used. Carbohydrates (final concentration 0.2 %, w/v), phenol (final concentration 0.025 %, w/v) and other carbon sources (final concentration 0.1 %, w/v; only anion considered for organic acids) were added as required. Alcohols, strongly acidic or alkaline carbon sources were handled aseptically and added to the molten hot medium. Insoluble carbon sources were heated at 80 °C for 20 min. Aqueous solutions of other carbon sources were filtered. The pH was adjusted to 7.2 if necessary. Sterility controls were included. Dispensed media were allowed to dry overnight at 37 °C. Inocula were obtained by washing 1-day-old culture from a nutrient agar slant (2-day-old for pectobacteria) with 10 ml sterile distilled water. Growth was recorded every other day for up to 16 days. Plates without carbon source served as controls for background growth. Plates with glucose as sole carbon source were included to check the viability of bacterial suspensions.

Carbon sources deleted from the study because growth on them was too often scored as 'doubtful' were amygdalin, D-arabinose, starch, maleate, malonate, 2-hydroxybenzoate, glycine, L-lysine, L-threonine, ethanolamine and hypoxanthine.

Coding of characters. All tests were scored as 1 or 0 on two columns in order to give equal weight to quantitative as well as qualitative characters. Carbon source utilization tests were scored as follows: 0, 0 (negative); 1, 0 (late positive); or 1, 1 (early positive). Tests that clearly showed two grades of reaction were scored in the same way. The Richard and O'Meara methods for the Voges Proskauer test were scored as above. Results of growth at different temperatures, pH and NaCl concentrations required more than two columns. They were coded additively (Sneath & Sokal, 1973) with two columns for the upper limit for pH, three columns for the lower limit for temperature, and four columns for the upper limit for temperature, the lower limit for pH and for growth in NaCl. Inapplicable or missing test results were scored 'no comparison' (NC).

Carbohydrate fermentation tests, which almost duplicated the corresponding carbon source utilization tests as regards ability to metabolize individual carbohydrates, were not included. Each culture was derived from a single selected colony, and because there may have been bias in choosing the colonies according to their appearance, colonial characteristics were omitted. Invariant character states were deleted before computation.

Computation. The simple matching coefficient, S_{SM} , of Sokal & Michener (1958) was computed and strains were clustered using both single linkage (Sneath, 1957) and the unweighted pair group method with averages (UPGMA; Sokal & Michener, 1958). Vigour and pattern statistics (Sneath, 1968) were also calculated. Centroids of the phenons formed at the 80 % similarity level by UPGMA were calculated, and the distribution of distances, $d = \sqrt{(1 - S_{SM})}$, of strains from the centroid of their own phenon was determined. The mean and standard deviation of these distances, $\bar{d}_{cj}$ and s_{dcj} , were then calculated. The limits of phenons are to some degree arbitrary because one can choose an envelope that encloses any given proportion of the strains of the phenon. The envelope chosen here is one that will in

theory enclose 97.5 % of strains of a phenon (assuming that the phenons are hyperspherical multivariate normal clusters, an assumption that appears to be a reasonable approximation, see Sneath, 1974). The envelope is defined by a radius, r_j , about the cluster centroid that equals $\bar{d}_{cj} + ks_{dcj}$ with $k = 1.96$. This gives a measure of the dimension (dispersion) of the cluster (there are some sources of bias in these estimates when the number of strains is very small, but the practical effects in the present study are of little consequence).

The estimation of overlap between clusters is a complex problem that has not been satisfactorily explored: it will not be considered in detail here. A rough indication of the overlap was obtained from histograms of the distances of strains from the centroids (Sneath, 1972) and showed that the clusters are well separated.

RESULTS

All the strains in the study were Gram-negative, straight rods with rounded ends, catalase positive, utilized glucose by fermentation, and utilized *N*-acetylglucosamine, aspartate, citrate, D-fructose, gluconate, D-glucose, L-malate, mannitol and D-ribose as sole carbon sources. Though all strains utilized gluconate for growth, not all produced detectable amounts of reducing compounds, possibly because in these cases the gluconate was catabolized by a different pathway (Fraenkel & Vinopal, 1973).

All strains were negative for the following tests: growth at pH 4; phenylalanine deaminase; urease; growth on acetamide, adipate, 4-aminobenzoate, DL- δ -aminovalerate, α -amylamine, azelate, benzylamine, butanol, isobutanol, isobutyrate, butylamine, citraconate, DL-citrulline, creatine, dulcitol, ethylene glycol, D-fucose, glycollate, L-leucine, L-isoleucine, DL-norleucine, laevulinate, D-mandelate, L-mandelate, mesaconate, methanol, methylamine, oxalate, pantothenate, phenol, phthalate, isophthalate, terephthalate, pimelate, propanol, isopropanol, propylene glycol, sebacate, spermine, suberate, testosterone, tryptamine, D-tryptophan, uracil, urea, isovalerate, L-valine, and L-xylose as sole carbon source.

Clustering

Clustering by unweighted average linkage (UPGMA) yielded eight phenons at the 80 % similarity (S) level (Fig. 1). The co-phenetic correlation, i.e. the correlation between the original similarity values and those shown in the phenogram, was 0.90, which was satisfactory. Characteristics of the phenons are given in Table 2. The relationships between phenons are not quite the same as those in earlier studies (Grimont, 1969; Grimont & Dulong de Rosnay, 1972) but to avoid confusion the same symbols have been retained here where applicable: this mainly affects the position of phenon B.

Phenon A. This contained 62 strains and could be subdivided (Fig. 1) into 11 subphenons (A1 to A11) though some are dubious: A7 was composed of strains 241 and 284 both derived from ATCC990; A10 comprised two strains, one of which (242) was scored as 'no comparison' for all carbon sources; using the pattern coefficient, subphenons A9 and A11 disappeared and their component strains were included in other subphenons. Seven subphenons may be taxonomically significant; the tests that differentiate them are given in Table 3. These subphenons were homogeneous with respect to red pigmentation. The pigments were non-diffusing but soluble in petroleum ether, and their visible spectra were similar to that of the pigment prodigiosin from strain NIMA which we have recently determined to be a typical strain of subphenon A2.

Phenon A corresponds to *Serratia marcescens* [the neotype suggested by Martinec & Kocur (1961*d*) is strain 504] and also to *Serratia* pattern 1 of Fulton *et al.* (1959), to the

single *Serratia* phenon of Colwell & Mandel (1965), to *Serratia* biotype 1 of Bascomb *et al.* (1971, 1973) and to phenon A of Grimont & Dulong de Rosnay (1972), as shown by the inclusion in phenon A of strains 287, 504, 290 and 5. We have recently identified *S. indica* (Eisenberg, 1886) Bergey *et al.* 1923 (monotype CIP53-98, ATCC4002) and *Erythrobacillus pyosepticus* Fortineau 1904 (monotype CIP53-91, ATCC275) as both belonging to subphenon A1. Strain ATCC8101 [labelled *S. marcescens* subsp. *kiliensis* in the American Type Culture Collection (1974) catalogue also belongs to phenon A. Later three strains of *S. anolium* Duran-Reynals and Clausen 1937 (B-1700 from the Northern Regional Research Laboratory, Peoria, Illinois, U.S.A., and s-4 and s-19 from the Division of Applied Biology, National Research Council of Canada, Ottawa, Canada) were found to belong to subphenon A4 although they produce very little pigment.

Phenon B. This contained 22 pigmented strains. Three subphenons were observed; their differential characteristics are given in Table 4. The pigments of all these strains were soluble in petroleum ether and gave spectra identical to that of prodigiosin from strain NIMA.

Phenon B corresponds to *Serratia marinorubra*, *Serratia* biotype 2 of Bascomb *et al.* (1971) and to phenon B of Grimont & Dulong de Rosnay (1972), as shown by the inclusion of strains 288, 377 and 20. We have recently studied the proposed neotype of *Serratia rubidaea* (see Discussion), CDC2199-72, and identify it as a strain of subphenon B2; its pigment is also indistinguishable from prodigiosin.

Phenon C1. Phenon C1 contained 24 non-pigmented strains, and was subdivided into four subphenons, whose differential characteristics are shown in Table 5. This phenon corresponds to *Enterobacter liquefaciens* (Grimes & Hennerty, 1931; Ewing, 1963) and to *Serratia liquefaciens* of Bascomb *et al.* (1971). It also corresponds to *Serratia* ecogroup V of Lockhart & Hartman (1963) and to part of phenon C of Grimont & Dulong de Rosnay (1972) as shown by the inclusion of strains of those groups. It also contained the strain received as *Serratia marcescens* var. *noctuarum* (strain 514). We have recently identified another strain of Grimes and Hennerty, ATCC14461, as a member of subphenon C1d (see Discussion).

Phenon C2. This contained 20 strains. Most were isolated from water and all but two produced red pigment. The pigments of all except one strain were soluble in petroleum ether, and the spectra were similar to that of prodigiosin from strain NIMA. Strain 329 was exceptional in producing a pigment soluble in water, but not in petroleum ether or benzene, showing maximum absorbance at 496 nm and a shoulder at 535 nm. There were three subphenons (Table 6).

Phenon C2 corresponds to *Serratia plymuthica* (Fischer's Plymouth bacillus), *S. kiliensis* (Breunig's Kiel bacillus), *S. marcescens* var. *kiliensis* of Martinec & Kocur (1961*d*) and to part of phenon C of Grimont & Dulong de Rosnay (1972), as shown by the inclusion of strains 510, 501, 502, 33 and 34. The atypical strains of Colwell & Mandel (1965), strains 500 and 501, also labelled *Serratia* III by Mandel & Rownd (1964), fell into this phenon, as did a strain which we have subsequently studied, CDC1210-70 (ATCC15928), that was reported by Ewing *et al.* (1973) to be an atypical strain of *Serratia rubidaea* (see Discussion). Four NCIB strains (512, 515, 516, 517) that were noted in the catalogue of the National Collection of Industrial Bacteria (1964) as being unclassifiable by the scheme of Ewing *et al.* (1973), fall into phenon C2.

We have recently found that the monotype of *Serratia esseyana* Combe 1933 (NCTC4618) falls into phenon C2. Although strains labelled *S. kiliensis* or *S. marcescens* var. *kiliensis* belonged to phenon C2, strain 242, identified by Ewing *et al.* (1962) as *S. marcescens* var. *kiliensis* on the sole basis of a negative Voges Proskauer reaction, clustered in phenon A.

Phenons A to C, comprising the *Serratia-Enterobacter liquefaciens* group, clustered at the

Table 2. *Characteristics of phenons (percentage of strains positive)*

A few reactions by strains 38 and 236 were doubtful, and are marked ? Numbers in parentheses indicate total numbers positive at the time of the final readings where these differed by more than 5 %. Times of all readings are given in Methods.

No. of strains	...	Carbon source utilization	Phenons										Strains		
			A 62	B 22	C ₁ 24	C ₂ 20	D 5	E 7	F 10	G 4	38 1	236 1			
16 d	97	Acetate		86	83	70	0	100	40	100		100	100		
4 d	95	Aconitate		64 (95)	8	75	80 (100)	43	10	75		100	0		
4 d	97	Adonitol		95	0	0	0	29	0	100		100	0		
16 d	93	β-Alanine		0	30	15	0	0	0	0		0	0		
4 d	97	D-Alanine		95	96	65 (95)	0 (60)	86 (100)	0	100		0	100		
4 d	100	L-Alanine		100	96	85 (100)	60 (75)	71 (100)	0	100		100	100		
4 d	92 (100)	4-Aminobutyrate		100	79 (100)	68 (94)	60 (100)	0 (14)	0	100		100	100		
4 d	0	5-Aminovalerate		0	0	0	0	0	0	25 (75)		0	0		
4 d	3 (10)	Anthranelate		9 (20)	0	0	0	0	0	0		0	0		
4 d	0	L-Arabinose		100	100	100	100	100	100	100		100	100		
4 d	2	D-Arabitol		100	0	0	60	29	0	100		0	0		
4 d	0	DL-Arginine		0 (11)	0	0	0	0	0	50 (100)		0	100		
4 d	16	Benzoate		73 (91)	8	0 (30)	0	0	0	100		0	0		
4 d	0 (7)	Benzylformate		5 (18)	0	10 (30)	0	0	0	0 (100)		0 (100)	0		
4 d	0	Betaine		95	0	16	0	0	0	0		0	0		
4 d	0	2,3-Butanediol		0 (10)	0	30 (74)	0	0	0	0		0	0		
16 d	0	Butyrate		0	0	0	0	0	0	0		0	100		
4 d	88 (98)	Caprate		68 (100)	82 (96)	42 (85)	0	0 (43)	0	0 (25)		0 (?)	0		
16 d	95	Caproate		100	100	89	0	0	0	0		100	100		
4 d	88 (98)	Caprylate		82 (100)	100	75 (90)	0	0	0	0		0 (100)	100		
4 d	56	DL-Carnitine		0	8 (42)	0	0	0	0	75		0	0		
4 d	0 (3)	D-Cellobiose		100	9 (95)	100	40 (60)	100	90	100		100	0		
4 d	72	meso-Erythritol		95	0	0	0	0	0	0		0	0		
4 d	61 (77)	Ethanol		18	32 (41)	50 (79)	0	0	10	100		0	0		
4 d	90 (100)	Fumarate		91 (100)	100	55 (100)	100	29 (100)	40 (90)	75 (100)		100	100		
4 d	90 (100)	D-Galactose		95	100	100	100	100	100	100		100	100		
4 d	97	D-Galacturonate		100	100	100	100	86	90 (100)	100		100	0		
4 d	95	D-Glucosamine		100	100	75 (84)	100	86	10	100		0 (?)	100		
4 d	97	D-Glucuronate		100	92	85 (100)	100	100	0	100		100	0		
4 d	97	L-Glutamate		86 (100)	96	90 (100)	100	43 (100)	0 (33)	100		100	?		
16 d	0	Glutarate		0	0	0	0	0	0	50		0	0		
4 d	97	Glycerate		91 (100)	96	70 (80)	20 (100)	86 (100)	90 (100)	100		100	100		
4 d	100	Glycerol		100	100	80	100	100	100	100		100	100		
4 d	46 (98)	Heptanoate		0	0	5 (44)	0	0	0	0		0	0 (?)		
4 d	10 (20)	Hippurate		14	4 (8)	0	0	0	0	100		0	0		
4 d	0	Histamine		36	0	0	0	0	0	75		0	0		
4 d	100	L-Histidine		100	100	95	80	86	0	100		100	100		
4 d	18	3-Hydroxybenzoate		0	0	0	0	0	0	50		0	0		
4 d	33	4-Hydroxybenzoate		0	25 (37)	15 (60)	0	0	0	100		0	0		

Table 2 (cont.)

No. of strains ...	Phenons										Strains		
	A	B	C ₁	C ₂	D	E	F	G			38	236	I
3-Hydroxybutyrate	62	22	24	20	5	7	10	4			I		
Inositol	18 (76)	0	0	0 (5)	0	0	0	0 (100)			0	0	0
Inulin	4 d 97	100	100	100	100	29 (57)	71 (100)	100			100	0	0
Itaconate	16 d 2	0	0	32	0	0	0	0			0	0	0
α -Ketoglutarate	4 d 15 (40)	64 (100)	79 (96)	35 (60)	0 (20)	0 (29)	0 (20)	60 (75)			100	0	0
Kynurenate	4 d 88 (100)	95	100	100	0	29 (57)	0	100			100	0	0
Lactate	4 d 98	95	96	50 (80)	60	71 (100)	10 (60)	100			100	100	0
D-Lactose	4 d 2	100	12 (29)	5 (100)	0	57 (100)	70 (80)	100			0 (100)	0	0
D-Malate	4 d 46 (81)	0 (53)	30 (75)	0 (25)	0 (100)	0 (33)	0 (10)	0			100	0 (?)	0
Maltose	4 d 97	100	100	100	100	100	0	100			100	100	0
D-Mannose	4 d 98	100	100	95	100	86 (100)	100	100			100	100	0
D-Melezitose	4 d 0	45	96	85	0	0 (14)	0	0			0	0	0
D-Melibiose	4 d 0	100	100	100	0	100	90 (100)	100			100	0	0
α -Methylglucoside	4 d 0	91 (100)	71 (92)	5 (60)	0	43 (57)	30	75 (100)			0	0	0
Mucate	4 d 0	91 (100)	0	15 (37)	80	57 (71)	0	100			0	0	0
Nicotinate	4 d 80 (90)	0	100	80 (90)	0	0	0	0			100	0	0
L-Ornithine	4 d 18 (77)	0 (9)	70 (87)	0 (5)	80 (100)	14	0	75			100	100	0
Pelargonate	16 d 100	91	58	42	0	0	0	0			0	0	0
Phenylacetate	4 d 87 (98)	82 (100)	79 (100)	45 (80)	0	57 (86)	0	100			100	0	0
L-Phenylalanine	4 d 28 (77)	95	4 (25)	5 (29)	0	0	0	50 (100)			100	0	0
L-Proline	4 d 100	95	100	100	100	100	0	100			100	100	0
Propionate	16 d 97	0	0	33	0	0	0	0			0	100	0
Putrescine	4 d 95	95	96	90	0	43 (57)	0	100			100	0	0
Pyruvate	4 d 100	100	96	100	60 (80)	86 (100)	100	100			100	100	0
Quinate	4 d 33 (42)	82 (95)	0	100	0	0	0	100			0	0	0
Raffinose	4 d 2	100	100	95	0	57 (100)	100	100			100	0	0
L-Rhamnose	4 d 0	0	0	5	100	100	70 (78)	100			100	0	0
Salicin	4 d 100	95	100	100	80	100	100	100			100	100	0
Sarcosine	4 d 0	14 (95)	0	0 (16)	0	0	0	0			0	0	0
L-Serine	4 d 36 (98)	23 (95)	83 (96)	50 (95)	0 (40)	29 (86)	50	100			0 (100)	100	0
D-Sorbitol	4 d 95	0	100	50	0	86	0	100			100	0	0
Sorbose	4 d 0	0	0	0	0	0	0	25			0	0	0
Succinate	4 d 85 (100)	68 (100)	100	60 (100)	80 (100)	57 (100)	70 (80)	75 (100)			0 (100)	100	0
Sucrose	4 d 97	100	100	100	100	71	100	100			100	100	0
D-Tartrate	4 d 0	64 (73)	0	0	40 (60)	0	0	25			100	0	0
L-Tartrate	4 d 0	18	0	0	0	0	0	25			0 (?)	0	0
<i>meso</i> -Tartrate	4 d 23	0 (4)	50 (75)	0	60	0	0 (20)	0 (25)			100	0	0
Trehalose	4 d 100	100	100	100	100	100	70	100			100	100	0
Trigonelline	4 d 23	100	4	5	0	0	0	25			100	0	0
L-Tryptophan	4 d 66 (82)	95	54 (86)	55 (82)	0	0	0	0			0	0	0
L-Tyrosine	4 d 92 (100)	100	87 (100)	82 (100)	0	0	0	50 (100)			0 (?)	100	0
Valerate	16 d 23	0	0	0	0	0	0	0			0	100	0
D-Xylose	4 d 0 (2)	100	87 (100)	100	80	57 (100)	100	100			100	0	0

Table 2 (cont.)

No. of strains ...	Phenons										Strains	
	A	B	C1	C2	D	E	F	G			38	236
Indole	62	22	24	20	5	7	10	4			1	1
Lysine decarboxylase	1 d	0	0	0	0	0	0 (22)	0			0	100
	98	73	100	0	0	0	0	100			100	100
Ornithine decarboxylase	4 d	81	100	0	0	0	0	100			100	100
Arginine decarboxylase		0	100	0	0	71	0	100			100	0
Malonate test	0	0	8	0	0	100	10	0			0	100
Gelatin liquefaction (stab)	4 d	82 (86)	0	0	0 (20)	86	20	0			0	0
Chitin hydrolysis	100	100	96	90	0	0	60	0			0	100
Tributylin hydrolysis	95	0	37	95	0	0	0	0			0	100
Marked	90	100	96	80	0	0	0	0			0	100
Marked and weak	100	100	100	95	0	0	0	0			0	100
Triacetin hydrolysis, acid												
Marked	3	0	83	5	0	0	33	0			0	0
Marked and weak	12	4	92	32	0	0	50	0			0	100
Tween 40 hydrolysis	100	95	100	100	20	57	67	25			0	100
Tween 60 hydrolysis	100	91	100	100	0	0	37	0			0	100
Tween 80 hydrolysis	100	91	100	100	0	0	20	0			0	100
Lecithin turbidity												
Marked	82	77	62	40	0	0	20	0			0	100
Marked and weak	92	86	71	40	0	0	30	0			0	100
Starch hydrolysis	0	0	0	0	0	0	0	0			0	100
DNAase												
Marked	89	82	79	95	0	0	10	0			100	100
Marked and weak	93	100	92	95	0	0	10	0			100	100
Pectin hydrolysis	0	0	0	0	0	0	100	0			0	0
β -Galactosidase (ONPG)	z h	85 (92)	100	100	60 (100)	100	100	100			100	100
β -Xylosidase (PNPX)	0	100	0	60	0	43	40	100			100	0
Growth on thallous acetate												
0.8 mg ml ⁻¹	97	91	100	30	20	57	0	25			0	0
1.6 mg ml ⁻¹	82	54	54	5	20	0	0	25			0	0
Growth on cetrimide												
1.5 mg ml ⁻¹	97	36	17	65	60	100	100	75			0	0
5 mg ml ⁻¹	21	0	0	5	40	14	50	0			0	0
Growth on tetracycline												
5 μ g ml ⁻¹	98	100	8	0	0	43	0	25			0	100
25 μ g ml ⁻¹	84	0	0	0	0	14	0	25			0	0
Growth on colistimethate												
10 μ g ml ⁻¹	100	59	96	85	0	14	30	25			100	100
100 μ g ml ⁻¹	92	36	92	60	0	0	0	0			100	0

Table 3. *Differentiation of subphenons within S. marcescens (numbers of strains positive within 4 days)*

	Subphenons						
	A1	A2	A3	A4	A5	A6	A8
No. of strains	9	9	8	7	5	2	5
Red pigment	9	9	0	0	0	2	0
Carbon source utilization							
<i>meso</i> -Erythritol	9	9	8	7	0	2	0
Quinate	0	0	0	7	5	1 (2)	5
4-Hydroxybenzoate	0	0	0	7	5	2	4*
Carnitine	7	5 (8)	3	6 (7)	5	1	0
Benzoate	9	0	0	0	0	0	0
Hippurate	5 (9)	0	0	0	0	0	0
3-Hydroxybenzoate	0	0	8	0	0	0	2
Trigonelline	0	0	2	0	1 (5)	0	5
Tetrathionate reduction	9	9	8	0	5	2	5

Numbers in parentheses indicate total numbers positive after 16 days, if different from the numbers after 4 days.

* The fifth strain gave a doubtful result.

Table 4. *Differentiation of subphenons within S. marinorubra (numbers of strains positive within 4 days)*

	Subphenons		
	B1	B2	B3
No. of strains	12	3	3
Carbon source utilization			
D-Melezitose	2	3	3
Sarcosine	(12)	3	(3)
Benzoate	10 (12)	3	1
Ethanol	0	3	0
D-Tartrate	11 (12)	0	2
Histamine	4	0	3
Benzylformate	0	2	0
Voges Proskauer (O'Meara)	11	0	2
Lysine decarboxylase	12	3	0
Malonate test	11 (12)	3	0
Lecithin turbidity in 5 days	12	3	2*

Numbers in parentheses indicate total numbers positive after 16 days, if different from the numbers after 4 days.

* Weak reaction.

70 % S level, together with the unidentified isolate 38 (see Discussion). Differential characters of *Serratia* species are given in Table 7. Included in this Table are the known GC base ratios; those of phenon A are about 58 % and those of phenons C1 and C2 are about 54 to 56 %. Only two values are available for strains of phenon B.

The representatives of other genera (phenons D to G) were clearly separated in the dendrogram from phenons A to C, and their identities are noted below.

Phenon D. This contained five yellow-pigmented strains including the proposed neotype of *Enterobacter agglomerans* (E20, *Chromobacterium typhiflavum*; see Ewing & Fife, 1972) also

Table 5. *Differentiation of subphenons within S. liquefaciens (numbers of strains positive within 4 days)*

	Subphenons			
	C1a	C1b	C1c	C1d
No. of strains	7	10	2	2
Carbon source utilization				
Benzoate	0	0	0	2
Aconitate	0	0	2	0
L-Ornithine	5 (7)	9 (10)	0	0
Methyl red test	0	8	1	1
Arginine decarboxylase	0	0	0	2
Chitin hydrolysis in 5 days	7	1	0	0
Triacetin hydrolysis, acid in 5 days	7	10	2*	0

Numbers in parentheses indicate total numbers positive after 16 days, if different from the numbers after 4 days.

* Weak reaction.

Table 6. *Differentiation of subphenons within S. plymuthica (numbers of strains positive within 4 days)*

	Subphenons		
	C2a	C2b	C2c
No. of strains	4	11	5
Carbon source utilization			
Inulin	4	1	1
Benzoate	(4)	(2)	0
Betaine	4	1	0
Benzylformate	4	2	0
Glycerol	4	11	1
Aconitate	4	11	0
Glycerate	4	11	1
Lactate	4	11	1
Acetate	4	10	0
2-Ketoglutarate	4	8	0
Sorbitol	4	2	4
Dense growth in nutrient broth in 24 h at 30 °C	4	9 (10)*	(3)*
β -Xylosidase	0	8	5

Numbers in parentheses indicate total numbers positive after 16 days.

* Dense and moderate growth.

commonly called *Erwinia herbicola* (Löhnis 1911) Dye 1964. This phenon formed at the 90 % S level and was homogeneous.

Phenon E. This contained seven strains at the 80 % S level. It could be divided into two subphenons, E1 and E2, and a single yellow-pigmented strain (CIP57-33). Subphenon E2 included the proposed neotype of *Enterobacter cloacae* (CIP60-86) and subphenon E1 was composed of two strains labelled *Erwinia nimipressuralis*. Differential characteristics of the subphenons are given in Table 8.

Phenon F. This was composed of 10 pectinolytic strains ('pectobacteria'), with subphenons corresponding to *Erwinia carotovora* var. *atroseptica* (F1), *E. carotovora* var. *carotovora* (F2) and *E. chrysanthemi* (F3). Differential characteristics are given in Table 9.

Phenon G. This was composed of four strains, including the proposed neotype of *Enterobacter aerogenes* (also called *Klebsiella mobilis* by Bascomb *et al.*, 1971).

Table 7. *Differential characters of Serratia species*

	Phenon A <i>S. marcescens</i>	Phenon B <i>S. marinorubra</i>	Phenon C1 <i>S. liquefaciens</i>	Phenon C2 <i>S. plymuthica</i>
Carbon source utilization				
meso-Erythritol	v	+	-	-
L-Arabinose	-	+	+	-
D-Arabitol	+	+	-	-
Adonitol	-	+	(+)	+
D-Xylose	-	+	-	v
Mucate	-	+	(+)	(v)
α -Methylglucoside	+	-	+	v
D-Sorbitol	-	+	(+)	+
Cellobiose	-	+	(+)	(+)
Lactose	-	+	v	+
Melibiose	-	+	+	+
Melezitose	-	v	+	v
Raffinose	-	+	+	v
Aconitate	+	(+)	-	v
Propionate	(+)	-	-	(v)
Heptanoate	(+)	-	-	(v)
Benzoate	v	(+)	-	(v)
Quinate	v	(+)	-	+
β -Alanine	(+)	-	-	(v)
Betaine	-	+	-	v
Sarcosine	-	(+)	-	(v)
Nicotinate	(+)	-	+	(+)
Trigonelline	v	+	-	-
Red pigment	v	+	-	v
Good growth at 5 °C	-	-	-	+
Growth at 40 °C	+	-	-	-
Growth in NaCl (8.5 % w/v)	v	+	-	-
Growth in KCN	(+)	-	v	(v)
Tetrazionate reduction	v	-	(+)	-
Gas from glucose (in agar)	-	-	+	v

Table 7 (cont.)

	Phenon A <i>S. marcescens</i>	Phenon B <i>S. marinorubra</i>	Phenon C1 <i>S. liquefaciens</i>	Phenon C2 <i>S. plymuthica</i>
Lysine decarboxylase	+	v	+	—
Ornithine decarboxylase	+	—	+	—
Chitin hydrolysis	+	—	v	+
Triacetin hydrolysis	(v)	—	(+)	(v)
β -Xylosidase	—	+	—	v
Growth on tetracycline ($5 \mu\text{g ml}^{-1}$)	+	+	—	—
% GC, strain and method	<div> <div>58</div> <div>285*</div> <div>T_m</div> </div> <div> <div>58</div> <div>290*</div> <div>T_m</div> </div> <div> <div>58.5</div> <div>241*</div> <div>T_m</div> </div> <div> <div>57.5</div> <div>504*</div> <div>T_m</div> </div> <div> <div>58</div> <div>296 </div> <div>T_m</div> </div>	<div> <div>58.3</div> <div>288†</div> <div>T_m</div> </div> <div> <div>53.5</div> <div>37 </div> <div>T_m</div> </div>	<div> <div>53.9</div> <div>503§</div> <div>T_m</div> </div> <div> <div>52.6</div> <div>503§</div> <div>d</div> </div> <div> <div>53.8</div> <div>275 </div> <div>T_m</div> </div>	<div> <div>53.5</div> <div>500*</div> <div>T_m</div> </div> <div> <div>55</div> <div>501*</div> <div>T_m</div> </div> <div> <div>57.5</div> <div>510*</div> <div>T_m</div> </div> <div> <div>56.3</div> <div>510†</div> <div>T_m</div> </div> <div> <div>54</div> <div>SM11‡</div> <div>T_m</div> </div> <div> <div>55.5</div> <div>SM12‡</div> <div>T_m</div> </div> <div> <div>54.6</div> <div>392 </div> <div>T_m</div> </div>

Unclustered strain 38 had a % GC of 54.6 || T_m

+, Over 90 % of strains positive (early reading); (+), over 90 % of strains positive (late reading); —, less than 10 % of strains positive (late reading); v, 10 to 90 % positive (early and late reading); (v), 10 to 90 % positive (late reading only).

The GC base ratios are from the following sources: * Colwell & Mandel (1965) and Hill (1966); † R. Owen (NCTC, personal communication); ‡ Recently identified by us as belonging to phenon C2 with GC ratios from Colwell & Mandel (1965) and Hill (1966); § Starr & Mandel (1969); || C. Barreau (Laboratoire de Biologie Moléculaire, INRA, Pont-de-la-Maye, France, personal communication).

Table 8. *Differentiation of subphenons within Enterobacter cloacae (numbers of strains positive within 4 days)*

	Subphenons	
	E1 <i>Ent. nimipressuralis</i>	E2 Typical <i>Ent. cloacae</i>
No. of strains	2	4
Carbon source utilization		
α -Methylglucoside	0	3 (4)
Kynurenate	0	2 (4)
Sucrose	0	4
Growth at 5 °C	2	0
Growth in NaCl (8.5 %, w/v)	0	4
Growth with thallos acetate (0.8 mg ml ⁻¹)	0	4

Numbers in parentheses indicate total numbers positive after 16 days, if different from the numbers after 4 days.

Table 9. *Differentiation of subphenons within the 'pectobacteria' (numbers of strains positive within 4 days)*

	Subphenons		
	F1 <i>Erwinia carotovora</i> var. <i>atroseptica</i>	F2 <i>Erwinia carotovora</i> var. <i>carotovora</i>	F3 <i>Erwinia chrysanthemi</i>
No. of strains	3	3	2
Carbon source utilization			
L-Rhamnose	3	3	0
α -Methylglucoside	3	0	0
L-Serine	0	3	2
meso-Tartrate	0	0	(2)
Trehalose	3	3	0
Acetate	0	(3)	(1)
Dense growth in nutrient broth in 24 h at 30 °C	0	2 (3)†	2
Growth at			
5 °C	3	(1)§	0
37 °C	0	3	2
40 °C	0	0	2
pH 5	(1)*	(1)	2
pH 9	(1)*	3	(2)*
Growth in NaCl (7 %, w/v)	0	3	0
Gas from glucose (in agar)	0	0	2
Voges Proskauer (Richard)	0	3	2
Indole production	0	0	(2)†
Malonate test	0	0	2
Gelatin hydrolysis	0	2	2
Tween 40 hydrolysis	0	3	2
Tween 80 hydrolysis	0	0	2
Lecithin turbidity	0	0	2
β -Xylosidase	0	2	2

Numbers in parentheses indicate total numbers positive after 16 days, except: * after 6 days; † after 5 days.

‡ Moderate and dense growth.

§ Absorbance between 0.03 and 0.30.

Table 10. *Differentiation of the higher ranked groups*

	Genus <i>Serratia</i>	<i>Erwinia</i> <i>herbicola</i>	<i>Enterobacter</i> <i>cloacae</i>	'Pectobacteria'	<i>Klebsiella</i> <i>mobilis</i>
Carbon source utilization					
L-Rhamnose	—	+	+	v	+
D-Glucosamine	+ or (+)	+	v	v	—
D-Glucuronate	+ or (+)	+	+	—	+
Maltose	+	+	+	—	+
Caprylate	+ or (+)	—	—	—	—
L- and D-Alanine	+ or (+)	v	(+)	—	+
4-Aminobutyrate	+ or (+)	(+)	(v)	—	(v)
L-Proline	+	+	+	—	+
L-Tyrosine	+ or (+)	—	—	—	(+)
Kynurenate	+ or (+)	—	v	—	+
Putrescine	+	—	v	—	+
Yellow pigment	—	+	v	—	—
Gluconate test	+	+	+	—	+
Iodoacetate test	+	+	(+)	—	(+)
Arginine decarboxylase	—	—	+	v	—
Gelatin hydrolysis	+	—	—	v	—
Tributyrin hydrolysis	+ or (+)	—	—	—	—
Tween 60 or 80	+	—	—	v	—
DNAase	+ or (+)	—	—	v	—
Pectin hydrolysis	—	—	—	+	—

+, Over 90 % of strains positive (early reading); (+), over 90 % of strains positive (late reading); —, less than 10 % of strains positive (late reading); v, 10 to 90 % positive (early and late reading); (v), 10 to 90 % positive (late reading only).

Table 11. *Vigour values of the clusters based on all characters excluding invariant ones*

	Range	Mean	S.D.
A. <i>Serratia marcescens</i>	0.439–0.631	0.568	0.038
B. <i>Serratia marinorubra</i>	0.548–0.647	0.611	0.026
C1. <i>Serratia liquefaciens</i>	0.519–0.594	0.567	0.021
C2. <i>Serratia plymuthica</i>	0.347–0.602	0.514	0.066
D. <i>Erwinia herbicola</i>	0.281–0.380	0.335	0.043
E. <i>Enterobacter cloacae</i>	0.369–0.479	0.419	0.050
F. 'Pectobacteria'	0.178–0.347	0.262	0.050
G. <i>Klebsiella mobilis</i>	0.564–0.621	0.598	0.028
Strain 38	—	0.513	—
Strain 236	—	0.435	—

Two strains were not included in any cluster. Strain 38 remains unidentified (see Discussion). Strain 236 was received as *Serratia* but was subsequently identified as *Aeromonas hydrophila*, and was typical of this species in a numerical study (Popoff & Véron, 1976). It was rather close to the *Serratia* group on the UPGMA phenogram with S_{SM} , but not on the other phenograms.

A summary of characteristics allowing differentiation of *Serratia* from other groups is given in Table 10. Starr & Mandel (1969) cite the GC base ratios of strains of phenons E and G as between 53 and 55.5, so no distinction can be made on this basis (see also Table 7).

Comparison of numerical methods

The single linkage method gave similar results to UPGMA except that phenons C1 and C2 were not so sharply separated (though they were still distinct). Subphenons were not clearly seen in phenon A, but could be recognized in other phenons. Strain 222 was not included

Table 12. *Parameters of phenons*

Values of $\bar{d}_{ej} = \Sigma d_{ej}/t_j$, $s_{dej} = \sqrt{[\Sigma(d_{ej} - \bar{d}_{ej})^2]/(t_j - 1)}$, and $r_j = \bar{d}_{ej} + k s_{dej}$ (with $k = 1.96$) were calculated by the methods given in Sneath (1974).

Phenon	No. of strains	No. of characters	$\bar{d}_{ej}$	s_{dej}	r_j
A	62	244	0.230	0.0488	0.326
B	22	244	0.210	0.0441	0.296
C1	24	244	0.202	0.0389	0.278
C2	20	244	0.266	0.0413	0.347
D	5	244	0.181	0.0309	0.242
E	7	244	0.241	0.0399	0.319
F	10	244	0.229	0.0377	0.303
G	4	244	0.194	0.0485	0.289
Strain 38	1	240	—	—	—
Strain 236	1	239	—	—	—

in phenon A, but was distantly attached to phenons A–C. Strain CIP57-33 was separated from phenon E, and distantly attached to phenon F. The co-phenetic correlation was 0.87.

The square of the complement of the simple matching coefficient can be partitioned into the square of the difference in vigour, D_V^2 , and the square of the difference in pattern D_P^2 (Sneath, 1968). Vigour is the frequency of positive characters. The lowest values of vigour are shown by the pectobacteria and the highest by phenon B (Table 11). Although there are marked differences in vigour, its removal did not greatly alter the resemblances between strains, so the differences between the clusters are not due solely to differences in vigour; UPGMA clustering on D_P gave a phenogram similar to Fig. 1. There were few changes within the phenons when the vigour component was removed. Subphenon A8 was divided into two minor groups depending on ability to utilize 3-hydroxybenzoate. Subphenons within phenon E were recovered with the exclusion of strain C2 (CIP57-33) from the phenon. Within phenon F a tighter grouping of strains labelled *Erwinia chrysanthemi* (F3) was observed. At the level of 0.30 D_P two major groups of phenons were formed: one contained phenons A, C1 and C2; and the other phenons B, D, E, F, G, and strain 38. Strain 236 was not closely related to any group. The co-phenetic correlation was 0.89. Single linkage on D_P gave very similar results to single linkage on S_{SM} .

Cluster parameters. The centrotpe of each cluster, i.e. the strain closest to the centroid, is noted in Table 1. The most dispersed phenon was C2 and the most compact was phenon D as shown by r_j (Table 12).

DISCUSSION

The results support a genus *Serratia* consisting of four taxa, corresponding to phenons A, B, C1 and C2, which group together in the UPGMA phenogram at the 70% S level. Although phenon B is separated from the other three on the pattern coefficient phenogram, the change in position is slight, and may not be significant; therefore the balance of evidence favours retaining phenon B with A, C1 and C2. Very little overlap occurs between any of these four phenons, so they should certainly be given species rank. The subphenons, or some of them, could represent valid taxa of low rank which we do not yet have good methods for separating. They may provisionally be referred to as biovars, and may be of epidemiological interest (P. A. D. Grimont, unpublished).

Phenon A corresponds unambiguously to the species presently known as *Serratia marcescens* Bizio, and no special points arise.

Three possible epithets for phenon B were found in the literature: *stercoraria*, *rubidaea* and *marinorubra*. Although *Serratia stercoraria* Jan 1939 *a, b* is rather poorly described, its characteristics are not in conflict with those of phenon B. *Bacterium rubidaeu* Stapp 1940 shows two discrepancies. Phenon B is the only *Serratia* species unable to produce acid from sorbitol, but *B. rubidaeu* was sorbitol positive. In addition, *B. rubidaeu* produced a red pigment which differed from prodigiosin: it was non-diffusing and soluble in alcohol and acetone but, unlike prodigiosin, it had an absorption band between 475 and 485 nm and was insoluble in chloroform, ether, benzene, xylene and toluene. All pigmented strains of phenon B produced a pigment soluble in the latter solvents, with an absorption spectrum similar to that of the pigment of strain NIMA. The pigment of strain 329 resembles that of *B. rubidaeu*, but strain 329 belongs to phenon C2. No authentic strains of *S. stercoraria* or *B. rubidaeu* are available, so we cannot be sure of their identity. For this reason we are unable to follow the opinions of Ewing *et al.* (1973) in using the species name *Serratia rubidaea* (Stapp) Ewing *et al.* The characters of the 49 cultures placed in *S. rubidaea* by Ewing *et al.* (1973) are generally in good accord with those of phenon B. However, four of their strains fermented sorbitol and five failed to ferment adonitol, and, as noted earlier, one strain they considered an atypical *S. rubidaea* is, in our hands, a typical strain of subphenon C2b.

The only species known to us which definitely corresponds to phenon B is *Serratia marinorubra* ZoBell and Upham 1944. A monotype (strain 288, ATCC27614, NCTC10912) has been preserved; and it agrees with the original description. Although it is not a typical strain of phenon B using S_{SM} , this appears to be due principally to its rather slow rate of growth and low vigour, because it is quite typical of phenon B as judged by the D_p coefficient. In the D_p phenogram strain 288 was attached to subphenon B1. We recommend *Serratia marinorubra* ZoBell and Upham 1944 for phenon B, which is an unambiguous name.

Although strains of phenon C1 are non-pigmented, we believe this phenon should be included in the genus *Serratia*. It is phenetically even closer to *S. marcescens* than are phenons B and C2, both of which contain red-pigmented strains. The correct name appears to be *Serratia liquefaciens* (Grimes and Hennerty 1931) Bascomb *et al.* 1971. It cannot be safely identified with *Bacillus noctuarum* White 1923 because the strain studied by Weiser & Lysenko (1956) and named *S. marcescens* var. *noctuarum* by Lysenko (1958) was not an original strain of White. Authentic strains of *Aerobacter liquefaciens* Grimes and Hennerty are available and fall into phenon C1. This organism was later named *Aerobacter lipolyticum* by Grimes (1961) because the earlier name was then thought to be a later homonym of *Aerobacter liquefaciens* Beijerinck; however, the Judicial Commission (1973) in Opinion 48 rejected Beijerinck's name as a *nomen dubium*, thus removing the bar to the validity of *A. liquefaciens* Grimes and Hennerty.

Ewing (1963) had earlier transferred Grimes and Hennerty's species to *Enterobacter* as *Ent. liquefaciens*, but Bascomb *et al.* (1971) recognized its close affinity to *Serratia* and transferred it to that genus. Strain ATCC14460 (our 503) is the type (co-type according to Sneath & Skerman, 1966). Ewing *et al.* (1973) believed that no type strain had been designated and considered ATCC14460 was atypical: they therefore proposed a neotype CDC1284-57 (our 280). However, there is no need to replace ATCC14460 as it is not atypical (it belongs to subphenon C1d, as does another original strain, ATCC14461, that we have recently examined, whereas strain 280 belongs to C1b), and Grimes indicated that it was the type strain in a personal communication to R. E. Buchanan (American Type Culture Collection, 1964).

Phenon C2 occurs most often in fresh water. Several pigmented, aerogenic and cold-tolerant species of *Serratia* from water have been described in the past. Although Hefferan (1906) found that *Bacillus miniae* Zimmermann 1890, *Bacillus plymouthensis* and *Bacter-*

ium kilense were agglutinated by high dilutions of the same sera, it is not certain whether Zimmermann's original strain was, indeed, used and, as no authentic culture now appears available, we cannot be sure of the identity of *Serratia miniacea* (Zimmermann 1890) Bergey *et al.* 1923 with phenon C2. Phenon C2 appears identical with Fischer's 'roter Bacillus der Wasserleitung von Plymouth' (Fischer, 1887) and Breunig's Kiel bacillus (Breunig, 1888; provided Novy's culture ATCC4261 is authentic). The latter was named *Bacterium kiliense* by Lehmann & Neumann (1896). It has been ranked as a subspecies, *S. marcescens* subsp. *kiliensis*, by Ewing *et al.* (1973), but this is confusing as it was defined only by a negative Voges Proskauer reaction, and phenon C2 strains give both positive and negative reactions. Fischer did not name his red-pigmented aerogenic culture which he isolated from the water supply in Plymouth. The earliest name seems to be *Bacillus plymuthensis* (Dyar, 1895) which is regarded as validly published (Buchanan, Holt & Lessel, 1966). Unfortunately, Dyar did not clearly state he was naming or renaming Fischer's bacillus and the strain he studied was of uncertain origin. This removes the priority of the epithet *plymuthensis*. Lehmann & Neumann (1896) validly published the name *Bacterium plymuthicum* as follows: '*Bacterium plymuthicum* (Fischer) Lehm. et Neum. "Roter Wasserbacillus von Plymouth" Fischer. Genauer beschrieben bei Voges (C.B. XIV, p. 314)'. They refer to Fischer and to a newer description by Voges (1893). The sixth edition of *Bergey's Manual of Determinative Bacteriology* (Breed, Murray & Hitchins, 1948) transferred this species to the genus *Serratia* as *S. plymuthicum*. The epithet can be corrected to *plymuthica* without change in priority and we prefer it for phenon C2 to *kiliensis* of the same date (*Serratia kiliensis* Bergey *et al.* 1923) because Fischer's bacillus is better authenticated and a cause of less confusion. ATCC183 is the monotype. This strain is now discarded from the ATCC, but cultures derived from it exist (CCM640, our 510).

Strain 38 is apparently a single representative of an unknown species. In the *D_F* phenogram, it was loosely attached to *Erwinia herbicola*. It resembles the 'lysine-positive Citrobacter' of Leclerc & Buttiaux (1965), whose DNA showed 47 % relatedness to DNA from *Serratia marcescens* (Crosa *et al.*, 1974), but little other taxonomic information is available.

The representatives of other genera were only included to provide some bounds to the genus *Serratia*. Phenon E corresponds to *Enterobacter cloacae* (Jordon) Hormaeche and Edwards 1960; distinctive properties of *Erwinia nimipressuralis* Carter 1945 are few (Table 8), so it is doubtful whether it deserves species rank. One yellow-pigmented strain of *Enterobacter cloacae* was included (strain C2, CIP57-33), and its low similarity to the other strains of this species would support the view of Brenner (1973) that the DNA of pigmented and non-pigmented *Enterobacter cloacae* are only distantly related. In phenon F the three organisms *Erwinia carotovora* (Jones) Bergey *et al.* 1923, *Erwinia atroseptica* (Van Hall) Jennison 1923, and *Erwinia chrysanthemi* Burkholder, McFadden and Dimock 1953 are very close but can be differentiated.

This study also raises a question of taxonomic usage: how strict should one be in reviving old, and often forgotten, names? As one looks for earlier and earlier names, one usually finds the descriptions become poorer, but equally there are also fewer properties recorded that definitely disagree with characters of the strains under study. The description of *Bacterium plymuthicum* was found to fit phenon C2, but *Bacillus miniaceus* would fit almost as well. If authentic cultures are not available we believe one should be cautious in making an identification, particularly if the original description is poor. In our opinion, the only sure criterion is comparison with an original type culture, and in the absence of such cultures identifications are only too often matters of faith.

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