Deoxyribonucleic Acid Relatedness Between *Serratia plymuthica* and Other *Serratia* Species, with a Description of *Serratia odorifera* sp. nov. (Type Strain: ICPB 3995)

P. A. D. GRIMONT,¹ FRANCINE GRIMONT,¹ C. RICHARD,¹ B. R. DAVIS,² A. G. STEIGERWALT,² AND DON J. BRENNER²

Enteric Section, Center for Disease Control, Atlanta, Georgia 30333,² and Service des Enterobactéries, Institut Pasteur, F-75724 Paris Cedex 15, France¹

Seven new isolates of *Serratia plymuthica* (including two from a human source) and 24 isolates resembling unclustered *Serratia* strain 38 (Grimont et al., J. Gen. Microbiol. 98:39-66, 1977) are described. Deoxyribonucleic acid relatedness studies, obtained with labeled reference deoxyribonucleic acid from *S. plymuthica* 392 confirm that *S. plymuthica* is a discrete species. Mean percent relatedness of *S. plymuthica* isolates to strain 392 was 88 ± 13.6 (standard deviation), whereas the mean percent relatedness of *S. liquefaciens* isolates (the closest species) to strain 392 was 52 ± 12.4 . Strain 38 and twenty-four "38-like" isolates constitute a new deoxyribonucleic acid hybridization group that is 28 to 43% related to *Serratia* species and to the "*Citrobacter*-like" group of Leclerc and Buttiaux, and 15 to 22% related to other known species of *Enterobacteriaceae*. This 38-like group constitutes a new species that is named *Serratia odorifera* sp. nov. (type strain, ICPB 3995). Two biotypes (1 and 2) are described. Strains of this species will grow on caprylate-thallos agar (selective for *Serratia* spp.), and they have a characteristic odor. Most strains of *S. odorifera* were recovered from clinical specimens.

The taxonomy of the genus *Serratia* has changed markedly in the past few years. The 8th edition of *Bergey's Manual of Determinative Bacteriology* (23), following earlier work (10, 11, 20), recognizes only one species, *Serratia marcescens*. However, since 1959 several workers (1, 12, 14) have challenged the concept of a monospecific genus *Serratia*. The second species to be formally recognized was *S. liquefaciens* (Grimes and Hennerty 1931) Bascomb et al. 1971 (1, 13). A third species, *S. rubidaea* (Stapp 1940) Ewing et al. 1973, was proposed and corresponds to *Serratia* biotype 2 of Bascomb et al. (1) and to phenon B of Grimont and Dulong de Rosnay (14). The same taxon is also called *S. marinorubra* ZoBell and Upham 1944. The reasons for using *S. marinorubra* rather than *S. rubidaea* have been discussed by some of us elsewhere (16). Finally, a fourth species, *S. plymuthica* (Lehmann and Neumann 1896) Breed, Murray, and Hitchens 1948 (2, 18), has been reintroduced (16). *S. plymuthica* is phenetically closest to *S. liquefaciens*, from which it is differentiated by pigmentation, growth on quinate as the sole carbon source, and lack of lysine, ornithine decarboxylases, and tetrathionate reductase (16). Many additional strains appear to belong in the genus *Serratia* but are distinct from the four described species. One of these, strain 38, was previously described (16).

Studies of deoxyribonucleic acid (DNA) relatedness among species of *Serratia* and *Enterobacter* (27) have shown that *S. marcescens*, *S. liquefaciens*, and *S. rubidaea* (*S. marinorubra*) are valid species. In this study we investigated the distinctness of *S. plymuthica* from *S. liquefaciens* and other *Serratia* spp. on the basis of DNA relatedness and described a group of strains that resemble *Serratia* sp. strain 38, which remained unclustered in a recent numerical taxonomic study (16).

MATERIALS AND METHODS

The strains used in this study are shown in Table 1. Organisms were maintained on meat extract agar or semisolid yeast extract-nutrient agar. Routine cultivation of organisms was carried out in brain heart infusion broth.

Carbon source utilization tests, pigment and odor production, growth at 4°C, β -xylosidase test (with o-nitrophenyl- β -xyloside), tetrathionate reduction, Voges-Proskauer reaction as modified by Richard (21), gelatin hydrolysis (plate method), Tween hydrolysis, and chitin hydrolysis were carried out as described by Grimont et al. (16). The composition of caprylate-thallos medium has been published (26). All other biochemical tests were carried out as described by Edwards and Ewing (8).

The medium used for labeling cells with $^{32}\text{PO}_4$, the preparation of both labeled and unlabeled DNA, and the shearing of DNA have been described (4, 6). In all

TABLE 1. *Origin of strains*

Strain no.	Biotype ^a	Source and history	References
Strains identified as <i>Serratia ply-</i>			
<i>muthica</i> :			
CDC 2000-73	C2a	Grimont 33, Brisou 3617, seawater	16
CDC 2001-73	C2a	Grimont 34, Brisou 3736, unknown	16
Grimont 299	C2b	Brisou 6912, seawater	16
Grimont 392	C2b	Spring water, centrotpe of phenon C2	16
CDC 1286-57	C2b	M. Grimes, Ireland, milk	27
CDC 2707-57	C2b	NCTC 8015, NCIB 8285, Grimont 517, water	16
CDC 2708-57	C2b	NCTC 8706, NCIB 8266, Grimont 516, soil	16
CDC 2709-57	C2b	NCTC 8900, laboratory contaminant	
CDC 3855-62	C2b	Le Minor 24-62, L. Le Minor, Institut Pasteur (Paris)	
CDC 1210-70	C2b	ATCC 15928, source unknown	9, 16
CDC 462-75	C2b	Sputum, Rhode Island	
CDC 3513-75	C2b	Sputum, Colorado	
Grimont 510	C2c	CCM 640, ATCC 183, type strain	16
CDC 4743-70	C2c	Y. Hamon, Institut Pasteur (Paris)	
Strains identified as <i>Serratia</i>			
<i>odorifera</i> sp. nov.:			
Grimont 38	1	Cultivated mushroom, Bordeaux, France	14, 16
Grimont 1073	1	Sputum, Bordeaux; ICPB 3995, holotype	
CDC 4409-66	1	Lung, Connecticut	
CDC 3252-68	1	Throat, Pennsylvania	
CDC 5112-69	1	Bile, Connecticut	
CDC 4321-70	1	Sputum, California	
CDC 4933-70	1	Sputum, Colorado	
CDC 1877-71	1	Sputum, Minnesota	
CDC 5248-71	1	Sputum, Colorado	
CDC 3138-72	1	Throat, California	
CDC 652-73	1	Sputum, New York	
CDC 2985-73	1	Mouth, Colorado	
CDC 133-74	1	Horse-bite, California	
Richard 2-73	1	Mussel, Saint-Etienne, France	
Richard 17-74	1	Blood, Lyon, France	
Richard 12-76	1	Pus, Paris	
Richard 11-77	1	Sputum, Paris	
CDC 292-74	2	Blood, California	
CDC 2346-74	2	Abdominal wound, Wisconsin	
CDC 2861-74	2	Tracheal secretion, California	
CDC 3472-74	2	Blood, Massachusetts	
CDC 1396-75	2	Bronchial washing, Vermont	
CDC 3567-75	2	Urine, New Jersey	
CDC 1517-76	2	Tracheal exudate, Pennsylvania	
CDC 1945-77	2	Sputum, Maryland	
Other strains used in DNA relat-			
edness studies:			
<i>S. liquefaciens</i>			
ATCC 14460	Cld	Grimes, type strain	16, 24, 27
" <i>E. liquefaciens</i> "	Cld	Derived from ATCC 14460	
CDC 446-68	Cld	Derived from ATCC 14460	27
CDC 6136-66	Clb	Centrotpe of phenon C1 (Grimont 275)	16, 27
CDC 1107-57		Dairy product, Minnesota	27
CDC 1284-57	Clb	Milk, Cork, Ireland	27
<i>S. marcescens</i>			
SM6	A2	Walter Reed Army Institute of Research	27
CDC 866-57	A4	Abscess	27
CDC 868-57	A4	Urine	27
CDC 5147-64		Blood, Pennsylvania	
CDC 1201-65	A5	Stool, Kentucky	27

TABLE 1—Continued

Strain no.	Biotype ^a	Source and history	References
<i>S. marinorubra</i> (<i>S. rubidaea</i>)			
CDC 4445-64		Blood, Bruhl, Hamburg, Germany	27
CDC 5474-68		H. Lautrop, Copenhagen, Denmark	27
CDC 4057-71		Blood, Colorado	27
CDC 934-72		River, Maryland	27
" <i>Citrobacter</i> -like"			
CDC 4552-71		H. Leclerc, Lille, France	7
CDC 4554-71		Leclerc, Lille, France	7
<i>Enterobacter aerogenes</i> CDC 1627-66		Sputum, Massachusetts	27
<i>Enterobacter cloacae</i>			
CDC 1347-71		Blood, Colorado	27
256-4 (Y)		National Institutes of Health	27
<i>Hafnia alvei</i>			
CDC 5632-72			
CDC 329-73		Wound, Connecticut	
CDC 4510-75			
<i>Enterobacter</i> sp. 76-01		Urine, Clermont-Ferrand, France	22
<i>Enterobacter agglomerans</i> CDC 2780-70		Wound, Washington	
<i>Erwinia carotovora</i> (<i>Pectobacterium carotovorum</i>) ATCC 495		Soft rot of carrot, Jones; co-type	24
<i>Erwinia amylovora</i> ICPB EA 178		M. P. Starr; ATCC 19381	
<i>Citrobacter freundii</i> CDC 460-61		Meat, South Carolina	
<i>Proteus mirabilis</i> PR 14		Center for Disease Control	
<i>Escherichia coli</i> K-12		University of Washington, Seattle	

^a Biotype of *Serratia* spp. according to Grimont et al. (16).

cases, 0.1 μ g of sheared, 32 PO₄-labeled DNA per ml was reacted with 150 μ g of sheared, unlabeled DNA per ml of 0.28 M phosphate buffer (pH 6.8) for 16 h at 60 or 75°C. After incubation, samples were diluted to 0.14 M phosphate buffer, and sodium dodecyl sulfate was added to 0.4%. Reassociated DNA was separated from unreacted DNA on hydroxyapatite by use of a batch procedure (5). Unreacted DNA did not bind to hydroxyapatite. The reassociated DNA bound to hydroxyapatite was eluted in a series of washes with 0.4 M phosphate buffer.

A homologous control and nine heterologous samples were processed simultaneously. Observed homologous DNA reassociation was arbitrarily deemed 100%. Heterologous DNA reassociation was evaluated relative to that of homologous DNA reassociation.

In thermal elution experiments, the reassociated DNA bound to hydroxyapatite was eluted by increasing the temperature in 5°C increments. In this case, bound DNA is denatured and eluted from hydroxyapatite as the temperature exceeds the $T_{m(e)}$ (that temperature at which 50% of given double-stranded DNA is eluted from hydroxyapatite). $\Delta T_{m(e)}$ is the decrease in thermal elution midpoint between heterologous DNA reactions and the homologous DNA reaction. A 1°C decrease in thermal stability is assumed to result from about 1% unpaired bases (19). Hence, $\Delta T_{m(e)}$ allows one to estimate the percentage divergence within related sequences.

RESULTS

The computer data bank of the Enteric Section at the Center for Disease Control (CDC) was surveyed to detect any "atypical" *Serratia* or *Enterobacter* species that closely or loosely corresponded to the description of either *S. plymuthica* or the unclustered strain 38 of Grimont et al. (16). The selected strains were subjected to a number of biochemical tests (see above). Eleven of these strains were identified as *S. plymuthica*, and 19 were close to strain 38 and to a similar strain isolated in Bordeaux (no. 1073). Four strains resembling strain 38 (38-like) were independently identified at the Pasteur Institute (Paris). To avoid any confusion, 38-like strains will now be called *Serratia odorifera* sp. nov., a step which is justified in the Discussion.

Biochemical characteristics of the *S. plymuthica* strains are shown in Table 2. As several *S. plymuthica* strains identified at CDC had been also studied (under other collection numbers) by Grimont et al. (16), only characteristics of newly studied strains are listed in Table 2. It can be seen that these strains can be assigned to one of the three biotypes described in *S. plymuthica*

TABLE 2. Biochemical characters of CDC strains newly identified as *Serratia plymuthica*

Biochemical character	Reaction of CDC strains:						
	1286-57	2709-57	3855-62	1210-70	462-75	3513-75	4743-70
Carbon source utilization ^a							
Acetate	(+)	(+)	(+)	(+)	(+)	(+)	-
Aconitate	+	+	+	+	+	+	-
Adonitol	-	-	-	-	-	-	-
L-Arabinose	+	+	+	+	+	+	+
D-Arabitol	-	-	-	-	-	-	-
Benzoate	-	(+)	-	-	-	-	-
Betaine	-	-	-	-	-	-	-
DL-Carnitine	-	-	-	-	-	-	-
Cellobiose	+	+	+	+	+	+	+
Erythritol	-	-	-	-	-	-	-
Glycerol	+	+	+	+	+	+	-
Histamine	-	-	-	-	-	-	-
3-Hydroxybenzoate	-	-	-	-	-	-	-
4-Hydroxybenzoate	+	-	-	(+)	+	+	(+)
Lactose	(+)	(+)	(+)	(+)	(+)	(+)	(+)
Melibiose	+	+	+	+	+	+	+
α -Methyl glucoside	(+)	(+)	+	-	(+)	+	(+)
Mucate	-	-	-	-	+	+	+
Nicotinate	-	-	+	+	+	+	+
L-Ornithine	-	-	-	-	-	-	-
Quinate	+	+	+	+	+	+	+
Rhamnose	-	-	-	-	+	-	-
Sorbitol	-	-	-	-	+	+	+
D-Tartrate	-	-	-	-	-	-	-
Trigonelline	-	-	-	-	-	-	-
Pigment (red)	-	+	+	-	-	-	+
Odor "38-like"	-	-	-	-	-	-	-
β -Xylosidase (ONPX) ^b	+	-	+	(+)	-	-	+
Tetrathionate reduction	-	-	-	-	-	-	-
Lysine decarboxylase	-	-	-	-	-	-	-
Arginine dihydrolase	-	-	-	-	-	-	-
Ornithine decarboxylase	-	-	-	-	-	-	-
Gas from glucose	-	-	+	+	-	-	+
Growth at 4°C ^c	+	+	+	+	+	+	(+)

^a +, Growth in 1 to 4 days; (+), growth in 5 to 14 days.^b +, Positive in 2 h; (+), positive in 24 h.^c +, Good growth in 4 days; (+), good growth in 5 to 10 days.

(16). It is interesting that two strains (CDC 462-75 and 3513-75) were recovered from human sputum.

Biochemical characteristics of *S. odorifera* (38-like strains) are listed in Table 3. Two biotypes were distinguished. Strains of biotype 1 can grow on sucrose and ornithine but not on erythritol (as the sole carbon source), and have an ornithine decarboxylase, whereas biotype 2 strains give the opposite reactions in these tests.

All *S. odorifera* strains are gram-negative, peritrichous rods that grow well on ordinary media (e.g., nutrient agar), ferment D-glucose (without production of gas), are oxidase negative, and reduce nitrates to nitrites. They clearly are members of the family *Enterobacteriaceae* (8).

S. odorifera strains are not pigmented. However, they grow on the caprylate-thallos selective medium for *Serratia* (26) and hydrolyze gelatin, corn oil, and DNA but not Tween 80 or chitin. With rare exceptions, on most agar media (e.g., brain heart, Trypticase soy, nutrient, Mueller-Hinton) they give off a characteristic odor that resembles the smell of vegetable matter (14, 16).

S. odorifera biotype 1 includes strain 38, which was close to the genus *Serratia* by numerical taxonomy (16).

A characteristic of *S. odorifera* that may be of practical importance is the variability of results with both indole and Voges-Proskauer tests. Indole production was often scored "weak," and different methods produced differ-

ent results for acetoin production. Only the Richard method (21) seemed sensitive enough to detect acetoin production reproducibly.

Results of DNA relatedness studies obtained with labeled reference DNA from *S. plymuthica* 392 and from *S. odorifera* 1073 are shown in Table 4.

The main question about *S. plymuthica* was whether it could be distinguished from *S. liquefaciens*. *S. plymuthica* strains were more than 73% related to strain 392 at 60°C. At 75°C relatedness did not drop below 52%. For the least related strain of this species (2001-73), divergence was only 4.7%. Relatedness of *S. liquefaciens* strains to *S. plymuthica* 392 was lower and did not exceed 60% (at 60°C). At 75°C, this relatedness dropped significantly (to 31% or lower), and divergence was about 9.8%. This is clearly illustrated by thermal elution profiles of DNA duplexes (Fig. 1). The thermal elution profiles (with *S. plymuthica* 392 labeled DNA) were almost superposable when the unlabeled DNA was from either *S. plymuthica* 392 (the centrotypic strain of phenon C2, reference 16) or *S. plymuthica* 510 (the type strain isolated in 1887). $T_{m(e)}$ values were respectively 91 and 90.4°C. However, when the unlabeled DNA was from either *S. liquefaciens* 14460 (the type strain) or *S. liquefaciens* 6136-66 (the centrotypic strain of phenon C1, reference 16), the thermal elution profiles shifted toward lower temperatures. $T_{m(e)}$ values were 81.3°C for both duplexes.

Other species of *Serratia* were 26 to 53% related to *S. plymuthica* and non-*Serratia* species showed less than 20% relatedness. The exception was the "Citrobacter-like" group of Leclerc and Buttiaux (7, 17), which showed relatedness to *S. plymuthica* similar to that of other *Serratia* species. A summary of relatedness values of *Serratia* species (plus the "Citrobacter-like" group) to *S. plymuthica* 392 is shown with calculated standard deviations in Table 5. These standard deviations, taking into account intra-strain (reproducibility) and interstrain (homogeneity) variations, allow a better interpretation of DNA relatedness values.

Members of *S. odorifera* were 60 to 98% related to strain 1073. Related sequences showed little divergence (0.3 to 4.0%), and the relative binding at 75°C was at least 58%. Strains assigned to biotype 1 were more related to strain 1073 (over 70%) than were the strains of biotype 2 (60 to 69%). However, the stability of reassociated sequences supports inclusion of biotype 2 strains in the same hybridization group with biotype 1. The average relative binding of DNA from all previously known *Serratia* species and

of *Citrobacter*-like group to *S. odorifera* 1073 was found in the range of 31 to 38%. The most related strain studied was *S. liquefaciens* 6136-66 (centrotypic), with 43% relatedness to strain 1073. The lowest relatedness of a *Serratia* strain to 1073 was that of *S. marcescens* 868-57 (28% relatedness). Members of other genera, except for the *Citrobacter*-like group, were not closely related to *S. odorifera* 1073. Divergence of related sequences in *S. odorifera*/other *Serratia* spp. hybridizations was in the range of 10 to 13%.

DISCUSSION

These results of DNA relatedness correlate well with those obtained by numerical taxonomy (16). Both types of data confirm that *S. plymuthica* and *S. liquefaciens*, although closely related, are distinct both phenetically and genetically. They also have different proteinase patterns in agar gel electrophoresis (15). *S. marcescens* is next most closely related to *S. plymuthica*, followed by the *Citrobacter*-like group, and finally by *S. marinorubra*. When the reference labeled DNA is from *S. liquefaciens* or *S. marcescens* (27), *S. marinorubra* is also the most distant *Serratia* species. These relationships among *Serratia* species, based on relatedness of DNA, are in complete agreement with phenetic data (16). Strain 1286-57 of Steigerwalt et al. (27), labeled *S. liquefaciens*, is now identified as *S. plymuthica*. When its DNA was compared with labeled DNA from *S. liquefaciens* 446-68, a low percent relatedness was found at 75°C, and divergence was as high as 9.8% (27). Recognition of *S. plymuthica* as a separate species from *S. liquefaciens* reduces the heterogeneity of *S. liquefaciens* that was apparent in the study of Steigerwalt et al. (27).

S. plymuthica has probably no clinical significance for humans. Among several thousand *Serratia* spp. identified at the CDC during the last 20 years, only two isolates of *S. plymuthica* were from humans. The major habitat of *S. plymuthica* is probably water (16).

The 25 strains that resemble strain 38 share a number of common biochemical characteristics, and the two biotypes differ in only four tests. Although DNA relatedness data at 60°C reflect this subdivision into two biotypes, DNA duplexes are stable (low percent divergence). These 25 strains constitute one hybridization group clearly distinct from 17 others included in this study. We conclude that these 38-like strains constitute a discrete bacterial species.

The hybridization groups closest to the 38-like species are *Serratia* spp. and the *Citrobacter*-

TABLE 3. *Biochemical characteristics of Serratia odorifera*^a

Characteristic	Biotype 1	Biotype 2	Strain(s) with opposite sign ^b
No. of strains	17	8	
Carbon source utilization			
Aconitate	+ ^c 17 ^d	+ 8	
Adonitol	+ 17	+ 8	
D-Alanine	+ 17	+ 8	
L-Alanine	+ 17	+ 8	
4-Aminobutyrate	+ 16 (1) ^e	+ 7 (1)	
L-Arabinose	+ 17	+ 8	
D-Arabitol	- 0	- 0	
Benzoate	- 0	- 0	
Betaine	- 0	- 0	
Caprylate	+ 12 (5)	+ 8	
DL-Carnitine	- 0	- 0	
D-Cellobiose	+ 17	+ 8	
meso-Erythritol	- 0	+ 8	
D-Glucosamine	+ 17	+ 8	
Histamine	- 0	- 0	
3-Hydroxybenzoate	- 0	- 0	
4-Hydroxybenzoate	- 0	- 0	
Kynurenate	+ 17	+ 8	
D-Lactose	(+) 2 (15)	(+) 1 (7)	
Maltose	+ 17	+ 8	
D-Melibiose	+ 17	+ 7 (1)	
α -Methyl glucoside	- 0	- 0	
Mucate	+ 17	+ 7 (1)	
Nicotinate	+ 14 (3)	+ 6 (1)	3472-74
L-Ornithine	(+) (13)	- 0	5248-71, 3138-72, 2-73, 17-74
L-Proline	+ 17	+ 8	
Putrescine	+ 17	+ 8	
Quinate	- 0	- 0	
L-Rhamnose	+ 17	+ 5 (3)	
D-Sorbitol	+ 17	+ 8	
Sucrose	+ 16	- 0	1877-71
D-Tartrate	(+) 2 (15)	(+) (6)	292-74, 1945-77
Trigonelline	+ 17	+ 8	
D-Tyrosine	+ 17	+ 8	
Growth on caprylate-thallos agar	+ 16 (1)	+ 8	
Pigment	- 0	- 0	
Odor "38-like"	+ 15	+ 7	2-73, 17-74; 2346-74
Motility	+ 17	+ 7	2861-74
Growth at 4°C	+ 15 (2)	+ 8	
Oxidase	- 0	- 0	
ONPG (β -galactosidase)	(+) 8 (9)	(+) 4 (4)	
ONPX (β -xylosidase)	+ 16 (1)	+ 8	
Tetrathionate reduction	- 0	- 0	
Indole	(+) (13)	(+) (5)	3252-68, 3138-72, 38, 652-73; 2861-74, 3472-74, 2346-74
Methyl red	(+) (17)	(+) (8)	
Voges-Proskauer	- (7)	(+) (7)	4321-70, 652-73, 2985-73, 2-73, 17-74, 12-76, 11-77; 2861-74
Voges-Proskauer (Richard)	+ 11 (5w)	+ 7 (1w)	3138-72
Citrate (Simmons)	(+) 4 (6)	(+) 1 (4)	3252-68, 4321-70, 1877-71, 5248-71, 3138-72, 133-74, 38; 2346-74, 3567-75, 1517-76
H ₂ S-triple sugar iron	- 0	- 0	
Urea	- 1 w	- 0	17-74
Phenylalanine	- 0	- 0	
Lysine decarboxylase	+ 17	+ 8	
Arginine dihydrolase	- 0	- 0	

TABLE 3—*Continued*

Characteristic	Biotype 1	Biotype 2	Strain(s) with opposite sign ^b
Ornithine decarboxylase	+ 15 (1)	— 0	5248-71
Malonate	— 0	— 0	
KCN	+ 7 (3)	— 1	4409-66, 3252-68, 5112-69, 4321-70, 4933-70, 1877-71, 5248-71; 292-74
Nitrate	+ 17	+ 8	
Gelatin (tube method)	+ 16	+ 8	38
Gelatin (plate method)	+ 16 (1w)	+ 8	
Tween 40	(+ w) (15)	(+ w) (7)	38, 17-74; 2346-74
Tween 60	(+ w) (13)	— (1 w)	38, 1073, 17-74, 11-77; (292-74)
Tween 80	— 0	— 0	
Corn oil	— 5 (2)	+ 8	3252-68, (5112-69), 4321-70, 1877-71, 2985-73, (133-74), 2-73
Deoxyribonuclease	+ 17	+ 8	
Chitin	— 0	— 0	
Pectate	— 0	— 0	
Gluconate test	+ 17	+ 8	
Iodoacetate test	+ 17	+ 8	
Glucose fermentation (oxidation/fermentation)	+ 17	+ 8	
Glucose, gas	— 0	— 0	
Acid from:			
Lactose	(+) (16)	(+) (8)	4409-66
Sucrose	+ 16 (1)	— 0	
Mannitol	+ 17	+ 7 (1)	
Dulcitol	— 0	— 0	
Salicin	+ 17	(+) (7)	1396-75
Adonitol	(+) (9)	(+) 1 (4)	1073, 5112-69, 4321-70, 1877-71, 5248-71, 652-73, 2985-73, 133-74; 2861-74, 1396-75, 1517-76
Inositol	(+) 6 (9)	+ 6 (2)	5112-69, 4933-70
Sorbitol	+ 17	+ 8	
Arabinose	+ 17	+ 8	
Raffinose	+ 17	— (2)	2346-74, 1945-77
Rhamnose	+ 13 (3)	+ 5 (2)	652-73; 1396-75
Maltose	+ 16 (1)	+ 8	
Xylose	+ 17	+ 8	
Trehalose	+ 17	+ 8	
Cellobiose	+ 17	+ 8	
α -Methyl glucoside	— 0	— 0	
Erythritol	— 0	(+) (6)	292-74, 3567-75
Melibiose	+ 17	+ 7 (1)	
D-Arabitol	— 0	— (1)	1396-75
Mannose	+ 17	+ 8	
Glycerol	(+) (12)	(+) 1 (6)	3252-68, 5248-71, 652-73, 2985-73, 133-74; 1396-75
Esculin	+ 17	(+) (4)	292-74, 3472-74, 3567-75, 1517-76
Mucate	— (5)	— 1 (3)	(3252-68, 1877-71, 3138-72, 652-73, 2985-73); 292-74, (2861-74, 1517-76, 2346-74)
Tartrate (Jordan)	+ 17	+ 8	
Acetate	(+) (9)	(+) (5)	38, 1073, 5112-69, 4321-70, 4933-70, 3138-72, 2985-73, 133-74; 292-74, 1517-76, 1945-77

^a Parentheses indicate delayed reaction: Carbon source utilization tests, growth in 5 to 14 days; Tweens, precipitation in 5 to 10 days; other tests, positive in 2 to 4 days. Abbreviation: w, weak reaction.

^b Strains that gave a reaction different from that indicated for their respective biotype. Biotype 1 strain numbers are in roman type; biotype 2 strain numbers are in italic type.

^c Symbols: +, most strains positive; (+), most strains delayed positive; —, most strains negative.

^d Number of positive reactions (excluding delayed positive).

^e Number of delayed positive reactions.

TABLE 4. Reassociation of DNA from *Serratia plymuthica* 392 and *S. odorifera* 1073

Source of unlabeled DNA	<i>S. plymuthica</i> 392			<i>S. odorifera</i> 1073		
	% Relative binding		$\Delta T_{m(e)}$ at 60°C	% Relative binding		$\Delta T_{m(e)}$ at 60°C
	60°C	75°C		60°C	75°C	
<i>S. plymuthica</i>						
392	100	100	0	38 ± 5.0	16	
299	91 ± 7.5	81		37 ± 3.0	16	
510	100 ± 5.7	85	0.6	36 ± 6.2	13	10.8
1286-57	88 ± 13.1	69		40 ± 2.5	10	
3855-62	85 ± 19.5	71		40 ± 9.7	14	
1210-70	82 ± 6.3	77		39 ± 7.8	13	
4743-70	103 ± 1.1	83		38 ± 4.0	15	
2000-73	86 ± 3.8	53		36 ± 2.6	14	
2001-73	73 ± 5.2	52	4.7	36 ± 2.0	12	
462-75	83 ± 17.6	65		40 ± 4.4	15	
3513-75	88 ± 7.9	88		34 ± 1.5	15	
<i>S. odorifera</i> (biotype 1)						
1073	36 ± 4.9	14	12.9	100	100	0
38	36 ± 4.9	12		98 ± 1.0	98	0.3
4409-66	36 ± 3.6	13		70 ± 3.0	85	
3252-68	41 ± 9.0	11		96 ± 4.4	91	
4321-70	41 ± 10.0	12		79 ± 4.9	115	
5248-71	34 ± 1.0	10		71 ± 3.1	95	
652-73	35 ± 2.5	15		81 ± 5.5	107	
<i>S. odorifera</i> (biotype 2)						
292-74	35 ± 6.0	11		60 ± 5.2	62	4.0
1396-75	34 ± 3.8	11		69 ± 1.5	58	
3567-75	37 ± 4.5	10		69 ± 7.5	64	
1517-76	36 ± 2.6	12		67 ± 5.5	59	
<i>S. liquefaciens</i>						
14460	59 ± 3.2	23	9.8	31 ± 4.6	12	13.3
" <i>E. liquefaciens</i> "	60 ± 12.5	27		33 ± 6.8	11	
446-68	49 ± 2.1	22		33 ± 6.8	5	
6136-66	58 ± 6.7	31	9.7	43 ± 5.9	9	11.3
1107-57	53 ± 0.7	—		39 ± 0.7	—	
1284-57	31 ± 2.1	—		36 ± 0.7	—	
<i>S. marcescens</i>						
SM6	53 ± 8.7	27	8.1	31 ± 9.3	20	10.4
866-57	43 ± 3.5	23		29 ± 8.5	15	
868-57	36 ± 8.7	22		28 ± 2.6	15	
5147-64	47 ± 6.6	26	10.2	34 ± 2.1	17	
1201-65	46 ± 3.1	26		34 ± 7.0	17	11.5
<i>S. marinorubra</i> (<i>S. rubidaea</i>)						
4445-64	29 ± 3.1	15		31 ± 6.0	15	
5474-68	31 ± 3.1	13		34 ± 10.4	15	
4057-71	30 ± 3.2	14	8.8	35 ± 4.0	20	10.0
934-72	26 ± 1.7	13		29 ± 3.6	15	
" <i>Citrobacter</i> -like"						
4552-71	36 ± 2.5	6		36 ± 8.1	8	13.0
4554-71	33 ± 3.1	12	14.2	40 ± 4.9	10	
<i>Enterobacter aerogenes</i>	15 ± 3.5			15 ± 0.7		
1627-66						
<i>Enterobacter cloacae</i>						
1347-71	17 ± 4.2			22 ± 4.9		
256-4 (Y)	13 ± 6.4			20 ± 0.0		
<i>Enterobacter agglomerans</i>	12 ± 2.1			18 ± 0.7		
2780-70						
<i>Enterobacter</i> sp. 76-01	15 ± 1.4			12 ± 3.5		
<i>Hafnia alvei</i>						
5632-72	16 ± 4.2			20 ± 2.8		
329-73	15 ± 0.7			21 ± 2.8		
4510-75	14 ± 0.0			17 ± 0.7		
<i>Erwinia carotovora</i> 495	15 ± 1.4			19 ± 2.8		
<i>Erwinia amylovora</i> EA 178	16 ± 0.7			17 ± 1.4		
<i>Citrobacter freundii</i> 460-61	19 ± 4.2			18 ± 2.1		
<i>Proteus mirabilis</i> PR 14	7 ± 2.1			7 ± 2.1		
<i>Escherichia coli</i> K-12	10 ± 2.8			18 ± 3.5

FIG. 1. Thermal elution profiles of *Serratia plymuthica* 392 DNA duplexes. ^{32}P -labeled DNA from *S. plymuthica* 392 was incubated with unlabeled DNA from *S. plymuthica* 392 (●), *S. plymuthica* 510 (○), *S. liquefaciens* CDC 6136-66 (▲), and *S. liquefaciens* ATCC 14460 (△). Incubations were carried out in 0.14 M phosphate buffer (PB) at 60°C. After incubation the reaction mixtures were added to a test tube held at 60°C and containing 10 ml of packed hydroxyapatite and subjected to thermal batch chromatography on hydroxyapatite and assayed as described in the text.

TABLE 5. Summary of reassociation of DNA at 60°C from *S. plymuthica* 392 and *S. odorifera* 1073

Species	Mean % relative binding $\pm$ SD ^a	
	<i>S. plymuthica</i> 392	<i>S. odorifera</i> 1073
<i>Serratia odorifera</i> ^b	36 $\pm$ 6.0	76 $\pm$ 13.3
<i>S. plymuthica</i> ^b	88 $\pm$ 13.6	38 $\pm$ 5.5
<i>S. liquefaciens</i>	52 $\pm$ 12.4	36 $\pm$ 6.8
<i>S. marcescens</i>	45 $\pm$ 9.0	31 $\pm$ 7.2
<i>S. marinorubra</i> (<i>S. rubidaea</i>)	29 $\pm$ 3.5	32 $\pm$ 7.2
" <i>Citrobacter</i> -like"	35 $\pm$ 3.5	38 $\pm$ 7.3

^a Standard deviations (SD) were calculated as the square root of the sum of between strain variance and mean within strain variance.

^b Excluding homologous reactions.

like group. The percent DNA relatedness of *Serratia* spp. to the 38-like species is generally considered to be at the genus level (3, 4). In a numerical taxonomic study (16), strain 38 clustered with *S. marcescens*, *S. liquefaciens*, *S. plymuthica*, and *S. marinorubra* (S. *rubidaea*) at 70% similarity level (unweighted average linkage clustering). Furthermore, strains 38 and 1073 were susceptible to several *Serratia* phages, whereas five *Citrobacter*-like strains were resistant (F. Grimont, unpublished data). We conclude that the 38-like species may reasonably belong to the genus *Serratia*.

We found no description in the literature that fits the 38-like species. We therefore treat it as a new species to be called *S. odorifera* species novum (Latin feminine adjective *odorifera*: bringing odors, fragrant). We designate strain 1073 (ICPB 3995) as the type strain for *S. odorifera*.

This new species may be of clinical significance in view of the source of strains studied (Table 1). With commercial identification systems, *S. odorifera* may look like atypical (gelatin-positive, anaerogenic) *Enterobacter aerogenes*. However, the typical odor of *S. odorifera*

cultures should help to make a correct identification. The ability of *S. odorifera* strains to grow on caprylate-thallos agar (26) makes selective isolation possible (together with other *Serratia* spp.) in epidemiological and ecological studies.

DNA relatedness studies on *Citrobacter*-like strains were previously reported (27). This organism is certainly most closely genetically related to *Serratia*. Phenotypically it is closer to *Citrobacter*. H. Leclerc (personal communication) is presently examining a large number of strains both phenotypically and genetically.

Description of *Serratia odorifera* sp. nov.

Type strain. Grimont 1073, CDC 1979-77, ICPB 3995. Isolated from human sputum.

Cell characteristics. Gram-negative, peritrichous, nonsporeforming, straight rods resembling other *Enterobacteriaceae*. A thin capsule is occasionally observed.

Surface colonies. Surface colonies on nutrient agar are whitish, often iridescent, and slightly mucoid. Colony diameter is about 2 to 2.5 mm after overnight growth. Growth on agar media gives off a musty, potato-like odor. Colonies on horse blood agar are nonhemolytic.

Broth. Turbid; may form a white ring at surface or slight pellicle; gray sediment.

Growth factor requirements. There is no growth factor requirement.

Culture conditions. Facultatively aerobic; good growth occurs at temperatures between 4 and 40°C. Growth on caprylate-thallos selective medium for *Serratia*.

Nutritional abilities. The following carbon sources are utilized: aconitate, adonitol, D-alanine, L-alanine, 4-aminobutyrate, L-arabinose, caprylate, D-cellobiose, D-glucosamine, kynurenate, maltose, D-melibiose, mucate, nicotinate, L-proline, putrescine, L-rhamnose, D-sorbitol, D-tartrate, trigonelline, and L-tyrosine. The following carbon sources are not utilized: D-arabitol, benzoate, betaine, DL-carnitine, histamine, 3-hydroxybenzoate, 4-hydroxybenzoate, α -methylglucoside, and quinate. Utilization of *m*-erythritol, L-ornithine, and sucrose serves to differentiate biotypes.

Biochemical characteristics. No oxidase, tetrathionate reductase, phenylalanine deaminase, or arginine dihydrolase (or decarboxylase). Presence of β -galactosidase (*o*-nitrophenyl- β -D-galactopyranoside test), β -xylosidase, and lysine decarboxylase. Malonate broth unchanged. Indole produced weakly. H₂S not produced from thiosulfate. Urea not hydrolyzed. Nitrates reduced into nitrites. Gelatin liquefied, DNA hydrolyzed. Corn oil and Tween 40 slowly

and irregularly hydrolyzed. Chitin, pectate, and Tween 80 not hydrolyzed. Reducing compounds produced from gluconate. Acid from glucose in the presence of iodoacetate. Acid from arabinose, cellobiose, glucose, inositol, maltose, mannitol, mannose, melibiose, rhamnose, salicin, sorbitol, trehalose, and xylose. Acid slowly produced from lactose. No acid from D-arabitol, dulcitol, and α -methylglucoside. No gas from glucose. Methyl red test positive. Acetylmethylcarbinol produced (Voges-Proskauer test variable according to the technique). Two biotypes differ by ornithine decarboxylase and fermentation of sucrose and raffinose.

Habitat. Found in human clinical specimens and in food.

ACKNOWLEDGMENT

During the period this study was effected, P. A. D. Grimont was the recipient of a Bourse de Recherche du Ministère des Affaires Étrangères (France).

REPRINT REQUESTS

Address reprint requests to: P. A. D. Grimont, Service des Entérobactéries, Institut Pasteur, F-75724 Paris Cedex 15, France.

LITERATURE CITED

1. Bascomb, S., S. P. Lapage, W. R. Willcox, and M. A. Curtis. 1971. Numerical classification of the tribe *Klebsiellae*. *J. Gen. Microbiol.* 77:291-315.
2. Breed, R. S., E. G. D. Murray, and A. P. Hitchens (ed.). 1948. *Bergey's manual of determinative bacteriology*, 6th ed. The Williams and Wilkins Co., Baltimore.
3. Brenner, D. J. 1973. Deoxyribonucleic acid reassociation in the taxonomy of enteric bacteria. *Int. J. Syst. Bacteriol.* 23:298-307.
4. Brenner, D. J., G. R. Fanning, K. E. Johnson, R. V. Citarella, and S. Falkow. 1969. Polynucleotide sequence relationships among members of the *Enterobacteriaceae*. *J. Bacteriol.* 98:637-650.
5. Brenner, D. J., G. R. Fanning, A. Rake, and K. E. Johnson. 1969. A batch procedure for thermal elution of DNA from hydroxyapatite. *Anal. Biochem.* 28:447-460.
6. Brenner, D. J., G. R. Fanning, F. J. Skerman, and S. Falkow. 1972. Polynucleotide sequence divergence among strains of *Escherichia coli* and closely related organisms. *J. Bacteriol.* 109:953-965.
7. Crossa, J. H., A. G. Steigerwalt, G. R. Fanning, and D. J. Brenner. 1974. Polynucleotide sequence divergence in the genus *Citrobacter*. *J. Gen. Microbiol.* 83:271-282.
8. Edwards, P. R., and W. H. Ewing. 1972. *Identification of Enterobacteriaceae*, 3rd ed. Burgess Publishing Co., Minneapolis.
9. Ewing, W. H., B. R. Davis, M. A. Fife, and E. F. Lessel. 1973. Biochemical characterization of *Serratia liquefaciens* (Grimes and Hennerty) Bascomb et al. (formerly *Enterobacter liquefaciens*) and *Serratia rubidaea* (Stapp) comb. nov. and designation of type and neotype strains. *Int. J. Syst. Bacteriol.* 23:217-225.
10. Ewing, W. H., B. R. Davis, and J. G. Johnson. 1962. The genus *Serratia*: its taxonomy and nomenclature. *Int. J. Syst. Bacteriol.* 12:47-52.
11. Ewing, W. H., B. R. Davis, and R. W. Reavis. 1959. Studies on the *Serratia* group. Center for Disease Control, Atlanta, Ga.

12. **Fulton, M., C. E. Forney, and E. Leifson.** 1959. Identification of *Serratia* occurring in man and animals. *Can. J. Microbiol.* **5**:269-275.
13. **Grimes, M., and A. J. Hennerty.** 1931. A study of bacteria belonging to the subgenus *Aerobacter*. *Sci. Proc. Roy. Dublin Soc.* **20**:89-97.
14. **Grimont, P. A. D., and H. L. C. Dulong de Rosnay.** 1972. Numerical study of 60 strains of *Serratia*. *J. Gen. Microbiol.* **72**:259-268.
15. **Grimont, P. A. D., F. Grimont, and H. L. C. Dulong de Rosnay.** 1977. Characterization of *Serratia marcescens*, *S. liquefaciens*, *S. plymuthica* and *S. marinorubra* by electrophoresis of their proteinases. *J. Gen. Microbiol.* **99**:301-310.
16. **Grimont, P. A. D., F. Grimont, H. L. C. Dulong de Rosnay, and P. H. A. Sneath.** 1977. Taxonomy of the genus *Serratia*. *J. Gen. Microbiol.* **98**:39-66.
17. **Leclerc, H., and R. Buttiaux.** 1965. *Les Citrobacter*. *Ann. Inst. Pasteur (Lille)* **16**:67-74.
18. **Lehmann, K. B., and R. Neumann.** 1896. Atlas und Grundriss der Bakteriologie und Lehrbuch der Speziellen bakteriologischen Diagnostik. Teil 2. Lehmann, Munich.
19. **McConaughy, B. L., and B. J. McCarthy.** 1970. Related base sequences in the DNA of simple and complex organisms. VI. The extent of base sequence divergence among the DNAs of various rodents. *Biochem Genet.* **4**:425-446.
20. **Martinec, T., and M. Kocur.** 1961. Taxonomická studie rodu *Serratia*. *Folia Fac. Sci. Nat. Univ. Purkynianae Brunensis.* **2**:1-77.
21. **Richard, C.** 1972. Méthode rapide pour l'étude des réactions de rouge de méthyle et Voges-Proskauer. *Ann. Inst. Pasteur (Paris)* **122**:979-986.
22. **Richard, C., B. Joly, J. Sirot, G. H. Stoleru, and M. Popoff.** 1976. Etude de souches de *Enterobacter* appartenant à un groupe particulier proche de *E. aerogenes*. *Ann. Microbiol. (Inst. Pasteur)* **127A**:545-548.
23. **Sakazaki, R.** 1974. Genus IX. *Serratia* Bizio 1823, 288, p. 326. In R. E. Buchanan and N. E. Gibbons (ed.), *Bergey's manual of determinative bacteriology*, 8th ed. The Williams and Wilkins Co., Baltimore.
24. **Sneath, P. H. A., and V. B. D. Skerman.** 1966. A list of type and reference strains of bacteria. *Int. J. Syst. Bacteriol.* **16**:1-133.
25. **Stapp, C.** 1940. *Bacterium rubidaenum* nov. spec. *Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 2* **102**:251-260.
26. **Starr, M. P., P. A. D. Grimont, F. Grimont, and P. B. Starr.** 1976. Caprylate-thallos agar medium for selectively isolating *Serratia* and its utility in the clinical laboratory. *J. Clin. Microbiol.* **4**:270-276.
27. **Steigerwalt, A. G., G. R. Fanning, M. A. Fife-Asbury, and D. J. Brenner.** 1976. DNA relatedness among species of *Enterobacter* and *Serratia*. *Can. J. Microbiol.* **22**:121-137.