Serratia fonticola, a New Species from Water

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Twenty strains of *Enterobacteriaceae* isolated from water are assigned to a new species in the genus *Serratia* on the basis of phenetic (numerical analysis) and genetic (deoxyribonucleic acid [DNA]-DNA hybridization) evidence. They are closely related to each other (75 to 91% DNA-DNA relatedness) and show 50% DNA relatedness with species of *Serratia* but only 20 to 30% relatedness with other members of the family *Enterobacteriaceae*. The strains are acetoin, gelatinase, and deoxyribonuclease negative, but lysine, ornithine decarboxylase, and Tween-esterase positive. They are metabolically very active, fermenting all the sugars and alcohols tested except melezitose, inulin and sorbose. The name *Serratia fonticola* is proposed for these strains. Strain 11 (= ATCC 29844) is the type strain of the species.

In 1965, Leclerc and Buttiaux (18) described a new group of *Enterobacteriaceae* which seemed related to the genus *Citrobacter* on the basis of its IMViC characters (indole positive, methyl red positive, Voges-Proskauer [acetoin] positive, Simmons citrate positive) but which differed from *Citrobacter* in other significant properties such as lysine decarboxylase production.

In a recent numerical taxonomy study (12), we confirmed the relationship of these strains to each other. Crosa et al. (3) and subsequently Steigerwalt et al. (25) in a deoxyribonucleic acid (DNA)-DNA hybridization study established that this group of "Citrobacter-like" bacteria, referred to as "class C," are more closely related to *Serratia* than to *Citrobacter* or any other *Enterobacteriaceae*.

Taking this into account, we carried out another numerical taxonomy study with the strains of class C and strains of *Serratia*, including type and neotype strains. Moreover, we evaluated the genetic relatedness of the class C strains through DNA-DNA hybridization and assessed the rate of relatedness of the class C reference strain with *serratiae* (*S. marcescens*, *S. liquefaciens*, *S. plymuthica*, and *S. rubidaea*). Finally, we have attempted to determine the taxonomic position of the class C strains.

MATERIALS AND METHODS

Bacterial strains. The phenetic study included 72 strains isolated from water and soil (20 of them cluster in the previously defined class C) and 20 *Serratia* strains from culture collections (Table 1).

The DNA-DNA hybridization studies were carried out with the following strains: 15 class C strains, 3 strains representative of class C as defined in the numerical taxonomy study, and 32 culture collection strains of the family *Enterobacteriaceae* (see Table 5).

Biochemical tests. The biochemical tests used were described previously (11, 12).

Numerical analysis. After elimination of the characters which were positive or negative for all of the strains, the remaining characters (117 of 219) were analyzed. Similarity between strains was calculated by means of the DICE index (11). Classification of the strains was based on the hierarchical aggregation method, using average distance (4).

DNA-DNA hybridization. DNA extraction was performed by Marmur's method (21) as slightly modified by Ferragut and Leclerc (9).

The purity and quality of each DNA preparation were checked by determination of the absorbancy ratio at 260/280 nm (1.8 to 1.9) and at 260/230 nm (2.0 to 2.3) (according to Marmur [21]) and by the melting (T_m) curves, which include hyperchromicity values from 30 to 40% (9, 20).

The strain chosen for DNA labeling was the reference strain of class C (strain 11). For the preparation of labeled DNA, 1 mCi of [³H]thymidine (specific activity, 10 to 25 Ci/mmol; Centre Energie Atomique, Gif sur Yvette, France) was added to 500 ml of a synthetic medium containing 4 g of glucose per liter, 4 g of Casmino Acids per liter, and 250 µg of deoxyadenosine per liter to inhibit the thymidylate synthetase. The cells were harvested in the late log phase. The specific activity was 80,000 cpm/µg for ³H-labeled DNA. The unlabeled DNA of high molecular weight was fixed and denatured on nitrocellulose filters by the technique described by De Ley and Tytgat (5).

Fragmentation of unlabeled and labeled DNA was

carried out in a French pressure cell (10) at a pressure of 20,000 lb/in².

DNA-DNA reassociation was done in a double-strength citrate salt solution (SSC; 0.15 M NaCl-0.015 M trisodium citrate, pH 7.0) with 30% dimethyl sulfoxide, as described by Legault-Demare et al. (19). The optimal temperature of renaturation in buffer 2 SSC-30% dimethyl sulfoxide (TorD) was calculated from De Ley and Tytgat's equation: $TorD = (0.51 \times \text{mol\% guanine plus cytosine [G+C]}) + 28.0$. The G+C content of labeled DNA of strain 11 equaled 51.5 mol%. Consequently, the optimal temperature of incubation was 54.2°C. The time of incubation was 16 h.

Radioactivity counting was done by scintillation with 10 ml of Bray solution (2) in vials. The rate of reassociation was calculated by the formula: (counts per minute without competitor DNA - counts per minute with heterologous DNA / counts per minute without competitor DNA - counts per minute with homologous DNA) $\times$ 100.

RESULTS

Numerical analysis of *Serratia* strains. The distance level for optimal delimitation of classes, calculated by the method of Véron (26), was 0.87. According to this criterion, distinction of three main classes is then possible: S_A (32 strains), C (20 strains), and S_B (46 strains); two strains remained unclassified. Classes S_A and S_B can each be divided into two subclasses: S_{A1} (2 strains) and S_{A2} (30 strains), and S_{B1} (13 strains) and S_{B2} (23 strains) for a level equal to 0.54.

The 92 strains selected for this study were all oxidase negative and β -galactosidase positive, attacked glucose by a fermentative pathway, and used citrate as a sole carbon source.

Classes S_{A1}, S_{A2}, and S_{B2} are identical with the three species recognized by Ewing et al. (7) within the genus *Serratia*—*S. rubidaea*, *S. marcescens*, and *S. liquefaciens*, respectively—and are encompassed by the single species, *S. marcescens*, recognized by Sakazaki (23) in the genus *Serratia* in *Bergey's Manual*. Class S_{B1} is similar to *S. plymuthica* as described by Grimont et al. (14).

The characters of the four subclasses (see Table 2) as a whole agree with those of the four species of the genus as defined by Edwards and Ewing (6), Ewing et al. (7), Johnson et al. (16), Grimont and Dulong De Rosnay (13), and Grimont et al. (14).

Phenetic study of class C strains. Table 3 gives the characters of class C and those of strain 11 which are conventionally determined for *Enterobacteriaceae*. Studies on the utilization of various additional substrates which led to the classification and clustering of these bacteria were presented in a previous study (12).

Genetic study of class C strains. (i) DNA-DNA hybridization. The DNA relatedness of

strain 11 of class C to 14 other isolates of this class ranged from 75 to 91%; relatedness with 13 of the strains exceeded 80% (see Table 4).

Table 5 presents the DNA relatedness between strain 11 and strains belonging to different species and genera of *Enterobacteriaceae*.

For strains within a species, the DNA relatedness results are given in descending order. The *Serratia* strains were the only ones which gave rates of hybridization of over 40% with strain 11. For all the other strains, the relatedness results were close to 25 to 30%.

(ii) **G+C content of DNA.** The G+C contents of the DNAs of the strains of class C were studied by Ferragut et al. (8); the results are summarized in Table 6. The mean G+C content, 50.9 mol%, varies greatly from the G+C content of most of the strains of the genus *Serratia* recorded in Table 6. However, it is close to those of strains of *S. liquefaciens*: 51.9 mol% for ATCC 14460 (24) and 50.9 mol% for the strain studied by Izard et al. (15).

DISCUSSION

The uniqueness of the class C strains among the *Enterobacteriaceae* was first suggested by Leclerc and Buttiaux (18) and was confirmed by the results of both our previous numerical taxonomy study (12) and the present investigation. Class C strains also represent a new entity from the genetic point of view. The DNA relatedness of the class C strains ranges between 75 and 91% (Table 4). The closeness of these values is substantiated by the results (DNA reassociation, 93 to 100%) obtained by Crosa et al. (3) with five strains of *Citrobacter*-like bacteria.

Table 5 presents the results of DNA-DNA reassociation between strain 11 and other *Enterobacteriaceae*. *Serratia* is the only genus whose members showed a taxonomically significant level of relatedness to strain 11. The very low results obtained with the strains of all the other species suggest that the strains of class C should be placed in the genus *Serratia*.

The genus *Serratia* was recently studied in detail by Steigerwalt et al. (25), who determined that *S. marcescens*, *S. liquefaciens*, and *S. rubidaea* are closely related (see Table 7). For the seven strains of *S. marcescens* analyzed, the DNA relatedness varied from 84 to 100%; for six strains of *S. liquefaciens*, it varied from 68 to 100%; and for four strains of *S. rubidaea*, it varied 10 to 100%. The relatedness between *S. marcescens* and *S. liquefaciens* is rather high, i.e., between 50 and 60%.

The DNA-DNA relatedness of *S. rubidaea* to other *Serratia* species is relatively low (hybridization levels below 50%) except for one strain,

TABLE 1. *Strains included in the phenetic study*^a

Taxon	Strain no.	Source	Culture collection no.	Name as received
S _{A1} (<i>S. rubidaea</i>)	22	?	CDC 2199-72	<i>S. rubidaea</i>
	91	?	S ₃₆	
S _{A2} (<i>S. marcescens</i>)	23	MA	CUETM 78-210	Original isolate
	24	MA	CUETM 78-200	Original isolate
	25	MA	CUETM 78-216	Original isolate
	26	MA	CUETM 78-211	Original isolate
	27	MA	CUETM 78-202	Original isolate
	28	MA	CUETM 78-199	Original isolate
	29	MA	CUETM 78-217	Original isolate
	30	MA	CUETM 78-215	Original isolate
	31	MA	CUETM 78-208	Original isolate
	32	MA	CUETM 78-207	Original isolate
	33	MA	CUETM 78-209	Original isolate
	34	MA	CUETM 78-213	Original isolate
	35	MA	CUETM 78-212	Original isolate
	36	MA	CUETM 78-203	Original isolate
	37	MA	CUETM 78-214	Original isolate
	38	MA	CUETM 78-198	Original isolate
	39	MA	CUETM 78-205	Original isolate
	40	MA	CUETM 78-206	Original isolate
	41	MA	CUETM 78-204	Original isolate
	42	MA	CUETM 78-201	Original isolate
	47	D	CUETM 78-248	Original isolate
	21	?	ATCC 13 880	<i>S. marcescens</i>
	44	?	LM 23-65	<i>S. marcescens</i>
	79	?	CUETM 78-163	<i>Serratia</i> sp.
	80	?	CUETM 78-164	<i>Serratia</i> sp.
	86	?	CUETM 78-165	<i>Serratia</i> sp.
	87	?	CUETM 78-166	<i>Serratia</i> sp.
	88	?	CUETM 78-167	<i>Serratia</i> sp.
	89	?	CUETM 78-168	<i>Serratia</i> sp.
	90	?	CUETM 78-169	<i>Serratia</i> sp.
S _{B1} (<i>S. plymuthica</i>)	45	D	CUETM 78-225	Original isolate
	46	D	CUETM 78-226	Original isolate
	50	D	CUETM 78-224	Original isolate
	62	D	CUETM 78-218	Original isolate
	65	D	CUETM 78-221	Original isolate
	68	D	CUETM 78-220	Original isolate
	72	D	CUETM 78-219	Original isolate
	73	D	CUETM 78-222	Original isolate
	74	D	CUETM 78-223	Original isolate
	75	?	B 3617	<i>S. plymuthica</i>
	84	?	CUETM 78-170	<i>Serratia</i> sp.
	85	?	CUETM 78-171	<i>Serratia</i> sp.
S _{B2} (<i>S. liquefaciens</i>)	43	Su	CUETM 78-242	Original isolate
	49	D	CUETM 78-238	Original isolate
	51	D	CUETM 78-244	Original isolate
	52	D	CUETM 78-240	Original isolate
	53	D	CUETM 78-229	Original isolate
	54	D	CUETM 78-246	Original isolate
	55	D	CUETM 78-230	Original isolate
	56	D	CUETM 78-236	Original isolate
	57	D	CUETM 78-243	Original isolate
	58	D	CUETM 78-241	Original isolate
	59	D	CUETM 78-239	Original isolate

TABLE 1. (continued)

Taxon	Strain no.	Source	Culture collection no.	Name as received
	60	D	CUETM 78-245	Original isolate
	61	D	CUETM 78-237	Original isolate
	63	D	CUETM 78-234	Original isolate
	64	D	CUETM 78-235	Original isolate
	66	D	CUETM 78-228	Original isolate
	67	D	CUETM 78-247	Original isolate
	69	D	CUETM 78-231	Original isolate
	70	D	CUETM 78-197	Original isolate
	71	D	CUETM 78-232	Original isolate
	76	So	CUETM 78-233	Original isolate
	77	?	CUETM 78-172	<i>Serratia</i> sp.
	78	?	CUETM 78-173	<i>Serratia</i> sp.
	81	?	CUETM 78-174	<i>Serratia</i> sp.
	92	?	ATCC 14 460	<i>E. liquefaciens</i>
Class C	1 (33) ^b	Se	CUETM 78-22	Original isolate
	5 (65)	Su	CUETM 78-7	Original isolate
	6 (80)	D	CUETM 78-43	Original isolate
	7 (81)	D	CUETM 78-9	Original isolate
	8 (89)	D	CUETM 78-11	Original isolate
	9 (92)	D	CUETM 78-15	Original isolate
	10 (93)	D	CUETM 78-26	Original isolate
	11 (95)	D	CUETM 78-165	Original isolate
	12 (98)	D	CUETM 78-28	Original isolate
	13 (99)	D	CUETM 78-12	Original isolate
	14 (100)	D	CUETM 78-45	Original isolate
	15 (101)	D	CUETM 78-25	Original isolate
	16 (102)	D	CUETM 78-6	Original isolate
	17 (104)	D	CUETM 78-49	Original isolate
	18 (105)	D	CUETM 77-143	Original isolate
	19 (106)	D	CUETM 78-20	Original isolate
	20 (107)	D	CUETM 77-168	Original isolate
	2 (40)	So	CUETM 78-14	Original isolate
	3 (45)	So	CUETM 78-16	Original isolate
	4 (52)	So	CUETM 78-10	Original isolate
Unclassified	82	?	CUETM 78-175	<i>Serratia</i> sp.
	83	?	CUETM 78-176	<i>Serratia</i> sp.

^a Abbreviations: B, J. Brisou, University of Poitiers, France; CDC, Center for Disease Control, Atlanta, Ga.; CUETM, Collection Unité d'Ecotoxicologie Microbienne, Villeneuve d'Ascq, France; LM, L. Le Minor, Institut Pasteur, Paris, France; D, drinking water; MA, medical analysis; Se, sewage; So, unpolluted soils; Su, surface water.

^b Numbers in parentheses are corresponding strain numbers of these strains used in a previous study (12).

which is 52% related to a strain of *S. liquefaciens*.

Some of the strains of the group of *Citrobacter*-like bacteria, four of which were studied here, were previously (25) assigned to one of three species of the genus *Serratia*. The DNA relatedness values of these bacteria ranged from 51 to 57% with *S. marcescens*, from 46 to 52% with *S. liquefaciens*, and from 41 to 44% with *S. rubidaea*. These values are very close to those obtained between species of the genus *Serratia*. The results of our determination of the DNA relatedness of the species *S. marcescens* (7

strains), *S. liquefaciens* (2 strains), *S. plymuthica* (2 strains), and *S. rubidaea* (3 strains) to labeled DNA of strain 11 of class C are given in Table 5. The data indicate a relationship of class C strains to the genus *Serratia*.

Two of the three analyzed strains of *S. rubidaea* (*S. rubidaea* CDC 934-72 and *S. marinorubra* (*S. rubidaea*) S 36) show very low hybridization levels with strain 11—24 and 30%, respectively. These data confirm the low level of relatedness of *S. rubidaea* with the other species of the genus. The identities of these two collection strains might also have to be revised. Except

TABLE 2. Characteristics useful in differentiating species of the genus *Serratia*^a

Characteristics	Class C (N = 20)			<i>S. rubidae</i> (S _{R1}) (N = 2)			<i>S. marcescens</i> (S _{M2}) (N = 30)			<i>S. plymuthica</i> (S _{P1}) (N = 13)			<i>S. liquefaciens</i> (S _{L2}) (N = 23)		
	Reaction	% +	% (+)	Reaction	No. of strains +	No. of Strains (+)	Reaction	% +	% (+)	Reaction	% +	% (+)	Reaction	% +	% (+)
Growth at pH 4	+	100		+	2		+	100		-	0		- or +	17	
Lysine decarboxylase	+	100		+	2		+	100		-	0		+	100	
Ornithine decarboxylase	+	100		+ or -	1		+	100		- or +	31		+	96	
Gelatin liquefaction (20°C)	-		5	+	2		+ or (+)	70	30	+	92		+	100	
Methyl red test (30°C)	+	100		-	0		-	3		-	0		- or +	17	
Methyl red test (37°C)	+	100		-	0		-	7		- or +	38		+ or -	86	
Voges-Proskauer reaction (30°C)	-	0		+	2		+	97		+	92		+	92	
Voges-Proskauer reaction (37°C)	-	0		+	2		+	97		+	100		+	100	
Mucate ^b	+	100		-	0		-	0		-	0		-	0	
D-Tartrate ^b	+ or -	65		-	0		-	0		-	0		-	0	
L-Tartrate ^b	+ or -	65		-	0		-	0		-	0		-	0	
Malonate	+	100		+	2		-	0		-	0		-	0	
Tetrathionate reductase	+	100		-	0		+	90		-	0		+	96	
Deoxyribonuclease	-	0		+	2		+	100		+	92		+	96	
β-Xylosidase	+	95		+	2		-	0		-	0		-	0	
Fermentation						2									
L-Arabinose	+	100		(+)			- or (+)		27	+	100		+	100	91
D-Glucose	+	100		-	0		-	0		(+) or +	15	55	(+)		91
D-Lactose	+	100		+	2		d	17	67	(+) or +	30	70	(+)		
L-Rhamnose	+ or -	86		-	0		-	0		d	8	23	- or +	40	
α-Methyl-D-glucoside	+	95		-	0		-	0		+ or (+)	85	8	-		4
Raffinose	+	100		+	2		- or +	27		+	92		+	96	
Melibiose	-	100		+	2		-	3		+	100		+	100	
D-Melezitose	-	0		+ or -	1		-	0		+	100		+	100	4
D-Sorbitol	+	100		-	0		+	100		- or +	23		+ or (+)	87	
D-Dulcitol	+	100		(+) or -	2		-	0		+	0		+	100	
D-Glycerol gas	+	95		+	1		-	7		+	100		+	100	
Adonitol	+	100		+	2		+ or (+)	87	13	-	9		-	8	
meso-Erythritol	(+) or +	5	85	-	0		-	7		-	0		-	0	
Stern glycerol	+ or -	80		-	0		-	0		-	0		-	0	
Carbon source utilization															
Fumarate	- or +	40		+	2		+	100		+ or -	77		+	91	
D-Lactate	+	100		+	2		+	93		- or +	38		+ or -	83	
Aconitate	- or +	40		+	2		+	100		+	92		+ or -	69	
2-Keto-glutarate	-	0		+	2		- or +	27		+ or -	69		+ or -	56	
D-Quinate	-	5		+	2		+ or -	60		+ or -	55		-	0	
Phenyl-acetate	+ or -	60		+	2		+ or -	83		+ or -	54		- or +	13	
Nicotinate	- or +	20		-	0		+ or -	80		+ or -	69		+ or -	87	
L-Aspartate	+ or -	80		-	0		-	3		-	0		+ or -	26	
n-Caproate	-	0		+ or -	1		- or +	20		- or +	15		+ or -	61	
L-Serine	- or +	25		+	2		+	100		+	100		+	100	
Betaine	-	0		+	2		+	0		-	8		-	0	
Trigonelline	- or +	20		+	2		+ or -	60		-	0		-	0	
Asparagine	+ or -	70		+	2		+	37		- or +	31		- or +	26	
Acetamide	+ or -	65		-	0		-	0		-	0		-	0	

^a Symbols: +, Positive reaction for at least 90% of the strains within 24 to 48 h; (+), slow positive reaction (example: between 2 and 30 days for fermentation reactions); + or -, - or +, the first sign corresponds to the most frequent result; -, negative reaction for at least 90% of the strains; d, different biochemical types [+, (+), -]; n, number of strains studied

^b Kauffmann's method (12)

TABLE 3. *Phenetic characterization of class C strains*

Characteristic	Class C (N = 20)			Strain 11 (= ATCC 29844)
	Reaction ^a	% +	% (+)	
Growth at 4°C	+4	100		+
Growth at 41°C	—	0		—
Growth at 44.5°C	—	0		—
Motility at 30°C	+	100		+
Motility at 37°C	+	100		+
KCN	+	95		+
Lysine decarboxylase	+	100		+
Arginine dihydrolase	—	0		—
Ornithine decarboxylase	+	100		+
Gelatin liquefaction (20°C)	—		5	—
Urease	—	0		—
Indole	—	0		—
Methyl red test (30°C)	+	100		+
Methyl red test (37°C)	+	100		+
Voges-Proskauer reaction (30°C)	—	0		—
Voges-Proskauer reaction (30°C)	—	0		—
Citrate (Simmons) 30°C	+	100		+
Citrate (Simmons) 37°C	+	100		+
Malonate	+	100		+
D-Tartrate ^b	+ or —	65		+
L-Tartrate ^b	+ or —	65		+
m-Tartrate ^b	—	0		—
Mucate ^b	+	100		+
Jordan tartrate	+	100		+
Phenylalanine deaminase	—	0		—
Nitrate to nitrite	+	100		+
H ₂ S production	—	0		—
Tetrathionate reductase	+	100		+
Tween-esterase	+	100		+
Deoxyribonuclease	—	0		—
Chitinolysis	—	0		—
β-Galactosidase	+	100		+
β-Xylosidase	+	95		+
Fermentation				
L-Arabinose	+	100		+
D-Xylose	+ or —	70		+
D-Ribose	+	100		+
Maltose	+	100		+
D-Glucose (gas)	+	100		+
D-Lactose	+	100		+
D-Galactose	+	100		+
L-Rhamnose	+ or —	80		+
D-Mannose	+	100		+
D-Fructose	+	100		+
α-Methyl D-glucoside	+	95		+
D-Trehalose	+	100		+
Raffinose	+	100		+
L-Sorbose	—	0		—
Melibiose	+	100		+
D-Melezitose	—	0		—
D-Sucrose	(+) or —	20	80	+7
D-Mannitol	+	100		+
D-Dulcitol	+	100		+2
meso-Inositol	+	95		+
D-Cellobiose (acid)	(+)		95	+6
D-Cellobiose (gas)	(+)		95	+5
D-Glycerol (gas)	+	95		+
Adonitol	+	100		+
meso-Erythritol	(+) or +	5	85	+5
Salicin	+	100		+
Inulin	— or (+)		20	—
Glycogen	(+) or —		60	+3
Esculin hydrolysis	+	100		+

^a Symbols as in Table 2. Numbers after symbols indicate number of days for reaction to take place.^b Kauffmann's method (6).

TABLE 4. DNA relatedness of 14 other strains of class C to strain 11

Class C strain (source of unlabeled DNA)	% Binding by competition with strain 11 DNA
11	100
8	91
7	91
4	90
6	90
12	89
21	89
9	88
10	88
2	87
15	87
18	85
16	84
20	81
1	75

for these two strains, the data on genetic information are in very good agreement. The relatedness between strain 11 and the other bacteria of the genus *Serratia* is between 43 and 57%, i.e., within the range of values for the recognized species of the genus. These results justify for class C the status of species within the genus *Serratia*.

A numerical analysis of the phenetic characters of the class C strains reveals the uniqueness of these strains with regard to the taxa identified as *S. marcescens*, *S. liquefaciens*, *S. plymuthica*, and *S. marinorubra*. The taxonomic distance between class C and the pairs *S. liquefaciens*—*S. plymuthica* and *S. marcescens*—*S. rubidaea* is equal to the distance (0.40) between these pairs. The species status of class C is confirmed by the results of both the genetic analysis and the phenetic study.

The strains of class C can only be located within the genus *Serratia* in spite of the discrepancies previously mentioned. Some of the characters of class C strains do not agree with the definition of the genus: Voges-Proskauer reaction negative, deoxyribonuclease not produced, gelatin not liquefied, and fermentation of rhamnose, arabinose, and raffinose. This necessitates an amendment of the definition of the genus *Serratia*. Characters useful in differentiating class C strains from strains of the named species of *Serratia* recognized here are given in Table 2.

The name *Serratia fonticola* (fon.ticó.la. L.n. fons, fontis: spring, fountain; L. suffix cola: dweller; M.L.n. fonticola: spring dweller) is proposed for this new species of bacteria, many strains of which have been isolated from well waters and springs. Strain 11 (ATCC 29844) is the type strain of the species.

Description of *S. fonticola* (based on 20 strains). Gram-negative, rod-shaped cells (0.5 by 30 μ m) which are motile and peritrichous.

Growth occurs on nutrient agar at 30 and 37°C but not at 41°C; growth occurs at 4°C.

All of the strains are positive for: citrate (Simmons), malonate, tartrate (Jordan), and mucate utilization; nitrate reduction to nitrite; lysine and ornithine decarboxylase production; production of tetrathionate reductase and Tween-esterase; production of β -galactosidase and β -xylosidase; and esculin hydrolysis.

All of the strains are negative for the production of indole, acetoin, H₂S, arginine dihydrolase, gelatinase, urease, phenyl alanine deaminase, and deoxyribonuclease.

Eight to 100% of the strains are positive within 24 to 48 h for the fermentation of L-arabinose, D-ribose, maltose, D-glucose, D-lactose, D-galactose, L-rhamnose, D-mannose, D-fructose, α -methyl-D-glucoside, D-trehalose, raffinose, melibiose, D-sucrose, D-mannitol, D-dulcitol, meso-inositol, D-glycerol, adonitol, and salicin.

Eighty to 100% of the strains were negative within 30 days for the fermentation of L-sorbose, D-melezitose, and inulin.

Carbon source utilization: Eighty to 100% of the strains utilize (within 5 days) the following as sole sources of carbon: L-arabinose, D-ribose, L-rhamnose, D-fructose, D-galactose, D-glucose, D-mannose, D-maltose, D-trehalose, gluconate, salicin, D-glycerol, meso-erythritol, adonitol, D-mannitol, D-dulcitol, meso-inositol, D-sorbitol, DL-lactate, DL-glycerate, pyruvate, citrate, succinate, L- α -alanine, D- α -alanine, L-aspartate, L-glutamate, L-proline, L-histidine, and putrescine.

Eighty to 100% of the strains do not utilize (within 5 days) the following as sole sources of carbon: D-arabinose, D-fucose, D-cellobiose, D-sucrose, inulin, ethanol, methanol, *n*-propanol, *iso*-propanol, geraniol, 2,3-butyleneglycol, ethylene-glycol, propylene-glycol, propionate, DL-3-hydroxy-butyrate, D-tartrate, L-tartrate, *m*-tartrate, citraconate, itaconate, oxalate, pimelate, suberate, azelate, glycolate, levulinate, 2-ketoglutarate, mesaconate, glutarate, sebacate, salicylate, adipate, butyrate, *iso*-butyrate, *n*-valerate, *iso*-valerate, *n*-caproate, heptanoate, pelargonate, caprate, benzoate, *p*-OH-benzoate, D-mandelate, L-mandelate, phthalate, *iso*-phthalate, *tere*-phthalate, phenol, benzyl-formate, glycine, β -alanine, L-leucine, L-isoleucine, L-valine, L-lysine, L-arginine, L-citrulline, L-threonine, L-methionine, DL-2-amino-butyrate, DL-3-amino-butyrate, DL-4-amino-butyrate, 5-amino-valerate, 2-amino-benzoate, 4-amino-benzoate, D-tryptophane, histamine, spermine, tryptamine, amylamine, ethanalamine, butylamine, benzylamine, diphenylamine, urea, urate, sarcosine, betaine,

TABLE 5. DNA homogeneity of *Citrobacter*-like strains and class C strains and DNA relatedness of reference strains of these taxa to members of the family *Enterobacteriaceae*

Source of unlabeled DNA ^a	<i>Citrobacter</i> -like strain 4556-71 (% binding at 60°C) ^b	Class C strain 11 (% bind- ing by competition)
<i>Citrobacter</i> -like strain 4556-71	100	
<i>Citrobacter</i> -like strains (5) ^c	93-96	
Class C strain 11		100
Class C strains (14) ^c		75-91
<i>Serratia marcescens</i> LM 1-63		57
<i>S. marcescens</i> LM 23-65		54
<i>S. marcescens</i> subsp. <i>kiliensis</i> ATCC 8101		53
<i>S. marcescens</i> ATCC 13880		49
<i>S. marcescens</i> CDC 868-57		47
<i>S. marcescens</i> SM6-W2	47	
<i>S. marcescens</i> strain 37 ^d		42
<i>S. liquefaciens</i> LM 3-70		43
<i>S. liquefaciens</i> strain 70 ^d		40
<i>S. plymuthica</i> strain 3617 B		48
<i>S. plymuthica</i> strain 62 ^d		45
<i>S. rubidaea</i> CDC 2199-72		59
<i>S. rubidaea</i> CDC 934-72		24
<i>S. marinorubra</i> S36		30
<i>Escherichia coli</i> K-12 1 UW	25	24
<i>Citrobacter freundii</i> strains	25-30	
<i>C. freundii</i> CUETM 77-166		18
<i>C. diversus</i> strains	28-30	
<i>Levinea malonatica</i> CDC 25 408	27	26
<i>L. amalonatica</i> strains	27-28	
<i>Enterobacter cloacae</i> CDC 1347-71	28	
<i>E. aerogenes</i> CDC 1627-66	28	
<i>E. agglomerans</i> CH 103	25	
<i>Escherichia coli</i> subsp. <i>adecarboxylata</i> ATCC 1783		18
<i>Hafnia alvei</i> CDC 4360-67	27	
<i>Klebsiella rhinoscleromatis</i> CDC 895-68		32
<i>K. pneumoniae</i> strain 2	31	
<i>K. pneumoniae</i> ATCC 13882		27
<i>K. ozaenae</i> CDC 276-71		24
<i>K. oxytoca</i> ATCC 13182		18
<i>Erwinia dissolvens</i> ATCC 23873		24
<i>E. amylovora</i> EA 178	22	
<i>Pectobacterium carotovorum</i> ATCC 495	24	
<i>Salmonella typhimurium</i> LT2	22	
<i>S. arizonae</i> CDC 62		19
<i>S. houtenae</i> CIS 498		5
<i>Shigella dysenteriae</i> A1 WRAIR		36
<i>S. sonnei</i> ATCC 9290		27
<i>S. flexneri</i> ATCC 12661		22
<i>S. boydii</i> 1 DP Wol. Paris		21
<i>Edwardsiella tarda</i> CDC 1795-62	23	
<i>Proteus vulgaris</i> ATCC 13315		18
<i>P. morganii</i> LM 11-70		16
<i>P. mirabilis</i> ATCC 9240		15
<i>P. stuartii</i> LM 1-70		14
<i>P. alcalifaciens</i> ATCC 13159		10
<i>Yersinia pseudotuberculosis</i> LM 48-1		5
<i>Y. enterocolitica</i> 377		0

^a Abbreviations: B, J. Brisou, University of Poitiers, France; CDC, Center for Disease Control, Atlanta, Ga.; CIS, Centre International des *Salmonella*, Institut Pasteur, Paris, France; CUETM, Collection Unité Ecotoxicologie Microbienne, Villeneuve d'Ascq, France; LM, L. Le Minor, Institut Pasteur, Paris; UW, University of Washington, Seattle, Wash.; WRAIR, Walter Reed Army Institute of Research, Washington, D.C.

^b Crosa et al. (3).

^c Number of strains.

^d Strain number as used in this study.

TABLE 6. *G + C content of the DNA of class C strains and of Serratia strains*

Strain	G + C content of DNA (mol%)
Class C	
CUETM 78-10	48.8 ^a
CUETM 77-143	49.0
CUETM 78-7	49.7
CUETM 78-12	50.3
CUETM 78-15	50.4
CUETM 77-168	50.5
CUETM 78-25	50.7
CUETM 78-43	50.8
CUETM 78-28	50.9
CUETM 78-45	50.9
CUETM 78-9	51.1
CUETM 78-11	51.1
CUETM 78-6	51.1
CUETM 78-22	51.5
CUETM 78-26	51.5
CUETM 77-165	51.5
CUETM 78-14	51.9
CUETM 78-49	51.9
CUETM 78-16	52.3
CUETM 78-20	52.5
<i>S. marcescens</i> ATCC 13880	
16 strains	60.9 ^b
	54.4–58.4 ^b
11 strains	53.6–61.4 ^b
<i>S. marcescens</i> subsp. <i>kiliensis</i>^b	
ATCC 11111	58.8–62.7 ^b
ATCC 8101	58.4–62.4 ^b
<i>S. marinorubra</i>	
2 strains	57.2 ^b –59.9 ^b
NCTC 10912	58.3 ^c
DSM 30124	53.5 ^c
<i>S. liquefaciens</i>	
ATCC 14460	51.9–53.9 ^b
CDC 6136-66	53.8 ^c
<i>S. plymuthica</i>	
ATCC 183	57.6–61.2 ^b
ATCC 992	59.5 ^c
ATCC 4261	55.0 ^c
DSM 3127	54.6 ^c

^a Data on all class C strains from reference 8.^b Data from reference 17.^c Data from reference 14.

creatine, hippurate, pantothenate, nicotinate, trigonelline, naphthalene, dodecane, and hexadecane.

Within the genus *Serratia* Bizio, we recognize the following species: (i) *Serratia marcescens* Bizio (type species), (ii) *Serratia liquefaciens* (Grimes and Hennerty) Bascomb et al. (1), (iii) *Serratia rubidaea* (Stapp) Ewing et al. (7), and (iv) *Serratia fonticola* sp. nov.

In addition to these Grimont et al. (14) recognize the species *Serratia plymuthica* (*Bacterium plymuthicum* (Fisher) Lehmann and Neumann).

TABLE 7. *DNA homologies among S. marcescens, S. liquefaciens, and S. rubidaea strains and strains of the Citrobacter-like group^a*

Taxon	<i>S. marcescens</i>	<i>S. liquefaciens</i>	<i>S. rubidaea</i>
<i>S. marcescens</i> (7 strains)	84–100 ^b	51–60	44–53
<i>S. liquefaciens</i> (6 strains)	52–63	68–100	41–47
<i>S. rubidaea</i> (4 strains)	47–51	40–49	70–100
<i>Citrobacter</i> -like (4 strains)	51–57	46–52	41–44

^a Data from Steigerwalt et al. (25).^b Percent binding at 60°C.

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