Classification of *Citrobacter* by DNA Hybridization: Designation of *Citrobacter farmeri* sp. nov., *Citrobacter youngae* sp. nov., *Citrobacter braakii* sp. nov., *Citrobacter werkmanii* sp. nov., *Citrobacter sedlakii* sp. nov., and Three Unnamed *Citrobacter* Genomospecies

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DNA relatedness studies (hydroxyapatite method) were done on 112 strains of *Citrobacter*. By using the recommended definition of a genomospecies 11 genomospecies were identified in the genus *Citrobacter*. These genomospecies were separable by their biochemical profiles. *Citrobacter koseri* (*Citrobacter diversus*) and *Citrobacter amalonaticus* proved to be homogeneous species, as previously described. *C. amalonaticus* biogroup 1, as described by Farmer et al. (J. Clin. Microbiol. 21:46–76, 1985), was shown to be a separate homogeneous species, which was named *Citrobacter farmeri* sp. nov. The *Citrobacter freundii* complex was quite heterogeneous. *C. freundii* sensu stricto, as represented by the type strain, contained only 9 of 66 strains in this complex. The remaining 57 strains were members of seven genomospecies. Genomospecies 5, containing 21 strains, was named *Citrobacter youngae* sp. nov. Genomospecies 6, containing 15 strains, was named *Citrobacter braakii* sp. nov. Genomospecies 7 and 8, each containing six strains, were named *Citrobacter werkmanii* sp. nov. and *Citrobacter sedlakii* sp. nov., respectively. Genomospecies 9, 10, and 11, each containing three strains, were not named.

The genus *Citrobacter* and the species *Citrobacter freundii* were designated in 1932 by Werkman and Gillen (13). *Citrobacter koseri* (8, 9) (*Citrobacter diversus* [4], *Levinea malonatica* [14]) and *Citrobacter amalonaticus* (5) (*Levinea amalonatica* [14]) are the other previously recognized *Citrobacter* species. Strains of the latter two species were initially identified as indole-positive, H₂S-negative biogroups of *C. freundii*. *C. koseri* also differs from *C. freundii* by its failure to grow in medium containing KCN and from both *C. freundii* and *C. amalonaticus* by its ability to utilize malonate and to produce acid from D-adonitol.

It has long been evident that a significant number of *Citrobacter* isolates from human clinical specimens do not exhibit the biochemical characteristics typical of these three previously described species (7). DNA relatedness studies have also revealed substantial heterogeneity within strains identified as *C. freundii* (3). Farmer et al. created the name *C. amalonaticus* biogroup 1 for strains, usually isolated from human feces, that differed from *C. amalonaticus* by their ability to usually ferment melibiose, α -methyl-D-glucoside, raffinose, and sucrose and their inability to utilize citrate (7).

The purpose of this study was to determine, by calculating levels of DNA relatedness, whether biochemically atypical *Citrobacter* strains in the culture collections of the Centers for Disease Control and Prevention (CDC) and the Institut Pasteur represent biotypes of previously described species or additional, undescribed species. Biochemical characteristics of the previously described species and each new

genomospecies detected on the basis of DNA relatedness levels were reexamined. (Genomospecies is a word that we have coined to use instead of genospecies. A genomospecies is a species defined by DNA relatedness [12]. Genomic species is another synonym for genomospecies and genospecies. The term genomovar was used by Rossello et al. "to denote genomic groups of a nomenclature" [10a]; "var" is traditionally used for differentiation below the species level, and we therefore prefer the term genomospecies or genomic species.) The traditional diagnostic laboratory tests were augmented by a battery of carbon source utilization tests to allow biochemical identification of each species and differentiation from all other species in the genus.

Eleven genomospecies were identified. Genomospecies 1 through 3 corresponded to *C. freundii*, *C. koseri*, and *C. amalonaticus*, respectively. Genomospecies 4, which corresponded to *C. amalonaticus* biogroup 1, was named *Citrobacter farmeri* sp. nov. Genomospecies 5 through 8 were named *Citrobacter youngae* sp. nov., *Citrobacter braakii* sp. nov., *Citrobacter werkmanii* sp. nov., and *Citrobacter sedlakii* sp. nov., respectively. Genomospecies 9 through 11, each containing three strains, were not named.

MATERIALS AND METHODS

Strains. The strains used in this study are listed in Table 1. All strains were originally sent for identification to diagnostic reference laboratories at the CDC or the Institut Pasteur. For consistency, CDC strain numbers are used in the Results and Discussion. The following type strains were used: *C. amalonaticus* (*L. amalonatica*) CDC 9020-77 (= ATCC

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<i>Citrobacter</i> genomospe- cies 4	41	11.5	59	6.0	48	100	0.0	100	34	13.5	37	13.0	19	42	13.0	40	12.5	39	12.0	14		
2991-81 ^T			69			100	1.0	99	46													
1604-79			65	6.0	53	100	1.0	94	40													
1263-79			68	6.5	53	98	0.5	100	42													
725-77			62			98	0.5	95	38													
2369-78			68			98	3.5	88	43													
415-80			62			92	0.5	94	38													
1402-82			64		54	92	3.5	82	40													
3295-78			57			89	0.5	85	34													
624-79			63	6.5	50	87	2.5	86	40	12.0						44						
3249-74	40		61			86	3.0	78	38													
2604-78			59			84	3.0	75	36													
2727-78			61			84	3.5	76	36													
530-79			57			76	2.0	76	35													
433-80																						
<i>C. freundii</i>																						
621-64 ^T	44	12.0	38	13.0		43	13.5		100	0.0	100	72	4.0	65		71	7.5	54	61	9.5	58	
4689-86			38	12.5					90	1.0	87	67	3.0	64								
4691-86									88	0.5	86	69	3.5	63	100	0.0			60	5.0	61	
4690-86			39	12.0					85	0.5	84	69	3.0	64								
4688-86			35	11.5					81	0.5	82	67	3.0	65			68	7.5	48	63	5.5	63
1243-78									75	4.0	67	92	1.0	90			71	9.0	47			
2929-78									71	4.0	62	73	3.0	70			63		61	5.5	56	
1242-78									68	3.0	60	100	0.0	100			63		67	5.5	55	
892-61	37		34						68	3.5	63	82	1.5	78			63					
<i>Citrobacter</i> genomospe- cies 5																						
460-61 ^T	43	12.0	36	13.0		37	13.0		61	6.5		57	6.5	46		100	0.0	100	59	7.0	42	
5118-60									67	5.5	52	62	8.0	43		98	0.5	89				
4692-86	41	12.5							69	5.5	54					97	0.5	92				
3062-62									67	7.0	53					97	1.0	91				
2974-50									68	7.5	49	63	7.5	43		95	0.5					
2611-80									65	6.0	52	62	7.5	48		95	2.5	87				
8107-85									69	5.5	48					95	2.5	85				
827-81									63	6.5	49	62	8.0	49		93	2.0	87				
1917-82									65	5.5	50	59	7.5	45		93	3.0	86				
4675-60									63	7.0	50					89	1.0	83				
8077-84	40		36						61	5.5	55	58	7.0	43		88	1.5	86				
8109-85									65	6.0	45	87	2.0									
6440-59									63	7.0	52					85	3.5	84				
3313-75			35						61	5.5	44	55	7.0	38		84	1.5	75				
8111-85									63	7.0		55	7.0	40		84	4.0	70				
8112-85									56	6.5	48	51	6.5	39		83	1.0					
4694-86			34						61	7.5						83	3.5	68				
2548-79									58	6.0	46					83	4.5	66				
5245-60									54	6.0						82	1.0	81				
8108-85									50	5.0						80	2.0	74				
8106-85									56	6.0		53	6.5	39		76	4.5	67				

Continued on following page

TABLE 1—Continued

Source of unlabeled DNA (CDC no.)	Labeled DNA from:											
	Citrobacter genomospecies 6 strain CDC 80-58 ^T			Citrobacter genomospecies 7 strain CDC 876-58 ^T			Citrobacter genomospecies 8 strain CDC 4696-86 ^T			Citrobacter genomospecies 8 strain CDC 4697-86		
	% Relat- edness at 60°C	D	% Relat- edness at 75°C	% Relat- edness at 60°C	D	% Relat- edness at 75°C	% Relat- edness at 60°C	D	% Relat- edness at 75°C	% Relat- edness at 60°C	D	% Relat- edness at 75°C
<i>Citrobacter</i> genomo- species 6												
4686-86	70	2.5	65									
3158-63	68	3.5	62									
2990-58	70	4.0	63									
3358-77												
4687-86	75	1.0	74									
8105-85	75	2.0	69									
8110-85	73	1.5	70									
80-58 ^T	100	0.0	100									
9115-81	73	0.5	72									
4685-86	75	1.5	73									
8113-85	71	1.5	68									
8114-85	71	1.5	72									
989-73	71	0.5	72									
2028-79	69	2.5	71									
4508-86	65	0.5	67									
<i>Citrobacter</i> genomo- species 7												
876-58 ^T	51	8.0	38									
3323-75	57			100	0.0	100	36		41	12.0	51	9.0
2444-78	56			100	0.0	100	40					
631-77	55			96	1.0	90	37					
2083-81	55			91	1.0	89	35					
2238-81	55			88	2.5	81	40					
<i>Citrobacter</i> genomo- species 8												
4696-86 ^T	34			49			100	0.0	86		41	12.0
3188-73	36						93	1.0	94			
190-81	34						89	1.0	79			
3659-74	31						84	0.5	85			
4697-86	29	11.0	10				84	1.0	79	100	0.0	100
305-74	31						77	0.5	76			

TABLE 2. Conventional biochemical reactions for *Citrobacter* genomospecies^a

Test	% of positive strains										
	Genomo- species 2	Genomo- species 3	Genomo- species 4	Genomo- species 1	Genomo- species 5	Genomo- species 6	Genomo- species 7	Genomo- species 8	Genomo- species 9	Genomo- species 10	Genomo- species 11
Indole	94	100	100	38	14	33	0	100	0	0	100
Citrate (Simmons)	100	100	7 (86)	88	76 (95)	87 (100)	100	83 (100)	0	33 (100)	100
H ₂ S produced on triple sugar-iron agar	0	13	0	75 (88)	67 (81)	60	100	0	0	67	67
Urease	56 (69)	81	36	38 (50)	76	47 (67)	83	100	100	0	67 (100)
Arginine	88 (94)	81 (100)	100	75 (100)	52 (90)	67 (100)	100	100	0	33 (100)	67 (100)
Ornithine	94 (100)	94 (100)	100	0	5	93	0	100	100	0	0
Motility	94	94 (100)	100	100	95	87	100	100	0	67	100
KCN	0 (6)	94 (100)	100	88 (100)	95 (100)	100	100	100	33	100	100
Malonate	94	13	0	13	5	0	100	100	100	100	0
D-Glucose (gas)	100	94	100	100	76	93	100	100	100	100	100
Acid produced from:											
Lactose	75 (94)	38 (88)	21 (100)	88 (100)	24 (90)	80 (87)	17 (100)	100	100	67	67 (100)
Sucrose	44	13	100	100	19	7	0	0	0	33	33
Dulcitol	38	0	0	13	86	33	0	100	0	0	100
Salicin	19 (94)	31 (94)	0 (93)	0 (13)	10	0 (7)	0	17 (50)	0 (100)	0 (67)	0 (67)
Adonitol	94	0	0	0	0	0	0	0	0	0	0
Raffinose	0	0	100	50 (100)	10	7 (13)	0	0	0	0	33
Cellobiose	94	100	100	25 (75)	43 (100)	73 (93)	20 (100)	100	100	67	100
α -CH ₃ -glucoside	40 (93)	14 (21)	92 (100)	0 (25)	0 (5)	29 (43)	0	0	0	0	0
Esculin	0 (33)	0 (29)	0 (54)	0	5	0	0	17 (50)	0 (33)	0 (33)	0 (100)
Melibiose	0	0	100	100	5	78 (100)	0	100	0	67	33 (100)
D-Arabitol	100	0	0	0	0	0	0	0	0	0	0
Glycerol	100	25 (31)	85	100	90 (100)	86	100	67	0	67 (100)	100
Acetate	86 (93)	93 (100)	77 (100)	50 (75)	67 (76)	50 (92)	80 (100)	83	33 (67)	0 (33)	33 (100)
NO ₃ reduced to NO ₂	100	93	100	100	86	100	100	100	100	100	100
ONPG ^b	100	100	100	100	95	85	100	100	100	67 (100)	100

^a Most preparations were incubated at 36 $\pm$ 1°C; the exceptions were the gelatin liquefaction preparation (22°C) and the DNase preparation (25°C). Most results were read after 7 days of incubation; the methyl red and Voges-Proskauer results were read after 2 days. Results are expressed as percentages of positive strains within 48 h; the values in parentheses are the percentages of strains which gave delayed-positive reactions. The following tests, except as noted, were positive for all strains tested: methyl red, acid production from D-glucose, acid production from D-mannitol, acid production from D-sorbitol (one genomospecies 2 strain was negative), acid production from L-arabinose, acid production from L-rhamnose, acid production from maltose (one genomospecies 3 strain was delayed positive and one genomospecies 5 strain was negative), acid production from D-xylose (one genomospecies 1 strain was delayed positive), acid production from trehalose, acid production from mucate (one genomospecies 10 strain was delayed positive), acid production from tartrate (one genomospecies 1 strain and one genomospecies 6 strain were negative), and acid production from mannose. The following tests, except as noted, were negative for all strains tested: phenylalanine deaminase, lysine decarboxylase (one genomospecies 6 strain was positive), gelatin liquefaction, acid production from myo-inositol (one genomospecies 1 strain was delayed positive), acid production from erythritol, lipase, DNase, oxidase, and pigment.

^b ONPG, o-nitrophenyl- β -D-galactopyranoside.

of isolation (61% from women) was seen in *C. amalonaticus* and *C. amalonaticus* biogroup 1. Most of the *Citrobacter* genomospecies were isolated from nonhuman as well as human sources; however, genomospecies 9 was isolated only from animals. Human isolates of all genomospecies except *C. koseri* were obtained predominantly from stools; *C. koseri* was most often isolated from urine and cerebrospinal fluid.

It is clear that clinical data are needed on all of the genomospecies formerly grouped in *C. freundii*. We hope that the delineation of 11 genomospecies in the genus *Citrobacter*, the means to identify these taxa biochemically (Tables 2 to 4), and the taxonomic proposals given below will aid in determining both the incidence and clinical significance of these organisms.

Description of *Citrobacter farmeri* sp. nov. *Citrobacter farmeri* (far'mer.i. N. L. gen. n. *farmeri*, to honor John J. Farmer III, an American microbiologist, who recognized and biochemically defined *C. amalonaticus* biogroup 1 and who has made many substantial contributions to the recognition and classification of new species and genera in the *Enterobacteriaceae* and other families [5-7]). Informally called *C. amalonaticus* biogroup 1 and *Citrobacter* genomospecies 4. Indole positive and citrate negative or delayed

positive. Produces arginine dihydrolase and ornithine decarboxylase. Other biochemical reactions useful in differentiation are acid production from α -CH₃-D-glucoside, melibiose, raffinose, and sucrose and the ability to utilize benzoate, 4-hydroxybenzoate, maltitol, D-melibiose, 1-O-CH₃- α -galactoside, palatinose, protocatechuate, D-raffinose, and sucrose but not *m*-coumarate, dulcitol, and malonate as sole carbon sources. Additional reactions useful in differentiation are given in Table 4. Full biochemical reactions are shown in Tables 2 and 3.

Isolated from human stools, urine, wounds, and blood. Presumptively pathogenic. The type strain is CDC 2991-81 (= ATCC 51112), which was isolated from the stool of a 1-year-old boy in New York.

Description of *Citrobacter youngae* sp. nov. *Citrobacter youngae* (young'ae. N. L. gen. n. *youngae*, to honor Viola M. Young, an American microbiologist, for her contributions to the genus *Citrobacter*, including the description of *C. amalonaticus* [*L. amalonatica*] [14]). Corresponds to *Citrobacter* genomospecies 5. Usually indole negative, delayed positive for citrate and arginine dihydrolase, and negative for ornithine decarboxylase. Other biochemical reactions useful in differentiation are acid production from dulcitol but not from melibiose and the ability to utilize

TABLE 3. Carbon source utilization reactions of *Citrobacter* genomospecies

Carbon source	% of positive strains ^a										
	Genomo- species 2	Genomo- species 3	Genomo- species 4	Genomo- species 1	Genomo- species 5	Genomo- species 6	Genomo- species 7	Genomo- species 8	Genomo- species 9	Genomo- species 10	Genomo- species 11
cis-Aconitate	100	93 (100)	57 (71)	88	78 (91)	89 (100)	100	100	0	33 (67)	67 (100)
trans-Aconitate	25 (63)	0	57	13 (38)	0	0	83	50 (100)	0	0	0
Adonitol	100	0	0	0	0	0 (6)	0	0	0	0	0
D-Alanine	100	100	71 (85)	100	100	100	100	100	100	67 (100)	100
4-Aminobutyrate	0 (6)	0 (13)	7 (21)	25 (50)	22 (39)	17 (56)	0 (67)	50	0	0	33 (67)
5-Aminovalerate	6 (44)	33 (53)	7 (36)	38 (88)	43 (56)	50	100	33 (50)	0	0	67 (100)
D-Arabitol	100	0	0	0	0 (4)	0	0	0	0	0	0
Benzoate	0	73	93	0	0	0	0	100	0	0	0
Caprate	0 (56)	0 (27)	0 (7)	0	0 (4)	0	0	0 (17)	0	0	0
D-Cellobiose	94	100	100	88	78 (87)	94 (100)	67 (100)	100	100	67	100
m-Coumarate	0	0	0	88 (100)	96	100	100	67	0	33 (67)	0 (67)
Dulcitol	44	0	0	13	87	33	0	100	0	0	100
Esculin	0	0	0	0	0 (9)	6	0	17 (50)	0	0	0 (33)
Ethanolamine	19 (82)	33 (67)	14	0 (25)	9 (22)	0	17 (34)	17 (33)	0	0	0 (33)
L-Fucose	100	100	100	100	91 (95)	94	100	100	33	67	100
Gentiobiose	88	100	93 (100)	88	52 (78)	89 (100)	67 (100)	100	0 (33)	67	100
Gentisate	100	100	93 (100)	100	0	94 (100)	100	100	67	0	67
L-Glutamate	100	100	86 (100)	75 (88)	83 (92)	94 (100)	83 (100)	83 (100)	67	0 (33)	100
Glycerol	100	100	100	100	100	100	100	100	0	100	100
3-Hydroxybenzoate	100	100	100	100	0	100	100	100	100	0	67
4-Hydroxybenzoate	0	100	100	0	0	0	0	100	0	0	0
3-Hydroxybutyrate	6 (12)	20 (33)	7	100	74 (87)	44 (66)	67 (100)	100	0	0	33 (67)
myo-Inositol	100	0	0	100	0	6	0	100	0	67	0
2-Ketogluconate	100	100	100	100	100	100	100	100	0 (67)	100	100
5-Ketogluconate	100	100	100	100	96 (100)	100	50	0	0	100	100
2-Ketoglutarate	6	7 (40)	7 (36)	13 (38)	0 (17)	22 (61)	33 (67)	33 (100)	0	33 (100)	0 (33)
DL-Lactate	100	100	100	100	100	100	100	100	33	100	100
Lactose	56 (87)	20 (87)	50 (86)	88 (100)	22 (44)	78 (84)	17 (34)	100	100	67	33 100
Lactulose	0	0	7	88	0	67 (78)	17	100	0 (33)	67	33 (100)
D-Lyxose	100	0	0 (7)	63 (88)	9 (52)	56 (78)	83 (100)	0 (67)	0	0	100
Malonate	81 (87)	0	0	0	0	0	50 (100)	67 (94)	67 (100)	0	0
Maltitol	100	7	93	25	0 (4)	44 (55)	0	0	0	0	0
D-Melibiose	0	7	100	88	0	94 (100)	0	100	0	100	33 (67)
1-O-CH ₃ -α-galactoside	0	0	100	88	0 (22)	100	0	100	0	67 (100)	0 (100)
1-O-CH ₃ -β-galactoside	100	7 (34)	14 (43)	100	65 (87)	83	100	100	100	67	100
3-O-CH ₃ -D-glucose	13	87	86 (100)	63	0	94 (100)	100	100	0	0	67
1-O-CH ₃ -α-D-glucoside	94 (100)	13	86 (93)	25	0	39 (45)	0	0	0	0	0
1-O-CH ₃ -β-D-glucoside	100	100	100	100	100	100	100	100	0 (33)	100	100
Palatinose	100	13	100	25	4	67	0	0	0	0	67
Phenylacetate	0	0	0	25	0	0	0	0	0	0 (33)	0
3-Phenylpropionate	0	0	0	75	96	83	100	0	0	0 (33)	0 (67)
L-Proline	100	87	57 (71)	100	87 (91)	100	100	100	100	33 (100)	100
Propionate	88	80 (87)	86	88	78 (87)	72 (78)	100	83 (100)	67 (100)	0	100
Protocatechuate	0	100	100	0	0	0	0	100	0	0	0
Putrescine	0	0	0	50 (75)	0	39 (61)	67	0	100	0	0 (67)
D-Raffinose	0	0	100	75 (88)	0	6 (12)	0	0	0	67	33
L-Sorbose	0	87	100	100	100	6	83	0	0	0	100
Sucrose	44	0	100	100	9 (13)	6	0	0	0	33	33
D-Tagatose	0	0	36 (43)	13	4	0	0	0	0	0	0
D-Tartrate	0	0	0	13	0	6	100	0	0	0	0
L-Tartrate	19 (75)	33 (73)	36 (65)	0 (63)	22 (74)	17 (67)	67 (84)	17 (50)	100	0	33 (67)
meso-Tartrate	0	40 (87)	7 (21)	50	78 (100)	72 (94)	100	67 (83)	100	0	67 (100)
Tricarballoylate	0	93	100	100	4 (17)	89 (95)	100	83 (100)	100	0	100
D-Turanose	6 (31)	0 (13)	36	0 (13)	0	11 (17)	0	0	0	0	0
L-Tyrosine	88	0	0	75 (88)	74 (87)	72 (100)	67 (100)	0	0	0 (33)	100
Xylitol	19 (57)	0	0	0	0	0	0	0	0	0	0

^a Percentages of strains which gave positive reactions within 48 h. The numbers in parentheses are the percentages of strains which gave positive reactions within 96 h. All strains, except as noted, utilized the following carbon sources within 48 h: *N*-acetyl-D-glucosamine, L-alanine, L-arabinose, L-aspartate (except one genomospecies 5 strain that was negative and one genomospecies 10 strain that was positive within 96 h), citrate (except one genomospecies 4 strain that was positive within 96 h), D-fructose, fumarate, D-galactose, D-galacturonate (except one genomospecies 10 strain), D-gluconate, D-glucosamine, D-glucose, D-glucuronate, DL-glycerate (except one genomospecies 2 strain), D-malate (except one genomospecies 4 strain and two genomospecies 10 strains that were positive within 96 h), L-malate, maltose (except one genomospecies 5 strain), maltotriose (except one genomospecies 4 strain and one genomospecies 5 strain), D-mannitol, D-mannose, mucate (except one genomospecies 5 strain that was positive within 96 h), L-rhamnose, D-ribose (except one genomospecies 5 strain), D-saccharate (except one genomospecies 5 strain and one genomospecies 10 strain), L-serine (except two genomospecies 4 strains), D-sorbitol, succinate, D-trehalose, and D-xylose (except two strains of genomospecies 1). All strains, except as noted, failed to utilize the following carbon sources within 96 h: L-arabitol, betaine, caprate, caprylate, i-erythritol, glutarate, histamine, L-histidine, HQ-β-glucuronide (except one genomospecies 6 strain), itaconate (except one genomospecies 7 strain that was positive within 96 h), D-melezitose, quinate, trigonelline, tryptamine, and tryptophan.

TABLE 4. Biochemical tests useful in differentiating *Citrobacter* genomospecies^a

Biochemical test	<i>C. amalonaticus</i> (genomo- species 3)	<i>C. farmeri</i> (genomo- species 4)	<i>C. freundii</i> (genomo- species 1)	<i>C. youngae</i> (genomo- species 5)	<i>C. braakii</i> (genomo- species 6)	<i>C. werkmanii</i> (genomo- species 7)	<i>C. sedlakii</i> (genomo- species 8)	Genomo- species 9	Genomo- species 10	Genomo- species 11
Indole	+ ^b	+	d	-	d	-	+	-	-	+
Citrate	+	+	+	(+)	+	+	+	-	d(+)	+
Urease	d	d	d	d	d	+	+	+	-	d(+)
Arginine dihydrolase	(+)	+	(+)	(+)	(+)	+	+	-	d(+)	d(+)
Ornithine decarboxylase	+	+	+	+	+	+	+	+	-	-
Motility	+	+	+	+	+	+	+	+	-	+
Malonate	-	-	-	-	-	-	-	-	+	-
Sucrose (acid)	-	+	+	d	-	-	+	-	d	d
Dulcitol (acid)	-	+	+	+	-	-	+	-	-	+
Raffinose (acid)	-	+	(+)	+	d	-	+	-	-	d
α -CH ₃ -D-glucoside (acid)	-(d)	+	-(d)	-	-	-	-	-	-	+
Esculin	-(d)	+	-	-	-	-	-(d)	-(d)	-	-
Melibiose (acid)	-	-(d)	+	-	d(+)	-	+	-	d	-(+)
Salicin (acid)	d(+)	-(+)	-	-	-	-	-(d)	-(+)	-(d)	-(d)
Acetate	+	d(+)	d	d	d(+)	d(+)	+	d	-(d)	d(+)
Carbon source utilization										
Benzoate	d	+	-	-	-	-	+	-	-	-
m-Coumarate	-	-	+	+	+	+	d	-	d	-(d)
Dulcitol	-	-	+	+	d	+	+	-	-	+
Gentisate	+	+	+	-	+	+	+	d	-	d
Glycerol	+	+	+	+	+	+	+	-	+	+
3-Hydroxybenzoate	+	+	+	+	+	+	+	+	-	d
4-Hydroxybenzoate	+	+	-	-	-	-	+	-	-	-
myo-Inositol	-	-	+	-	-	-	+	-	-	-
5-Ketogluconate	+	-	+	-	-	-	+	-	-	-
Lactulose	-	+	+	+	+	d	+	-(d)	+	d(+)
D-Lyxose	-	-	d(+)	-(d)	d	+	-(d)	-	-	+
Maltitol	-	+	d	-	d	-	-	-	-	-
D-Melibiose	-	+	+	-	+	-	+	-	+	d
1-O-CH ₃ - α -galactoside	-	+	+	-	+	-	+	-	d(+)	-(+)
3-O-CH ₃ -D-glucose	+	+	d	-(d)	+	+	+	-	-	d
Palatinose	-	+	d	-	d	-	-	-	-	d
3-Phenylpropionate	-	-	d	+	+	+	-	-	-(d)	-(d)
Protocatechuate	+	+	-	-	-	-	+	-	-	-
D-Raffinose	-	+	d(+)	-	-	-	+	-	d	d
L-Sorbose	+	+	+	+	-	+	-	-	-	+
Sucrose	-	+	+	+	-	+	-	-	-	d
D-Tartrate	-	-	+	-	-	-	-	-	-	-
Tricarballoylate	+	+	+	-	-	+	+	+	-	+
L-Tyrosine	-	-	d(+)	d(+)	d(+)	d(+)	-	-	-(d)	+

^a Genomospecies 2 (*C. koseri*) is not included in this table. This taxon is easily differentiated from all other genomospecies by its ability to produce acid from adonitol and D-arabitol and its ability to utilize these substrates as sole carbon sources; it is also unable to grow in the presence of KCN.

^b +, 83% or more of the strains are positive within 2 days; -, 17% or less of the strains are positive within 2 days. Parentheses indicate that a test is positive between 3 and 4 days (for carbon source utilization tests) or between 3 and 7 days (for other tests).

TABLE 5. Clinical information on *Citrobacter* isolates for which data were available

Taxon	No. of strains isolated from:		No. of strains isolated from the following human sources:							No. of strains isolated from the following nonhuman sources:			No. of strains isolated from humans of the following ages:				
	Males	Females	Stools	Urine	Cerebro-spinal fluid	Blood	Wounds	Sputum	Other	Animals	Food	Soil	<2 yr	2-15 yr	16-40 yr	41-60 yr	>60 yr
<i>C. koseri</i>	29	19	4	18	14	6	3	7	2	1			10	1	1	3	16
<i>C. amalonaticus</i>	18	28	32	3		5	3	1		1			7	1	3	5	8
<i>C. amalonaticus</i> biogroup 1	16	25	25	6		2	6					1	8	1	1	5	11
Genomospecies 1	1	1	5			1				1	1				1	1	
Genomospecies 5	3	3	11	1			2			3	1		3	1			
Genomospecies 6	4	1	7							2					1	1	1
Genomospecies 7		2	3			1						1	1	1	1		
Genomospecies 8	3	1	3			1	1										
Genomospecies 9										3							
Genomospecies 10			1								1						
Genomospecies 11		2	1			1										2	

dulcitol, 3-phenylpropionate, and L-sorbose but not gentisate, 3-hydroxybenzoate, malonate, D-melibiose, 1-O-CH₃-α-galactoside, 3-O-CH₃-D-glucose, and tricarballic acid as sole carbon sources. Additional reactions useful in differentiation are given in Table 4. Full biochemical reactions are shown in Tables 2 and 3.

Isolated from human stools and blood and from animals and food. Presumptively pathogenic. The type strain is CDC 460-61 (= ATCC 29935), which was isolated from meat scraps in South Carolina.

Description of *Citrobacter braakii* sp. nov. *Citrobacter braakii* (braak'i.i. N. L. gen. n. *braakii*, to honor Hendrik R. Braak, a Dutch microbiologist, who worked on glycerol fermentation by enteric bacteria and isolated a glycerol fermenter that he called "*Bacterium freundii*" [1]). Corresponds to *Citrobacter* genomospecies 6. Production of indole is variable. Positive or delayed positive for citrate, arginine dihydrolase, and ornithine decarboxylase. Other biochemical reactions useful in differentiation are lack of acid production from salicin and sucrose and the ability to utilize *m*-coumarate, 1-O-CH₃-α-galactoside, 3-phenylpropionate, and L-tyrosine (delayed) but not malonate and L-sorbose as sole carbon sources. Additional reactions useful in differentiation are given in Table 4. Full biochemical reactions are shown in Tables 2 and 3.

Isolated from human stools, urine, and wounds and from animals and food. Presumptively pathogenic. The type strain is CDC 80-58 (= ATCC 51113), which was isolated from a snake in France.

Description of *Citrobacter werkmanii* sp. nov. *Citrobacter werkmanii* (werk'man'i.i. N. L. gen. n. *werkmanii*, to honor Chester H. Werkman, an American microbiologist, who studied the fermentative production of trimethylene glycol from glycerol and proposed the genus *Citrobacter* [13]). Corresponds to *Citrobacter* genomospecies 7. Negative for indole production and ornithine decarboxylase; citrate positive and positive for arginine dihydrolase. Other biochemical reactions useful for differentiation are lack of acid production from dulcitol, melibiose, and sucrose and the ability to utilize *m*-coumarate, D-lyxose, malonate, 3-phenylpropionate, L-sorbose, and D-tartrate but not dulcitol, 4-hydroxybenzoate, D-melibiose, and 1-O-CH₃-α-galactoside as sole carbon sources. Additional reactions useful in differentiation

are given in Table 4. Full biochemical reactions are shown in Tables 2 and 3.

Isolated from human stools and urine and from food. Presumptively pathogenic. The type strain is CDC 876-58 (= ATCC 51114), which was isolated from a human stool in France.

Description of *Citrobacter sedlakii* sp. nov. *Citrobacter sedlakii* (sed.lak'i.i. N. L. gen. n. *sedlakii*, to honor Jiri Sedlak, a Czechoslovakian microbiologist, who developed a serotyping scheme for *C. freundii* [11]). Corresponds to *Citrobacter* genomospecies 8. Positive for indole production, arginine dihydrolase, and ornithine decarboxylase. Delayed citrate positive. Other biochemical reactions useful for differentiation are production of acid from dulcitol and melibiose but not from sucrose and the ability to utilize benzoate, dulcitol, 4-hydroxybenzoate, *myo*-inositol, lactulose, malonate, 1-O-CH₃-α-galactoside, and protocatechuate but not 5-ketogluconate and L-sorbose as sole carbon sources. Additional reactions useful in differentiation are given in Table 4. Full biochemical reactions are shown in Tables 2 and 3.

Isolated from human stools, blood, and wounds. Presumptively pathogenic. The type strain is CDC 4696-86 (= ATCC 51115), which was isolated from a human stool in France.

The following genomospecies, although biochemically separable from each other and from all other *Citrobacter*, each contain only three strains. While it is not likely that additional strains will have markedly different biochemical characteristics, it is prudent to examine additional strains before naming these taxa.

Description of *Citrobacter* genomospecies 9. *Citrobacter* genomospecies 9 is negative for indole production, citrate, and arginine dihydrolase and is ornithine decarboxylase positive. Other biochemical reactions useful for differentiation are lack of motility, inability to produce acid from melibiose and sucrose, and ability to utilize malonate but not *m*-coumarate, glycerol, 5-ketogluconate, D-melibiose, 1-O-CH₃-α-galactoside, and 3-O-CH₃-D-glucose as sole carbon sources. Additional reactions useful for differentiation are given in Table 4. Full biochemical reactions are shown in Tables 2 and 3.

This organism has been isolated only from rodents and is presumptively pathogenic for these animals. The type strain

is CDC 1843-73 (= ATCC 51116), which was isolated from a hamster in Connecticut.

Description of *Citrobacter* genomospecies 10. *Citrobacter* genomospecies 10 is negative for indole production and ornithine decarboxylase and delayed positive for citrate and arginine dihydrolase. Other biochemical reactions useful for differentiation are a negative urease reaction and the inability to utilize gentisate, 3-hydroxybenzoate, 3-O-CH₃-D-glucose, L-sorbose, and tricarballoylate as sole carbon sources.

This organism has been isolated from human stools and food; there is insufficient information to speculate on its clinical significance. The type strain is CDC 4693-86 (= ATCC 51117), which was isolated from a human stool in France.

Description of *Citrobacter* genomospecies 11. *Citrobacter* genomospecies 11 is indole and citrate positive, delayed positive for arginine dihydrolase, and ornithine decarboxylase negative. Other biochemical reactions useful for differentiation are acid production from dulcitol and the ability to utilize dulcitol, D-xylose, 1-O-CH₃-α-galactoside (delayed), and L-tyrosine but not malonate and protocatechuate as sole carbon sources.

This organism has been isolated from human stools and blood; there is insufficient information to speculate on its clinical significance. The type strain is CDC 2970-59 (= ATCC 51118), which was isolated from an unknown source in Illinois.

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