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Attempts to Classify Herbicola Group-*Enterobacter agglomerans* Strains by Deoxyribonucleic Acid Hybridization and Phenotypic Tests

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There are seven names on the Approved Lists of Bacterial Names that have been treated as partial or total synonyms for strains belonging to the *Enterobacter agglomerans*-Herbicola group of *Erwinia* species complex. A total of 124 strains belonging to this complex, isolated mainly from plant and human sources, were studied by deoxyribonucleic acid relatedness and by a variety of biochemical tests. Ninety of these strains fell into 13 deoxyribonucleic acid hybridization groups (2 to 13 strains per group), and the remaining 34 strains did not fit into any hybridization group. Nine of the hybridization groups could be separated biochemically, whereas four hybridization groups could not. Our results point out the inadequacy of the classification schemes presently used for these organisms, the inadequacy of the present nomenclature, the extreme diversity of the strains presently classified in the *Enterobacter agglomerans*-Herbicola group of *Erwinia* species complex, and the need for additional, in-depth studies of these organisms.

Strains belonging to the Herbicola group of *Erwinia* species-*Enterobacter agglomerans* complex (Herbicola-Agglomerans group) were described as early as 1888 (2, 14). The Herbicola-Agglomerans group contains organisms that are found as pathogens or saprophytes on plants (12, 18, 21), in soil and water (18, 22), in at least one insect (23), in animals (18), and as pathogens or secondary invaders in a variety of human infections (5, 24, 27). The name first given to these strains apparently was "*Bacterium agglomerans*" (2, 14); subsequently, they have been placed in 13 different genera under 27 species names, resulting in a total of at least 56 different nomenclatural combinations (Table 1). Studies by Graham et al. (6, 18, 19) and Dye (8–12) resolved much of the nomenclatural confusion surrounding the Herbicola-Agglomerans group. Largely as a result of the efforts of these workers, there are now only seven species names to consider.

In *Bergey's Manual of Determinative Bacteriology*, 8th ed. (23), three species, one of which has two varieties, are listed in the Herbicola group of *Erwinia* species; these are *Erwinia herbicola* (with the varieties *Erwinia herbicola* var. *herbicola* and *Erwinia herbicola* var. *ananas*), *Erwinia stewartii*, and *Erwinia uredovora*. After comparing human and plant isolates, Ewing and Fife (13, 14) proposed the name *Enterobacter agglomerans* for the Herbicola-Agglomerans group. These authors described seven anaerogenic and four aerogenic biogroups of *Enterobacter agglomerans*, which they concluded was a senior subjective synonym for the three *Erwinia* species in the Herbicola group. The three *Erwinia* species and *Enterobacter agglomerans* are on the Approved Lists of Bacterial Names (28). Three additional species on the Approved Lists are also thought to belong to the Herbicola-Agglomerans group. These are *Escherichia adecarboxylata*, *Erwinia ananas* (as a species rather than as a variety of *Erwinia herbicola*), and *Erwinia milletiae*.

Through 1965 there were only three reports that the Herbicola-Agglomerans group was associated with human disease (6, 7, 25, 27). Only a few human isolates were included in the studies of the Herbicola-Agglomerans group done by plant and agricultural bacteriologists. Reports of Herbicola-Agglomerans group isolates from humans increased beginning in the late 1960s. Biochemical characterization of groups of human strains was first reported by von Graevenitz (32, 33) and Bottone et al. (3, 17).

Interest in these organisms peaked in 1971, when they were implicated in a nationwide nosocomial septicemia outbreak due to contaminated intravenous products in which 40 of 378 patients died (24). Ewing and Fife studied the outbreak strains, compared them with the Herbicola group of *Erwinia*, and concluded that the two sets of strains belonged to the same species (13, 14). Reports of clinical isolates belonging to the Herbicola-Agglomerans group and of biochemical characterization of these isolates subsequently appeared from workers in many countries (5, 16, 26, 27, 31).

The purpose of this study was to determine whether any or all of the Herbicola group of *Erwinia* species and *Enterobacter agglomerans* comprise a single species or whether these taxa represent more than one species.

(Some of the results were taken from a thesis presented by J.K.L.K. to the University of North Carolina, Chapel Hill.)

MATERIALS AND METHODS

Nomenclature. *Erwinia herbicola*, *Erwinia stewartii*, *Erwinia uredovora*, *Erwinia ananas*, *Erwinia milletiae*, *Escherichia adecarboxylata*, and *Enterobacter agglomerans* all appear on the Approved Lists (28). There seems to be agreement that each of these names represents one or more species in the same group, but disagreement as to the number of species in this group and as to which, if any, of these names are synonyms. We use the term Herbicola-Agglomerans group to refer to all of the organisms in this group. Species names were used to denote the names under which specific strains were received and to compare various taxonomic proposals for these organisms. Names not on the Approved Lists are written in quotation marks (Table 1).

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TABLE 1. Presumed synonyms for members of the Herbicola-Agglomerans group

Name	Reference(s)[a]
Escherichia adecarboxylata	22
Enterobacter agglomerans	14
"Bacterium agglomerans"	14
"Bacillus ananas"	12
"Bacterium ananas"	23
Erwinia ananas	12
"Pectobacterium ananas"	23
"Erwinia herbicola var. *ananas"*	12
"Xanthomonas annamalaiensis"	11, 12
"Xanthomonas balsamivorum"	11, 12
"Bacterium cassavae"	12
"Erwinia cassavae"	12, 20
"Bacillus citrimaculans"	12, 20
"Erwinia citrimaculans	12, 20
"Xanthomonas cosmosicola"	11, 12
"Erwinia erivanensis"	12
"Xanthomonas esculenti"	11, 12
"Bacillus flavidus"	12
"Erwinia flavida"	12
"Agrobacterium gypsophilae"	19
"Bacterium herbicola"	10, 12
"Bacterium herbicola aureum"	10, 12
Erwinia herbicola	12
"Erwinia mangiferae"	12, 20
"Xanthomonas maydis"	11, 12
"Bacillus milletiae"	12
Erwinia milletiae	12, 20
"Xanthomonas penniseti"	11, 12
"Flavobacterium rhenanum"	23
"Xanthomonas rubrisorghi"	11, 12
"Xanthomonas tagetis"	11, 12
"Flavobacterium trifolii"	10, 12
"Flavobacterium trifolium"	10, 12
"Pseudomonas trifolii"	10, 12
"Xanthomonas trifolii"	10, 12
"Chromobacterium typhi-flavum"	12
"Bacterium typhi flavum"	12
"Bacillus vitavorus"	12
"Erwinia vitavora"	12, 20
"Aplanobacter stewarti"	9
"Bacillus stewarti"	9
"Bacterium stewarti"	9
Erwinia stewartii	9, 12
"Pseudobacterium stewarti"	23
"Pseudomonas stewarti"	9, 12
"Flavobacterium herbicola"	10, 12
"Pseudomonas herbicola"	12
"Xanthomonas herbicola"	18
"Xanthomonas indica"	11, 12
"Bacillus lathyri"	12
"Erwinia lathyri"	10, 12
"Bacillus mangiferae"	12
"Phytobacterium stewartii"	9
"Xanthomonas stewarti"	9
Erwinia uredovora	8, 12
"Xanthomonas uredovorus"	8, 12

[a] Reference(s) in which the name was proposed or in which the original work is cited.

Strains. The bacterial strains received as members of the Herbicola-Agglomerans group are listed in Table 2. The strains of other members of the *Enterobacteriaceae* from which deoxyribonucleic acid (DNA) was prepared have been listed previously (30).

DNA relatedness. The methods used to prepare DNA labeled with ^{32}P and unlabeled DNA, the methods used in the hydroxyapatite procedure for DNA hybridization, and the method used for calculating DNA relatedness have been described previously (4).

Biochemical tests. The conventional biochemical test media and conditions used have been described previously (21). The API 20E biochemical kit system (Analytab Products, Inc.) was used according to the directions of the manufacturer, except that incubations were done at both 25 ± 1 and 36 ± 1°C. The API 50 research system (API 50R; Analytab Products, Inc.) was used according to the instructions of the manufacturer, with the following exceptions. Incubations were done at 36 ± 1°C. A conventional methyl red test was done in parallel with the API 50 methyl red test; both were read after 48 h, and the results of the conventional reaction were used if the results differed. All other tests were read after 24 and 48 h. Reactions were graded on a scale from 0 to 5 depending on the intensity of the color produced, as directed by the manufacturer; on this scale a score of 0 was negative, 1 was weak or doubtful, 2 was doubtful positive, 3 was weakly positive, 4 was positive, and 5 was strongly positive. A reaction with a score of 2 was arbitrarily deemed negative, and a reaction with a score of 3 was arbitrarily deemed positive.

Computer-assisted phenetic analysis. All computations were performed by using the programs described by Walczak and Krichevsky (34–36). Interstrain similarities generated from 44 conventional biochemical tests were calculated by using both the simple matching and Jaccard coefficients. Clustering was done by using both single linkage and unweighted average linkage (29).

Antimicrobial agent susceptibility tests. Antibiograms were done on Mueller-Hinton agar by the disk method of Bauer et al. (1), as previously described (15).

RESULTS

We determined the level of DNA relatedness of 124 strains of the Herbicola-Agglomerans group to each of 17 reference strains chosen from this group. The reference strains were chosen arbitrarily, and their DNAs were labeled with ^{32}P. We also determined the level of relatedness of each reference strain to representative species of the *Enterobacteriaceae*. At least four strains from each of the 11 biogroups of *Enterobacter agglomerans* (14) were included in the study, as were strains received as *Erwinia herbicola, Erwinia ananas, Erwinia stewartii, Erwinia uredovora, Erwinia milletiae, "Erwinia mangiferae," "Erwinia lathyri," "Erwinia cassavae," "Erwinia maydis,"* and *"Xanthomonas trifolii"* (Table 2).

A total of 13 DNA hybridization groups (groups of strains whose DNAs were 70% or more related) containing from 2 to 13 strains each were identified from the DNA hybridization reactions (Tables 2 and 3). Altogether, 90 strains were classified into these DNA hybridization groups, whereas the remaining 34 strains did not belong to any group (Table 2). DNAs from four of these ungrouped strains (strains 486-51, 4908-71, ATCC 23372, and ICPB SS 11) were labeled, but they showed less than species level relatedness to unlabeled DNAs from the remaining ungrouped strains and to representative DNAs from each of the 13 previously established groups (data not shown). All of the hybridization groups were unique at the species level; no strain showed 70% or greater relatedness to strains representing more than one hybridization group. The levels of relatedness between pairs of the 13 hybridization groups ranged from 17 to 64% (Table 4). Hybridization groups I through VI, VIII, and XIII were 40% or more interrelated, as were hybridization groups VII,

TABLE 2. Herbicola-Agglomerans group strains

DNA relatedness group[a]	Biogroup[b]	Strain designation[c]	Name(s) as received	Source	Sent by:[d]
I	1	2780-70	*Enterobacter agglomerans*	Human, foot wound	Washington SHD
I	1	5967-70	*Enterobacter agglomerans*	Human, finger wound	Montana SHD
II	1	238-70	*Enterobacter agglomerans*	Human, blood	New York SHD
II	G1	1778-70	*Enterobacter agglomerans*	Human, blood	Oklahoma SHD
II	2	2548-70	*Enterobacter agglomerans*	Human, urine	Illinois SHD
II	2	2553-70	*Enterobacter agglomerans*	Human, leg wound	Hawaii SHD
II	2	3123-70	*Enterobacter agglomerans*	Human, stool	New Jersey SHD
II	1	198-71	*Enterobacter agglomerans*	Human, urine	Montana SHD
II	1	217-71	*Enterobacter agglomerans*	Human, skin	U. Va. Hosp.
II	2	258-71 (= ATCC 29000)	*Enterobacter agglomerans*	Human	Arizona SHD
II	1	303-71	*Enterobacter agglomerans*	Human, sputum	Virginia SHD
II	ND[e]	876	*Enterobacter agglomerans*	Human	Brenner
III	1	56-71	*Enterobacter agglomerans*	Human, blood	VA Hosp., Pittsburgh
III	2	226-71	*Enterobacter agglomerans*	Human, leg wound	Connecticut SHD
III	1	247-71	*Enterobacter agglomerans*	Human, blood	Florida SHD
III	2	1429-71	*Enterobacter agglomerans*	Intravenous fluid cap	Neblett
III	1	ATCC 14589 (= ICPB EH118)	*Erwinia herbicola*		Colwell
IV	3	2671-70	*Enterobacter agglomerans*	Human, urine	New Hampshire SHD
IV	6	3638-70	*Enterobacter agglomerans*	Human, cyst	Ft. McPherson, Ga.
IV	6	5795-70	*Enterobacter agglomerans*	Human, urine	PHS Hosp., Norfolk
IV	3	6148-70	*Enterobacter agglomerans*	Human, spinal fluid	Hawaii SHD
IV	3	1741-71 (= ATCC 27998)	*Enterobacter agglomerans*	Human, trachea	Connecticut SHD
V	6	2928-68	*Enterobacter agglomerans*	Human, sputum	Wisconsin SHD
V	6	2525-70	*Enterobacter agglomerans*		Quebec, Canada
V	3	4155-70	*Enterobacter agglomerans*	Human, nose	Connecticut SHD
V	2	4787-70	*Enterobacter agglomerans*	Human, throat	Pennsylvania SHD
V	3	3482-71	*Enterobacter agglomerans*	Human, urethra	Montana SHD
V	3	3518-71	*Enterobacter agglomerans*	Human, throat	U.S. Army, California
V	G1	3527-71	*Enterobacter agglomerans*	Human, blood	Institut Pasteur, Paris, France
V	4	3737-71	*Enterobacter agglomerans*	Human, sputum	NIH
VI	4	3768-69	*Enterobacter agglomerans*	Environment	UNC
VI	4	5448-69	*Enterobacter agglomerans*	Human, blood	VA Hosp., Bronx
VI	4	6070-69	*Enterobacter agglomerans*	Human, wound	Georgia SHD
VI	4	5748-70	*Enterobacter agglomerans*	Human, finger wound	California SHD
VI	7	XU104	"*Xanthomonas uredovorus*," *Erwinia uredovora*		Starr
VI	4	EA181	*Erwinia ananas*, *Erwinia herbicola* subsp. *ananas*		Starr
VI	4	B3526	*Enterobacter agglomerans*	Human, blood	Weaver
VI	4	ATCC 11530 (= ICPB EA197)	*Erwinia ananas*, *Erwinia herbicola* subsp. *ananas*		Colwell
VI	4	ATCC 14536 (= ICPB EH120)	*Erwinia ananas*, *Erwinia herbicola* subsp. *ananas*		Colwell
VI	7	ATCC 19321 (= ICPB XU102)	*Erwinia uredovora*		Colwell
VI	4	ATCC 23822 (= ICPB EH119)	*Erwinia ananas*, *Erwinia herbicola* subsp. *ananas*	Banana	Colwell
VII	5	166-70	*Enterobacter agglomerans*	Human, hand wound	NYCHD
VII	5	1469-70	*Enterobacter agglomerans*	Human, stool	Missouri SHD
VII	5	3970-70	*Enterobacter agglomerans*	Human, stool	North Carolina SHD
VII	5	4172-70	*Enterobacter agglomerans*	Human, stool	Alabama SHD
VII	5	5526-70	*Enterobacter agglomerans*	Human, eye	Louisiana SHD
VII	5	5656-70	*Enterobacter agglomerans*	Human, foot wound	Virginia SHD
VII	5	5685-71	*Enterobacter agglomerans*	Human	Massachusetts SHD
VII	5	6003-71	*Enterobacter agglomerans*		New Jersey SHD
VIII	6	5422-69	*Enterobacter agglomerans*	Human, urine	Washington SHD
VIII	6	4707-72	*Enterobacter agglomerans*	Human, pulmonary infection	Mississippi SHD
IX	G1	459-71A	*Enterobacter agglomerans*	Human, skin	U.S. Navy, Portsmouth, Va.

TABLE 2—*Continued*

DNA relatedness group[a]	Biogroup[b]	Strain designation[c]	Name(s) as received	Source	Sent by:[d]
IX	G1	459-71B	*Enterobacter agglomerans*	Human, skin	U.S. Navy, Portsmouth, Va.
IX	G1	2710-71	*Enterobacter agglomerans*	Mouse chow	NNMC
IX	G4	3525-71	*Enterobacter agglomerans*	Human, nose	Institut Pasteur, Paris, France
IX	G1	4388-71	*Enterobacter agglomerans*	Human, throat	NIH
X	G2	1599-71	*Enterobacter agglomerans*	Chicken livers	New Hampshire SHD
X	G2	1600-71	*Enterobacter agglomerans*	Chicken livers	New Hampshire SHD
XI	G3	1744-71 (= ICPB 3423)	*Enterobacter agglomerans*	Human, leg wound	Connecticut SHD
XI	G3	2709-71	*Enterobacter agglomerans*	Mouse chow	NIH
XI	G3	2711-71	*Enterobacter agglomerans*	Human, urine	California SHD
XI	G3	4519-71	*Enterobacter agglomerans*	Human, skin	Colorado SHD
XI	G3	5378-71	*Enterobacter agglomerans*	Human, vagina	Missouri SHD
XI	G3	2674-72 (= ATCC 27984)	*Enterobacter agglomerans*	Human, urine	Ft. McPherson, Ga.
XII	G3	185-71	*Enterobacter agglomerans*	Human, blood	Pennsylvania SHD
XII	G4	219-71	*Enterobacter agglomerans*	Intravenous fluid	U. Va. Hosp.
XII	G4	299-71	*Enterobacter agglomerans*	Human, blood	New Jersey SHD
XII	G4	509-71	*Enterobacter agglomerans*	Intravenous fluid	Michigan SHD
XII	G3	934-71	*Enterobacter agglomerans*	Human, blood	Colorado SHD
XII	G4	1083-71	*Enterobacter agglomerans*	Intravenous fluid	Neblett
XII	G4	1309-71	*Enterobacter agglomerans*	Human, blood	Neblett
XII	G4	1348-71	*Enterobacter agglomerans*	Human, blood	Colorado SHD
XII	G4	1426-71	*Enterobacter agglomerans*	Intravenous fluid cap	Neblett
XII	G4	3621-71	*Enterobacter agglomerans*	Human, hand wound	Florida SHD
XII	G3	4176-71	*Enterobacter agglomerans*	Human, sputum	Colorado SHD
XII	G4	4610-71	*Enterobacter agglomerans*	Human, sputum	VA Hosp., Durham
XII	G4	5764-71	*Enterobacter agglomerans*	Human, urine	Georgia SHD
XIII	1	1645-71	*Enterobacter agglomerans*		CDC
XIII	1	2774-71 (= ICPB 3424)	*Enterobacter agglomerans*	Human, leg wound	Connecticut SHD
XIII	1	EM101 (= ATCC 23374)	"*Erwinia mangifera*," *Erwinia herbicola* subsp. *herbicola*		Starr
XIII	1	EL102	"*Erwinia lathyri*," *Erwinia herbicola* subsp. *herbicola*		Starr Starr
XIII	1	EM102 (= ATCC 23375)	*Erwinia milletiae*, *Erwinia herbicola* subsp. *herbicola*		Starr
XIII	1	EH103	*Erwinia herbicola* subsp. *herbicola*		
XIII	1	EL107	"*Erwinia lathyri*," *Erwinia herbicola* subsp. *herbicola*		Starr
XIII	1	XT109	"*Xanthomonas trifolii*," *Erwinia herbicola* subsp. *herbicola*		Starr
XIII	1	EM114	*Erwinia milletiae*, *Erwinia herbicola* subsp. *herbicola*		Starr
XIII	1	ATCC 12287 (= ICPB EH117)	"*Xanthomonas trifolii*," *Erwinia herbicola* subsp. *herbicola*		Colwell
XIII	1	ATCC 23374 (= ICPB EM101)	"*Erwinia mangiferae*," *Erwinia herbicola* subsp. *herbicola*		Colwell
XIII	1	ATCC 23375 (= ICPB EM102)	*Erwinia milletiae*, *Erwinia herbicola* subsp. *herbicola*		Colwell
U	7	5257-64	*Enterobacter agglomerans*	Human, urine	PHS Hosp. Norfolk
U	6	2116-68	*Enterobacter agglomerans*	Human, eye	South Carolina SHD
U	6	4132-68	*Enterobacter agglomerans*	Hospital environment	Yale University
U	6	26-69	*Enterobacter agglomerans*	Human, blood	California SHD
U	4	3618-69	*Enterobacter agglomerans*	Human, leg wound	California SHD
U	4	4953-69	*Enterobacter agglomerans*	Sheep	Mayo Clinic
U	1	831-70	*Enterobacter agglomerans*	Human, finger wound	New Hampshire SHD
U	3	893-70	*Enterobacter agglomerans*	Human	Connecticut SHD
U	2	6012-70	*Enterobacter agglomerans*	Human	Georgia SHD
U	5	184-71	*Enterobacter agglomerans*	Human, urine	Pennsylvania SHD
U	2	314-71	*Enterobacter agglomerans*	Human, urine	Colorado SHD
U	G1	1376-71	*Enterobacter agglomerans*	Human, blood	New Jersey SHD
U	G2	1379-71	*Enterobacter agglomerans* (actually *Erwinia cypripedii*)	Intravenous fluid	Louisiana SHD
U	G2	2499-71	*Enterobacter agglomerans*	Human	Pennsylvania SHD

TABLE 2—*Continued*

DNA relatedness group[a]	Biogroup[b]	Strain designation[c]	Name(s) as received	Source	Sent by:[d]
U	2	2750-71	*Enterobacter agglomerans*	Human, vagina	Illinois SHD
U	1	2862-71	*Enterobacter agglomerans*	Mouse chow	NIH
U	G4	4524-71	*Enterobacter agglomerans*	Human, skin	Colorado SHD
U	G2	4908-71	*Enterobacter agglomerans*		Hawaii SHD
U	3	4990-71	*Enterobacter agglomerans*	Rabbit, stool	NIH
U	7	5379-71	*Enterobacter agglomerans*	Human, spinal fluid	Illinois SHD
U	G1	5380-71	*Enterobacter agglomerans*	Human, abdominal fluid	Montana SHD
U	7	ATCC 23637	*Erwinia uredovora*	Uredia of cereal rust	Colwell
U	1	EC11 (= ATCC 23372)	"*Erwinia cassavae*," *Erwinia herbicola* subsp. *herbicola*		Starr
U	1	ATCC 23372 (= ICPB EC11)	"*Erwinia cassavae*," *Erwinia herbicola* subsp. *herbicola*		Colwell
U	G3	486-51	*Enterobacter agglomerans*		Brenner
U	1	ICPB 3163	*Erwinia herbicola*		Starr
U	ND	NIH 457	*Enterobacter agglomerans*		Brenner
U	1	EM110	"*Erwinia maydis*"		Starr
U	2	ICPB 2553	*Erwinia herbicola*		Starr
U	4	EA101	*Erwinia ananas*		Starr
U	7	B3295	*Enterobacter agglomerans*	Human, urine	Weaver
U	6	B8006	*Enterobacter agglomerans*	Human, urine	Weaver
U	6	ATCC 14537 (= ICPB EH116)	*Erwinia herbicola*		Colwell
U	3	SS11 (= ATCC 8199)	*Erwinia stewartii*		Starr

[a] A total of 13 DNA relatedness groups were identified in this study (see text). Strains that did not fit into any of these DNA groups are designated U.

[b] Biogroups of Ewing and Fife (14). G, Aerogenic.

[c] Strain designations with a hyphen (e.g., 2780-70) or beginning with a single letter (e.g., B3526) are Centers for Disease Control strains. Designations beginning with two letters (e.g., XU104) or with ICPB were obtained directly from the International Collection of Phytopathogenic Bacteria.

[d] SHD, State Health Department; U. Va. Hosp., University of Virginia Hospital, Charlottesville; Brenner, V. Brenner, National Institutes of Health, Bethesda, Md.; VA Hosp., Pittsburgh, Veterans Administration Hospital, Pittsburgh, Pa.; Neblett, T. R. Neblett, University of Michigan, Ann Arbor; Colwell, R. R. Colwell, University of Maryland, College Park; PHS Hosp., Norfolk, Public Health Service Hospital, Norfolk, Va.; NIH, National Institutes of Health, Bethesda, Md.; UNC, University of North Carolina, Chapel Hill; VA Hosp., Bronx, Veterans Administration Hospital, Bronx, N.Y.; Starr, M. P. Starr, International Collection of Phytopathogenic Bacteria; Weaver, R. E. Weaver, Centers for Disease Control, Atlanta, Ga.; NYCHD, New York City Health Department; NNMC, National Naval Medical Center, Bethesda, Md.; VA Hosp., Durham, Veterans Administration Hospital, Durham, N.C.; CDC, Centers for Disease Control, Atlanta, Ga.

[e] ND, Not determined.

IX, XI, and XII; hybridization group X was 20 to 30% related to the former hybridization groups and 30 to 39% related to the latter hybridization groups.

The reference strain from each hybridization group was tested against 25 to 30 strains which represented all of the recognized genera in the *Enterobacteriaceae* as of 1976. The levels of relatedness were between 4 and 56% (Table 4). The highest levels of relatedness were observed between *Erwinia cypripedii* and hybridization groups II through VI (41 to 47%) and between *Enterobacter* species and hybridization group VII.

Strains isolated from humans were present in all 13 hybridization groups. A total of 20 strains isolated from humans or associated with human infections and 2 animal isolates did not fit into any of the hybridization groups. Strains isolated from plants (or presumed to have been isolated from plants by virtue of their *Erwinia* species names) were found in hybridization groups III, VI, and XIII, and nine such strains were ungroupable (Table 2).

The DNA hybridization groups did not correlate with the biogrouping scheme of Ewing and Fife (14). Seven hybridization groups contained strains from two or more biogroups, and strains from all biogroups except 5, 7, and G2 were found in more than one hybridization group. Biogroup 5 strains were found only in hybridization group VII, and biogroup G2 strains were found only in hybridization group 10 (only two strains). Strains belonging to all 11 biogroups were found among the strains that were ungroupable by DNA relatedness. Plant isolates were mainly found in biogroups 1, 4, and 7, although single strains that were ungroupable by DNA relatedness were present in biogroups 2, 3, and 6.

Strains from aerogenic and anaerogenic biogroups were rarely in the same hybridization group. Hybridization groups IX through XII contained only aerogenic strains. A single aerogenic strain was included among the anaerogenic strains contained in hybridization groups II and V. The other hybridization groups contained only anaerogenic strains (Table 2). The aerogenic strain in hybridization group II (strain 1778-80) was only 69% related to the group II reference strain, and the aerogenic strain in hybridization group V (strain 3527-71) was 73% related to the group V reference strain; the related sequences showed 6% divergence (6°C lower thermal stability than that observed for the homologous DNA from the reference strain). The other strains in hybridization group V were 83 to 100% related to

TABLE 3. Levels of relatedness of the Herbicola-Agglomerans group strains in the 13 DNA hybridization groups

DNA hybridization group	Strain	Relative binding ratio at 60°C[a]	Divergence (%)[b]	Relative binding ratio at 75°C[a]
I	2780-70[c]	100	0.0	100
	5967-70	93 (93)[d]	0.0 (0.0)	94 (94)
II	3123-70[c]	100	0.0	100
	2548-70	100	0.5	96
	217-71	96	0.0	96
	2553-70	88	1.5	84
	876	88	2.5	84
	198-71	83	2.0	72
	258-71	82	3.5	78
	238-70	80	2.5	76
	303-71	80	2.5	76
	1778-70	69 (85)	2.5 (2.0)	66 (80)
III	1429-71[c]	100	0.0	100
	226-71	94	0.5	90
	56-71	92	1.0	86
	247-71	90	0.5	84
	ATCC 14589	89 (91)	1.0 (1.0)	82 (86)
IV	1741-71[c]	100	0.0	100
	6148-70	94	1.0	89
	5795-70	91	0.0	92
	2671-70	88	1.0	87
	3638-70	87 (90)	1.0 (1.0)	88 (89)
V	3482-71[c]	100	0.0	100
	2928-68	100	0.0	100
	4787-70	99	0.0	100
	3518-71	99	0.0	100
	3737-71	99	0.0	98
	2525-70	99	0.0	97
	4155-70	84	0.0	84
	3527-71	73 (93)	6.0 (1.0)	67 (92)
VI[e]	6070-69[c]	100	0.0	100
	B3526	90	0.0	90
	3768-69	90	0.0	84
	5748-70	89	0.0	84
	ATCC 19321	88	1.0	89
	XU104	85	0.5	85
	5448-69	85	0.5	79
	ATCC 11530	83 (87)	0.0 (0.5)	90 (85)
VI[e]	ATCC 19321[c]	100	0.0	
	XU104	98	0.5	
	EA181	84	1.0	
	ATCC 23822	81	0.5	
	ATCC 14536	75 (85)	0.0 (0.5)	
VII	6003-71[c]	100	0.0	100
	4172-70	91	1.5	83
	5526-70	91	2.0	84
	3970-70	90	1.0	84
	166-70	88	2.0	79
	5656-70	87	1.5	82
	1469-70	86	1.5	82
	5685-71	84 (88)	1.5 (1.5)	81 (82)
VIII	5422-69[c]	100	0.0	100
	4707-62	97 (97)	0.5 (0.5)	91 (91)
IX	4388-71[c]	100	0.0	100
	3525-71	100	0.0	100
	2710-71	91	1.5	83
	459-71A	88	2.5	80
	459-71B	85 (91)	3.0 (2.0)	74 (84)
X	1600-71[c]	100	0.0	100
	1599-71	100 (100)	0.0 (0.0)	97 (97)
XI	5378-71[c]	100	0.0	100
	2711-71	75	5.5	57
	4519-71	74	5.5	63
	2709-71	74	5.5	61
	2674-72	73	6.0	59
	1744-71	71 (73)	6.0 (5.5)	62 (60)
XII	219-71[c]	100	0.0	100
	1083-71	100	0.0	99
	1309-71	99	0.0	98
	5764-71	92	0.5	93
	4610-71	90	0.5	92
	299-71	88	1.5	84
	3621-71	86	2.0	86
	1426-71	84	6.0	70
	185-71	83	6.0	66
	1348-71	79	6.5	78
	509-71	79	1.0	65
	934-71	79	6.0	68
	4176-71	75 (86)	6.0 (3.0)	66 (82)
XIII	1645-71[c]	100	0.0	100
	EM102	97	0.0	94
	XT109	96	0.5	91
	2774-71	96	1.0	91
	EL107	95	1.0	93
	EM114	93	1.0	94
	ATCC 23375	92	1.0	92
	EL102	92	2.0	89
	EH103	88	1.0	90
	ATCC 12287	87	0.0	83
	EM101	80	6.0	69
	ATCC 23374	75 (90)	6.5 (2.0)	68 (87)

[a] Relative binding ratios were calculated by using the following expression: [(percentage of heterologous DNA bound to hydroxyapatite)/(percentage of homologous DNA bound to hydroxyapatite)] × 100.

[b] Divergence was the decrease in the thermal stability of the heterologous DNA duplexes compared with the thermal stability of the homologous DNA duplexes. Divergence values were calculated on the assumption that each 1°C decrease in thermal stability was due to approximately 1% unpaired bases in double-stranded DNA.

[c] Source of labeled DNA.

[d] The numbers in parentheses are averages. For each group the homologous DNA reaction was arbitrarily defined as 100%. Therefore, the values for the homologous DNA reactions (labeled and unlabeled DNAs from the same strain) were excluded when the averages were calculated.

[e] Two labeled DNAs were used for DNA hybridization group VI. All reactions were done two or more times.

the reference strain, and their related sequences showed no divergence (Table 3). Therefore, one might argue that the aerogenic and anaerogenic biogroups are totally separable at the species level.

In hybridization group XI all strains were 71 to 75% related to the reference strain. The related sequences of these strains showed 5.5 to 6.0% divergence, and the level of relatedness fell to between 57 and 63% in reactions done at the stringent incubation temperature (75°C). It is possible that all strains except the reference strain are very highly related (Table 3). The only other "heterogeneous" hybridization group was group XII, in which the level of relatedness was between 75 and 100%, divergence was as high as 6.5%, and the level of relatedness at 75°C was between 65 and 99% (Table 3).

Table 5 shows the results of 33 biochemical tests that were

TABLE 4. Levels of relatedness of the Herbicola-Agglomerans group DNA hybridization groups to each other and to other members of the *Enterobacteriaceae*

Source of unlabeled DNA	Relative binding ratio at 60°C with DNA hybridization group:[a]				
	I	II	III	IV	V
HG I[b]	93	45 (1)[c]	44 (1)	42 (1)	38 (1)
HG II	49 ± 1.8 (10)[e]	85	56 ± 1.4 (2)	53 ± 4.9 (2)	53 ± 4.9 (2)
HG III	47 ± 0.9 (5)	51 ± 3.5 (5)	91	59 ± 2.3 (5)	50 ± 4.1 (5)
HG IV	43 ± 4.1 (5)	44 ± 3.8 (5)	56 (1)	90	44 ± 5.4 (5)
HG V	44 ± 2.0 (8)	50 ± 1.4 (8)	56 (1)	50 ± 2.4 (8)	93
HG VI	36 ± 2.9 (11)	38 ± 3.4 (11)	47 ± 2.8 (2)	46 ± 2.1 (2)	51 ± 0.7 (2)
HG VII	36 ± 2.0 (8)	30 ± 1.2 (8)	37 (1)	34 ± 2.1 (2)	33 (1)
HG VIII	49 ± 0.7 (2)	42 ± 0.7 (2)	46 ± 3.5 (2)	39 ± 4.9 (2)	45 ± 1.4 (2)
HG IX	41 ± 3.3 (5)	33 ± 0.0 (2)	38 ± 4.9 (2)	29 ± 7.1 (2)	36 ± 0.7 (2)
HG X	31 ± 2.8 (2)	24 (1)	30 (1)	23 (1)	24 (1)
HG XI	35 ± 2.4 (6)	33 ± 1.6 (6)	39 (1)	30 ± 5.1 (3)	34 ± 6.1 (3)
HG XII	31 ± 2.9 (13)	27 ± 2.1 (13)	30 (1)	25 ± 2.1 (4)	29 ± 4.3 (4)
HG XIII	42 ± 1.8 (12)	48 ± 1.9 (4)	50 ± 0.6 (3)	46 ± 5.2 (3)	64 ± 2.0 (3)
Erwinia cypripedii	—[f]	41	47	41	41
Erwinia salicis	—	36	—	—	—
Erwinia amylovora	—	—	—	—	—
Escherichia, Shigella, Salmonella, Enterobacter, Klebsiella, Citrobacter[g]	25–35	25–31	25–35	27–36	25–32
Erwinia, Serratia, Hafnia, Yersinia, Edwardsiella, Morganella[g]	17–25	13–23	16–24	21–26	19–25
Providencia, Proteus[g]	7–9	5–8	8–10	9–11	6–9

[a] See Table 3, footnote *a*. Standard errors of the mean are given except for reactions among strains of the same hybridization group.
[b] HG, DNA hybridization group.
[c] The numbers in parentheses are the numbers of strains tested.
[d] ND, Not determined.
[e] Data are expressed as mean ± standard error.
[f] —, Value in the range given for the genus.
[g] Labeled DNA from each DNA hybridization group was tested for its level of relatedness to 25 to 30 species of the genera indicated.

helpful in distinguishing among the hybridization groups. Only one API 50R test helped differentiate among hybridization groups. Several API 50R tests were not done in the conventional system; the tests which gave uniformly positive or negative results are shown in Table 5, footnote *a*. Groups I, VIII, and X, which contained only two strains each, were omitted, as were strains that did not fit into any hybridization group. Table 5 shows that it was difficult to distinguish biochemically among several of the hybridization groups, especially groups II through V. One simplistic approach is presented in Fig. 1. Groups VI, VII, IX, XI, XII, and XIII are separable from one another and from the other hybridization groups on the basis of gas production and several commonly used tests. Hybridization group IV is separable from groups II, III, and V by means of its negative malonate and rhamnose reactions. Groups II, III, and V are only partially separable on the basis of their reactions for cellobiose and acetate. The fermentation of dextrin in the API 50R system was helpful, as group V strains were positive and group II and III strains were negative in this test.

A total of 72 strains representing all 13 hybridization groups were compared by numerical taxonomy. There were two types of relationship between hybridization groups and phenotypic clustering behavior. All aerogenic strains (hybridization groups IX through XII) and the anaerogenic strains of hybridization groups I, VI, and VII formed distinct phenotypic clusters, whereas the strains of the other hybridization groups did not form homogeneous clusters. Strains that were not grouped by DNA hybridization did not cluster together.

Antimicrobial agent susceptibility tests were performed on 74 strains chosen from all of the hybridization groups, as well as from strains that could not be grouped by DNA hybridization. All of the strains tested were susceptible to chloramphenicol, polymyxin b, and cefamandole; all but one to three strains were susceptible to amikacin, cefoxitin, gentamicin, kanamycin, tetracycline, tobramycin, and trimethoprim-sulfamethoxazole. Resistance to other antimicrobial agents was as follows: ampicillin, 17 strains (plus 2 strains showing zone sizes intermediate between susceptibility and resistance); carbenicillin, 20 strains (plus 15 strains with intermediate zone sizes); cephalothin, 6 strains (plus 6 strains with intermediate zone sizes); nalidixic acid, 1 strain (plus 5 strains with intermediate zone sizes); nitrofurantoin, 23 strains (plus 4 strains with intermediate zone sizes); and penicillin G, 57 strains (plus 4 strains with intermediate zone sizes). Resistant strains were found in all hybridization groups except groups VII, X, and XI. The antimicrobial agent susceptibility patterns were not helpful in distinguishing among the hybridization groups.

DISCUSSION

The DNA relatedness studies on the Herbicola-Agglomerans group were completed during 1974 and were reported preliminarily in 1975 (G. R. Fanning, A. G. Steigerwalt, P. Klykken, E. Cadet, and D. J. Brenner, Abstr. Annu. Meet. Am. Soc. Microbiol. 1975, C52, p. 35).

The phenotypic characteristics described here were determined in 1976 and 1977. Although the hybridization groups that contained aerogenic strains and several of the groups containing anaerogenic strains could be identified phenotypically, four hybridization groups (groups II through V) were difficult, if not impossible, to define and separate biochemically. Analyzing the phenotypic data by numerical taxonom-

TABLE 4—*Continued*

Relative binding ratio at 60°C with DNA hybridization group:[a]							
VI	VII	VIII	IX	X	XI	XII	XIII
36 (1)	32 (1)	41 (1)	35 (1)	27 (1)	20 (1)	29 (1)	ND[d]
45 ± 4.9 (2)	34 ± 0.7 (2)	44 ± 2.1 (2)	33 ± 2.8 (2)	26 ± 1.4 (2)	21 ± 0.0 (2)	32 ± 1.4 (2)	49 (1)
47 ± 1.7 (5)	33 ± 2.8 (5)	44 ± 2.3 (5)	38 ± 1.9 (5)	27 ± 0.4 (5)	21 ± 3.0 (5)	33 ± 4.7 (5)	45 (1)
43 ± 2.3 (5)	27 ± 5.6 (5)	39 ± 3.2 (5)	34 ± 1.9 (5)	24 ± 0.4 (5)	19 ± 3.3 (5)	28 ± 4.0 (5)	ND
51 ± 2.0 (8)	30 ± 3.8 (8)	44 ± 1.4 (8)	36 ± 3.5 (8)	27 ± 2.6 (8)	22 ± 2.3 (8)	30 ± 3.6 (8)	ND
87	27 ± 1.4 (2)	38 ± 1.4 (2)	32 ± 2.1 (11)	24 ± 1.6 (11)	17 ± 4.1 (8)	28 ± 5.7 (8)	43 ± 3.4 (4)
31 ± 2.0 (8)	88	34 ± 2.0 (8)	49 ± 1.2 (8)	38 ± 1.4 (8)	54 ± 2.6 (8)	45 ± 1.1 (8)	ND
42 ± 0.7 (2)	27 ± 1.4 (2)	97	36 ± 1.4 (2)	30 ± 1.4 (2)	21 ± 2.1 (2)	38 ± 7.1 (2)	ND
30 ± 0.0 (2)	48 ± 3.5 (2)	35 ± 6.4 (2)	91	36 ± 0.7 (2)	39 ± 2.8 (2)	49 ± 2.8 (2)	ND
26 (1)	39 (1)	31 (1)	37 (1)	100	23 (1)	34 (1)	ND
30 ± 2.6 (11)	58 ± 1.7 (3)	32 ± 0.6 (2)	48 ± 1.5 (6)	37 ± 2.6 (6)	73	44 ± 1.5 (6)	ND
31 ± 4.1 (4)	44 ± 3.6 (3)	29 ± 3.0 (4)	49 ± 2.4 (13)	36 ± 1.8 (13)	36 ± 2.8 (4)	86	ND
48 ± 5.9 (3)	28 ± 0.6 (3)	40 ± 1.5 (3)	34 ± 1.0 (4)	26 ± 0.8 (4)	19 ± 2.3 (3)	29 ± 1.5 (3)	90
42	27	31	30	26	20	28	39
—	—	—	—	—	—	—	—
—	—	41	—	—	—	—	—
24–30	39–56	24–32	37–49	33–39	31–45	38–44	23–29
17–23	17–28	17–23	19–34	20–29	12–26	15–28	16–23
7–12	8–11	7–8	8–13	8–11	4–8	10–16	

ic techniques did not improve our ability to distinguish among these hybridization groups.

Diagnostic bacteriologists were still faced with the problem of identifying clinical isolates of this complex on the basis of a biochemical data base that clearly contained data from at least several different species, and there was concern that one or more of the newly defined species, such as *Escherichia vulneris* (4), in the family *Enterobacteriaceae* might be a synonym for one of the names on the Approved Lists. For these reasons we decided to present the data here, to point out the shortcomings of this study, and to try to define the scope of further studies.

Of the 124 strains of the Herbicola-Agglomerans group which we studied by DNA relatedness, 90 formed 13 distinct hybridization groups (average relatedness, 73% or more for each group). The remaining 34 strains did not fit into any of these hybridization groups. The levels of relatedness among the 13 hybridization groups were 17 to 64%. The levels of relatedness between the Herbicola-Agglomerans group strains and other *Enterobacteriaceae* strains were highest with some species of *Erwinia* and *Enterobacter*, followed by species of *Escherichia, Shigella, Salmonella, Citrobacter*, and *Klebsiella*. In no case was the level of relatedness between a hybridization group strain and another *Enterobacteriaceae* strain as high as the level of relatedness between that hybridization group and at least one other hybridization group in the Herbicola-Agglomerans complex. From these data we are confident that each hybridization group represents a single, unique species within the family *Enterobacteriaceae*.

A total of 26 plant isolates were studied. A single *Erwinia herbicola* strain was in hybridization group III. Hybridization group VI contained two strains of *Erwinia uredovora* and four strains of *Erwinia ananas*. Ten plant strains were in hybridization group XIII. These included strains received as *"Erwinia lathyri," "Erwinia mangiferae," "X. trifolii,"* and *Erwinia milletiae* (all considered to be synonyms of *Erwinia herbicola* subsp. *herbicola*), as well as *Erwinia herbicola* subsp. *herbicola*. Nine plant isolates did not fit into any of the 13 hybridization groups. These strains were received as *Erwinia herbicola* subsp. *herbicola, Erwinia herbicola* subsp. *ananas, Erwinia uredovora, Erwinia stewartii, "Erwinia maydis,"* and *"Erwinia cassavae."* These results show that the present classification of members of the Herbicola-Agglomerans group as three species plus *Erwinia herbicola* subsp. *ananas* is inadequate.

The classification scheme of Ewing and Fife with all of the strains in *Enterobacter agglomerans* and subdivision of the strains into 11 biogroups (14) is also not supported by the present data since there are multiple species, since more than one biogroup is present in 7 of the 10 hybridization groups that contain more than two strains, and since strains from almost all biogroups are present in more than one hybridization group. However, aerogenic strains and anaerogenic strains were not found in the same hybridization group.

Previous studies showed that 70% or greater interrelatedness was characteristic of the strains within any species of the *Enterobacteriaceae* (4, 15, 30). By this criterion there are 13 species among the grouped strains and 5 or more species among the 34 strains that do not fit into any of the groups (4 of these strains were labeled and were not related at the species level to any of the other ungroupable strains).

If we dismiss the ungroupable strains and hybridization group X (two strains isolated simultaneously from chicken livers), we are left with 12 species that contain two or more independently isolated strains. Seven of these hybridization groups are separable on the basis of phenotypic characteristics and, except for group VIII, form highly related clusters as determined by numerical taxonomic analysis (groups I, VI, VII, IX, XI, and XII). Groups II through V and XIII cannot be separated with certainty on the basis of biochemical tests, although phenylalanine deaminase, malonate, rhamnose, cellobiose, acetate, and dextrin reactions are helpful in distinguishing them.

Since 1980, a species has standing in nomenclature only if it appears on the Approved Lists (28). Seven species names that represent members of the Herbicola-Agglomerans group

TABLE 5. Differential reactions among DNA hybridization groups of the Herbicola-Agglomerans group

Test	% Of positive reactions in DNA hybridization group:[a]									
	II (8 strains)	III (7 strains)	IV (5 strains)	V (8 strains)	VI (4 strains)	VII (8 strains)	IX (3 strains)	XI (6 strains)	XII (14 strains)	XIII (3 strains)
Urea	25	0	20	37	25	75	33	100	28	33
Indole	0	14	0	12	100	100	0	100	7	33
Methyl red	75	71	80	62	0	100	0	100	35	33
Voges-Proskauer	50	28	40	50	100	0	100	0	78	100
Citrate (Simmons)	100	87	100	87	75	25	100	16	100	66
Growth in KCN	0	14	20	25	25	100	66	83	78	33
Motility	75	100	80	75	100	100	100	100	7	100
Gelatin (22°C)	75	82	60	100	100	75	100	33	85	100
Phenylalanine deaminase	0	33	20	25	0	0	33	0	14	100
Gas from D-glucose	0	14	0	12	0	0	66	100	100	0
Lactose	37	42	20	37	100	100	33	100	100	100
Sucrose	87	57	100	75	100	0	66	66	100	100
Dulcitol	0	0	0	25	0	0	66	66	21	0
Salicin	62	100	20	50	75	100	100	100	100	100
Adonitol	0	0	0	12	0	0	0	83	0	33
i-Inositol	62	42	20	50	100	0	33	16	0	0
D-Sorbitol	0	14	0	12	100	0	100	16	93	0
L-Arabinose	100	100	100	87	100	100	100	100	100	100
Raffinose	0	0	0	25	100	12	66	83	42	0
L-Rhamnose	100	100	20	75	100	100	100	100	100	100
Malonate	62	71	0	87	75	100	66	83	93	66
Mucate	62	28	20	25	25	0	100	0	100	33
Tartrate (Jordan)	0	28	0	0	0	0	0	16	7	0
Acetate	12	85	40	37	100	62	66	100	93	33
D-Xylose	100	85	80	100	100	100	100	100	93	100
Trehalose	100	100	100	100	0	87	100	100	93	0
Cellobiose	25	71	80	87	100	100	100	100	100	66
Glycerol	37	14	0	50	100	75	100	66	64	33
α-Methyl glucoside	0	0	0	0	25	0	0	0	64	0
Esculin	100	100	60	87	50	100	100	100	100	100
Citrate (Christensen)	100	100	100	100	0	62	100	16	93	0
$NO_3^- \rightarrow NO_2^-$	100	100	60	25	0	100	100	100	78	100
Pigment (yellow)	87	42	60	87	100	87	66	83	0	66

[a] All results are for conventional biochemical tests performed on conventional media and incubated at 36 ± 1°C for 48 h. All strains gave positive reactions in tests for growth on MacConkey agar and acid production from D-glucose, D-mannitol, D-arabitol, L-arabinose, (API 50R test), ribose (API 50R test), L-xylose (API 50R test), galactose (API 50R test), D-levulose (API 50R test), and D-mannose (API 50R test) and negative reactions in tests for gram staining, oxidase, H_2S, pectate (API 50R test), lysine decarboxylase, arginine dihydrolase, ornithine decarboxylase, lipase (corn oil), deoxyribonuclease (API 50R test), and acid production from erythritol, methyl xyloside (API 50R test), L-sorbose (API 50R test), alpha-methyl-D-mannoside (API 50R test), *N*-acetylglucosamine (API 50R test), inulin (API 50R test), D-melizitose (API 50R test), amylose (API 50R test), and glycogen (API 50R test).

appear on the Approved Lists. Two of these, *Erwinia stewartii* (strain ATCC 8199 [= SS11]) and *Erwinia uredovora* (strain ATCC 19321) were used in this study. *Erwinia stewartii* ATCC 8199 was not grouped, and *Erwinia uredovora* ATCC 19321 was in hybridization group VI. The other type strains used were either not designated when the study was initiated, were poorly known, or, for *Enterobacter agglomerans* ATCC 27155, were inexplicably excluded. Species are defined in terms of their type strains. For example, if the type strain of *Erwinia herbicola* were in hybridization group A and 10 other strains called *Erwinia herbicola* were in hybridization group B, hybridization group A would be *Erwinia herbicola*. Alternatively, if two type strains were in the same hybridization group, the first name to be published would have priority and the second name would lose standing in nomenclature. There are more than enough hybridization groups to accommodate all of the species names, but it will be necessary to examine all type strains before making any nomenclatural proposal.

Another problem in classification is the designation of one or more genus names for members of the Herbicola-Agglomerans group. The designated species are presently in three genera, *Erwinia*, *Enterobacter*, and *Escherichia* (*Escherichia adecarboxylata*). This problem can be approached logically only after the species problems have been answered.

The present study suffered from several problems in addition to the lack of type strains. We thought that the total number of strains chosen for study (124 strains) was more than adequate. Unfortunately, we were wrong. There were not enough plant strains, and *Escherichia adecarboxylata* strains were not included. The human strains were mainly from a nationwide outbreak of septicemia (24). The strains certainly showed genetic diversity, so much that 90 strains formed 13 hybridization groups and 34 other strains were ungroupable. This resulted in a small number of strains with which to define the biochemical characteristics of most hybridization groups. The lack of a sufficient number of strains does not account for our inability to separate each hybridization group on the basis of biochemical tests. It is possible that some, but not all, of the problem was technical.

Since we could not separate all hybridization groups with common biochemical tests, additional tests should be investigated. The possibilities include substrate utilization tests, specific enzyme substrate tests, and tests for differences in

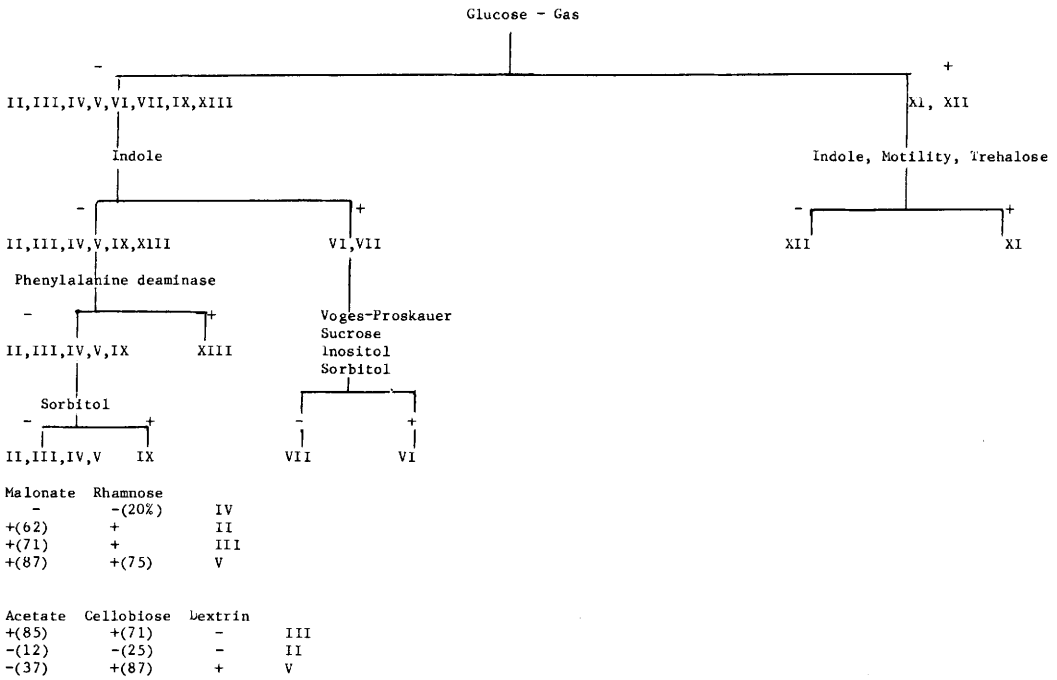

FIG. 1. Key for biochemical differentiation of the hybridization groups of *Enterobacter agglomerans*. The numbers in parentheses indicate the percentages of positive reactions after 48 h of incubation at 36 ± 1°C; where there are no numbers in parentheses, 100% of the reactions gave the results shown.

growth temperature range. On a practical note, several of the biochemical tests that helped identify members of the Herbicola-Agglomerans group are not included in many of the commercially available identification systems. These tests include trehalose, cellobiose, malonate, and acetate.

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LITERATURE CITED

1. **Bauer, A. W., W. M. M. Kirby, J. C. Sherris, and M. Turck.** 1966. Antibiotic sensitivity testing by a standardized disk method. Am. J. Clin. Pathol. **45**:493–496.
2. **Beijerinck, M. W.** 1888. Cultur des *Bacillus radicicola* aus den Knollchen. Bot. Zeitung **46**:740–750.
3. **Bottone, E., and S. S. Schneierson.** 1972. *Erwinia* species: an emerging human pathogen. Am. J. Clin. Pathol. **57**:400–405.
4. **Brenner, D. J., A. C. McWhorter, J. K. Leete Knudson, and A. G. Steigerwalt.** 1982. *Escherichia vulneris*: a new species of *Enterobacteriaceae* associated with human wounds. J. Clin. Microbiol. **15**:1133–1140.
5. **Cooper-Smith, M. E., and A. von Graevenitz.** 1978. Nonepidemic *Erwinia herbicola (Enterobacter agglomerans)* in blood cultures: bacteriological analysis of fifteen cases. Curr. Microbiol. **1**:29–32.
6. **Cruickshank, J. C.** 1935. A study of the so-called *Bacterium typhi flavum*. J. Hyg. **35**:354–371.
7. **Dresel, E. G., and O. Stickl.** 1928. Uber reversible Mutationsformen der Typhusbazillen beim Menschen. Dtsch. Med. Wochenschr. **54**:517–519.
8. **Dye, D. W.** 1963. The taxonomic position of *Xanthomonas uredovorus* Pon et al. 1954. N. Z. J. Sci. **6**:146–149.
9. **Dye, D. W.** 1963. The taxonomic position of *Xanthomonas stewartii* (Erw. Smith 1914) Dowson 1939. N. Z. J. Sci. **6**:495–506.
10. **Dye, D. W.** 1964. The taxonomic position of *Xanthomonas trifolii* (Huss, 1907) James, 1955. N. Z. J. Sci. **7**:261–269.
11. **Dye, D. W.** 1966. A comparative study of some atypical "xanthomonads." N. Z. J. Sci. **9**:843–854.
12. **Dye, D. W.** 1969. A taxonomic study of the genus *Erwinia*. III. The "Herbicola" group. N. Z. J. Sci. **12**:223–236.
13. **Ewing, W. H., and M. A. Fife.** 1971. *Enterobacter agglomerans*, the Herbicola-Lathyri bacteria. Center for Disease Control, Atlanta, Ga.
14. **Ewing, W. H., and M. A. Fife.** 1972. *Enterobacter agglomerans* (Beijerinck) comb. nov. (the Herbicola-Lathyri bacteria). Int. J. Syst. Bacteriol. **22**:4–11.
15. **Farmer, J. J., III, G. R. Fanning, G. P. Huntley-Carter, B. Holmes, F. W. Hickman, C. Richard, and D. J. Brenner.** 1981. *Kluyvera*, a new (redefined) genus in *Enterobacteriaceae*: identification of *K. ascorbata* sp. nov. and *K. cryocrescens* sp. nov. in clinical specimens. J. Clin. Microbiol. **13**:919–933.
16. **Geere, I. W.** 1977. *Enterobacter agglomerans*: the clinically important plant pathogen. Can. Med. Assoc. J. **116**:517–519.
17. **Gilardi, G. L., and E. W. Bottone.** 1971. *Erwinia* and yellow-pigmented *Enterobacter* isolates from human sources. Antonie von Leeuwenhoek J. Microbiol. Serol. **37**:529–535.
18. **Graham, D. C., and W. Hodgkiss.** 1967. Identity of gram negative, yellow pigmented, fermentative bacteria isolated from plants and animals. J. Appl. Bacteriol. **30**:175–189.
19. **Graham, D. C., and C. E. Quinn.** 1974. Identification of *Agrobacterium gypsophilae* strains NCPPB 179 and NCPPB 1948 as *Erwinia herbicola*. Int. J. Syst. Bacteriol. **24**:238–241.
20. **Hayward, A. C., and W. Hodgkiss.** 1961. Taxonomic relationships of *Xanthomonas uredovorus*. J. Gen. Microbiol. **26**:133–140.
21. **Hickman, F. W., and J. J. Farmer III.** 1978. *Salmonella typhi*: identification, antibiograms, serology, and bacteriophage typing. Am. J. Med. Technol. **44**:1149–1159.
22. **Leclerc, H.** 1962. Etude biochimique d'Enterobacteriaceae pigmentées. Ann. Inst. Pasteur Paris **102**:726–741.
23. **Lelliott, R. A.** 1974. Genus XII. *Erwinia* Winslow, Broadhurst, Buchanan, Krumwiede, Rogers and Smith 1920, 209, p. 332-339. *In* R. E. Buchanan and N. E. Gibbons (ed.), Bergey's manual of determinative bacteriology, 8th ed. The Williams & Wilkins Co., Baltimore.
24. **Maki, D. G., F. S. Rhame, D. C. Mackel, and J. V. Bennett.**

1976. Nationwide epidemic of septicemia caused by contaminated intravenous products. I. Epidemiologic and clinical features. Am. J. Med. **60**:471–485.

25. **Muraschi, T. F., M. Friend, and D. Bolles.** 1965. *Erwinia*-like microorganisms isolated from animal and human hosts. Appl. Microbiol. **13**:128–131.
26. **Richard, C.** 1974. Une nouvelle espéce d'entérobactérie rencontrée en bactériologie médicale *Enterobacter agglomerans* (ex-*Erwinia herbicola*). Ann. Biol. Clin. (Paris) **32**:341–346.
27. **Schneierson, S. S., and E. J. Bottone.** 1973. *Erwinia* infections in man. Crit. Rev. Clin. Lab. Sci. **4**:341–355.
28. **Skerman, V. B. D., V. McGowan, and P. H. A. Sneath (ed.).** 1980. Approved lists of bacterial names. Int. J. Syst. Bacteriol. **30**:225–420.
29. **Sneath, P. H., and R. R. Sokal.** 1973. Numerical taxonomy. W. H. Freeman and Co., San Francisco.
30. **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.
31. **Ursing, J.** 1977. Characterization of *Enterobacter agglomerans* (*Erwinia* spp.) from clinical specimens. Acta Pathol. Microbiol. Scand. Sect. B **85**:61–66.
32. **von Graevenitz, A.** 1970. *Erwinia* species isolates. Ann. N.Y. Acad. Sci. **174**:436–443.
33. **von Graevenitz, A.** 1971. Recognition and differential diagnosis of *Erwinia herbicola* strains isolated in the hospital. Pathol. Microbiol. **37**:84–88.
34. **Walczak, C. A., and M. I. Krichevsky.** 1980. Computer methods for describing groups from binary phenetic data: preliminary summary and editing of data. Int. J. Syst. Bacteriol. **30**:615–621.
35. **Walczak, C. A., and M. I. Krichevsky.** 1980. Computer methods for describing groups from binary phenetic data: modification of numerical taxonomy programs to increase flexibility. Int. J. Syst. Bacteriol. **30**:622–626.
36. **Walczak, C. A., and M. I. Krichevsky.** 1982. Computer-aided selection of efficient identification features and calculation of group descriptors as exemplified by data on *Capnocytophaga* species. Curr. Microbiol. **7**:199–204.