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Separation of *Kluyvera* and *Buttiauxella* by Biochemical and Nucleic Acid Methods

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We propose to maintain the genera *Buttiauxella* and *Kluyvera*, which are phenotypically similar, as separate genera in the *Enterobacteriaceae*. This separation is supported by the following findings: (i) strains of *Kluyvera ascorbata* and *Kluyvera cryocrescens* were related to *Buttiauxella agrestis* ATCC 33320^T (T = type strain) at levels of 32 to 36% and 30 to 31%, respectively, as determined by deoxyribonucleic acid relatedness (nitrocellulose filter method at 52.8°C); (ii) the guanine-plus-cytosine ratios of *Buttiauxella* and *Kluyvera* deoxyribonucleic acids 48 to 50 mol% and 55 to 57 mol%, respectively; and (iii) production of indole and lysine decarboxylase and fermentation of sucrose in 2 days could differentiate *Kluyvera* from *Buttiauxella*.

Kluyvera and *Buttiauxella* are two recently named genera in the family *Enterobacteriaceae* (4, 6). These two genera were proposed independently.

The purpose of this study was to compare strains of *Kluyvera* and *Buttiauxella* by using phenotypic characteristics, guanine-plus-cytosine (G+C) ratios, and deoxyribonucleic acid (DNA)-DNA hybridization so that a recommendation could be made for the classification of these organisms. From our findings we conclude that *Kluyvera* and *Buttiauxella* should remain separate genera.

Strains. The two complete sets of strains which we studied have been described previously (4, 6). Additional DNA hybridization experiments were done in France with three strains of *Kluyvera ascorbata* (ATCC 33433^T [T = type strain], ATCC 33434 and ATCC 14236) and three strains of *Kluyvera cryocrescens* (ATCC 33435^T, ATCC 14237, and ATCC 14238). The strains of *Buttiauxella* studied at the Centers for Disease Control were strains CUETM 77-167^T (= ATCC 33320^T = CIP 80-31^T), CUETM 78-19, CUETM 78-3, CUETM 78-34, and CUETM 78-8.

DNA-DNA hybridization and G+C ratios. The hybridization method used has been described previously (5, 7). DNA extraction was performed by the method of Marmur (8). DNA reassociation was performed with nitrocellulose membrane filters by using the technique of De Ley and Tyjtgat (3). The optimal temperatures of renaturation were 52.8, 57.4, and 55.4°C for *Buttiauxella agrestis* ATCC 33320^T, *K. ascorbata* ATCC 33433^T, and *K. cryocrescens* ATCC 33435^T, respectively. The G+C ratios of the *Kluyvera* type strains were measured by the average melting point method, as described by De Ley (2) and as used previously by Ferragut et al. (6) to study 17 strains of *Buttiauxella*.

Biochemical reactions. Biochemical reactions were studied at both of our laboratories by methods described previously (4, 6).

The levels of relative binding of DNAs from *K. ascorbata* and *K. cryocrescens* strains to the DNA of *B. agrestis* type strain ATCC 33320 are presented in Table 1.

The strains within each of the species examined were closely related as determined by DNA-DNA hybridization; *K. ascorbata* strains were more than 80% related to each other (26 strains tested), *K. cryocrescens* strains were 75 to 96% related (10 strains), and *B. agrestis* strains were 82 to 96% related (17 strains). *K. cryocrescens* was 60 to 72% related to *K. ascorbata* (4, 6) (Table 1).

The DNA values obtained for relatedness between *B. agrestis* (labeled DNA) and *Kluyvera* species were low (30 to 31% and 32 to 36% for *K. cryocrescens* and *K. ascorbata*, respectively).

Similar relatedness values between the two genera were obtained when DNAs from type strains of *Kluyvera* species were labeled (Table 1).

The G+C ratios of *K. cryocrescens* ATCC 14237 and CDC 0546-78 and *K. ascorbata* ATCC 33433^T and CDC 1058-74, as determined by the buoyant density method (cesium chloride centrifugation), were 55, 55, 56, and 57 mol% respectively (Table 1). According to the method of Ferragut and Leclerc (7) and Ferragut et al. (5)

TABLE 1. G+C content and DNA relatedness between species of *Kluyvera* and *Buttiauxella*

Source of unlabeled DNA	G+C content (mol%), as determined by the method of:		Relative binding (%) to labeled DNA from:				
	Farmer et al.[a]	Ferragut et al.[b]	*B. agrestis* ATCC 33320^T	*K. ascorbata* ATCC 14236[c]	*K. ascorbata* ATCC 33433^T	*K. cryocrescens* ATCC 14237[c]	*K. cryocrescens* ATCC 33435^T
K. ascorbata strains							
ATCC 33433^T (= CDC 648-74^T)	56	58	36 ± 7[d]	90	100	67	53
ATCC 33434 (= CDC 2221-78)			36 ± 8	93		66	
ATCC 14236 (= CDC 408-78)			32 ± 6	100		65	
CDC 1058-74	57			92		68	
Range for 26 strains				69–100		55–72	
K. cryocrescens strains							
ATCC 33435^T (= CDC 2065-78^T)		54	31 ± 5	69	52	94	100
ATCC 14237 (= CDC 409-78)	55		30 ± 10	62		100	
ATCC 14238 (= CDC 410-78)			30 ± 9	65		80	
CDC 0546-78	55			65		80	
Range for 10 strains				60–69		75–100	
B. agrestis strains							
ATCC 33320^T (= CUETM 77-167^T)		49	100[e]		14		14
CUETM 78-41		48	87		26		31
CUETM 78-39		47	94		16		15
CUETM 78-4		48	89		22		22
CUETM 77-157		48	91		31		18
CUETM 78-19		48	96		28		35
Range for 17 strains		48–50	82–94[e]				

[a] See reference 4.
[b] See reference 5.
[c] Data from Farmer et al. (4).
[d] Mean ± standard deviation.
[e] Data from Ferragut et al. (6).

TABLE 2. Biochemical reactions of *Kluyvera* and *Buttiauxella* species

Test	Days of incubation	Source(s) of data[a]	Reaction of:		
			B. agrestis (17 strains)	*K. ascorbata* (73 strains)	*K. cryocrescens* (16 strains)
Indole production	2	CDC + CUETM	−[b]	+	(+)
L-Lysine decarboxylase	2	CDC + CUETM	−	+	V
Ascorbate test[c]	2	CDC	−	+	−
Growth and D-glucose fermentation at 5°C	21	CDC	+	−	+
Sucrose fermentation	2	CDC + CUETM	−	+	+
D-Sorbitol fermentation	2	CDC + CUETM	−	V	V
Tetrathionate reductase	1	CUETM	V	−	−

[a] CDC indicates that the data are based on all of the *Kluyvera* strains examined and only five strains of *Buttiauxella*. CUETM indicates that data are based on all 17 strains of *Buttiauxella* and only 6 strains of *Kluyvera*. CDC + CUETM indicates that the data are based on the whole collection of strains.
[b] +, 90 to 100% positive; (+), 75 to 89.9% positive; V, 25.1 to 74.9% positive; −, 0 to 10% positive.
[c] See reference 4.

the G+C contents of the DNAs of *Kluyvera* species are very similar (54 mol% for *K. cryocrescens* ATCC 33435^T and 58 mol% for *K. ascorbata* ATCC 33433^T).

Ferragut et al. (5) studied the G+C contents of 17 strains of *B. agrestis*, and the mean value was 49 mol%. Therefore, the G+C contents of the DNAs of *Kluyvera* species were different enough from those of *B. agrestis* to differentiate the two groups.

The separation of *Buttiauxella* and *Kluyvera* was confirmed by the low level of DNA relatedness between members of these two genera.

In addition to the genomic differences between *Kluyvera* and *Buttiauxella*, there were also some phenotypic differences (Table 2). Most *Kluyvera* strains produced indole and L-lysine decarboxylase (Møller) and rapidly fermented sucrose. Strains of *Buttiauxella* were negative or exhibited delayed reactions for these properties.

We believe that the genomic and phenotypic differences between these genera are such that continued separation is warranted at this time, pending further study.

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