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Ability of Commercial Identification Systems To Identify Newly Recognized Species of *Citrobacter*

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The genus *Citrobacter* was recently determined to contain 11 genetically distinct species. In addition, the International Committee on Systematic Bacteriology no longer recognizes *C. diversus* and has, instead, validated the name *C. koseri* in its place. The 11 species are *C. freundii*, *C. koseri*, *C. amalonaticus*, *C. farmeri*, *C. youngae*, *C. braakii*, *C. werkmanii*, *C. sedlakii*, and three unnamed groups, genomospecies 9, 10, and 11. To determine the ease with which some identification systems could respond to these changes, we evaluated five systems for their potential ability to recognize current species in the genus *Citrobacter*. A simple dichotomous key using conventional biochemicals is presented that may be helpful to presumptively identify *Citrobacter* strains.

The genus *Citrobacter* was designated in 1932 and included seven species: *Citrobacter freundii* (type strain), *C. diversum*, and five others (6). Over time, only *C. freundii* and *C. diversum* (later described as *C. diversus* [2]) remained as valid names. *C. amalonaticus* and *C. amalonaticus* biogroup 1 (3) completed the four groups that have been recognized for the past several years.

In 1990, Frederiksen (4) pointed out a potential error that had occurred in 1972 when *C. diversus* was published. He requested that the incorrect name *C. diversus* be rejected and replaced by *C. koseri*. At that time, three valid names, *C. diversus*, *C. koseri*, and *Levinea malonatica*, were apparently the same taxon. His request was later granted by the Judicial Commission of the International Committee on Systematic Bacteriology (5). The Centers for Disease Control and Prevention has adopted the decision of the commission and will use the recognized name *C. koseri.*

Microbiologists around the world recognized that many strains of *Citrobacter* isolated from humans were difficult to characterize and classify into the three recognized species. Reference laboratories maintained stock strains of atypical *Citrobacter* spp. Recently, Brenner et al. (1) responded to the historical issue of problems within the *Citrobacter* group and examined 112 typical and atypical strains by DNA relatedness studies (hydroxyapatite method). Their work resulted in the recognition of 11 *Citrobacter* species, including five newly named species and three unnamed genomospecies, in addition to *C. freundii* (type species), *C. koseri*, and *C. amalonaticus.*

Identifying 11 species and groups of *Citrobacter* in the clinical laboratory could pose a problem, especially since the manufacturers of identification systems have not yet had time to respond. Clearly, the new names are not included in existing databases. In addition, reference laboratories that use conventional biochemical testing may have difficulty separating the new strains. Our laboratory examined the potential ability of some commercial identification systems to separate these *Citrobacter* species with the existing configuration of the system that generates a biocode for each isolate. We also present a simplified dichotomous key designed to assist in the recognition of all 11 species of *Citrobacter.*

Organisms. The 112 strains described by Brenner et al. (1) were removed from storage at −70°C and passed twice on 5% sheep blood agar (Becton Dickinson Microbiology Systems, Cockeysville, Md.) prior to inoculation into commercial identification systems. When the isolate was to be inoculated into the MicroSCAN Walk/Away panels, it was passed once onto MacConkey's agar (Becton Dickinson Microbiology Systems). Although more organisms for testing would strengthen the evaluation reported here, the availability of hybridized strains with genetically confirmed identifications is limited.

Commercial identification systems. The Vitek system (bioMérieux Vitek, Hazelwood, Mo.) was used with the GNI card and version R08.1 software. The MicroSCAN Walk/Away (Baxter Diagnostics, Inc., West Sacramento, Calif.) was used with the Rapid Neg Combo 3 panel and version 19.11 software. The Biolog MicroStation System (Biolog, Inc., Hayward, Calif.) was used with the Biolog GN Microplate and version 3.0 software. The RapID onE system (Innovative Diagnostic Systems, Inc., Norcross, Ga.) used Version onE V1.93 Code Compendium. The API 20E (bioMérieux Vitek, Hazelwood, Mo.) was used with its current Profile Index. Profile numbers not in the index were entered into the telephone computer database. Each product was used according to each manufacturer's directions.

Identification key. The biochemical data for the dichotomous key (Fig. 1) were derived from Table 1. Where possible, only those reactions exhibiting a 100 or 0% response were used. Because of the limited number of biochemicals incorporated and because some species demonstrate variable reactions, this dichotomous key is not intended to be used for reference identification but rather as a method for determining a presumptive identification with conventional biochemicals to be confirmed by more-extensive testing.

The commercial systems used in this study were evaluated for their ability to accurately identify *C. freundii*, *C. diversus*, *C. amalonaticus*, and *C. amalonaticus* biogroup 1, using the database profiles currently published (the old nomenclature).

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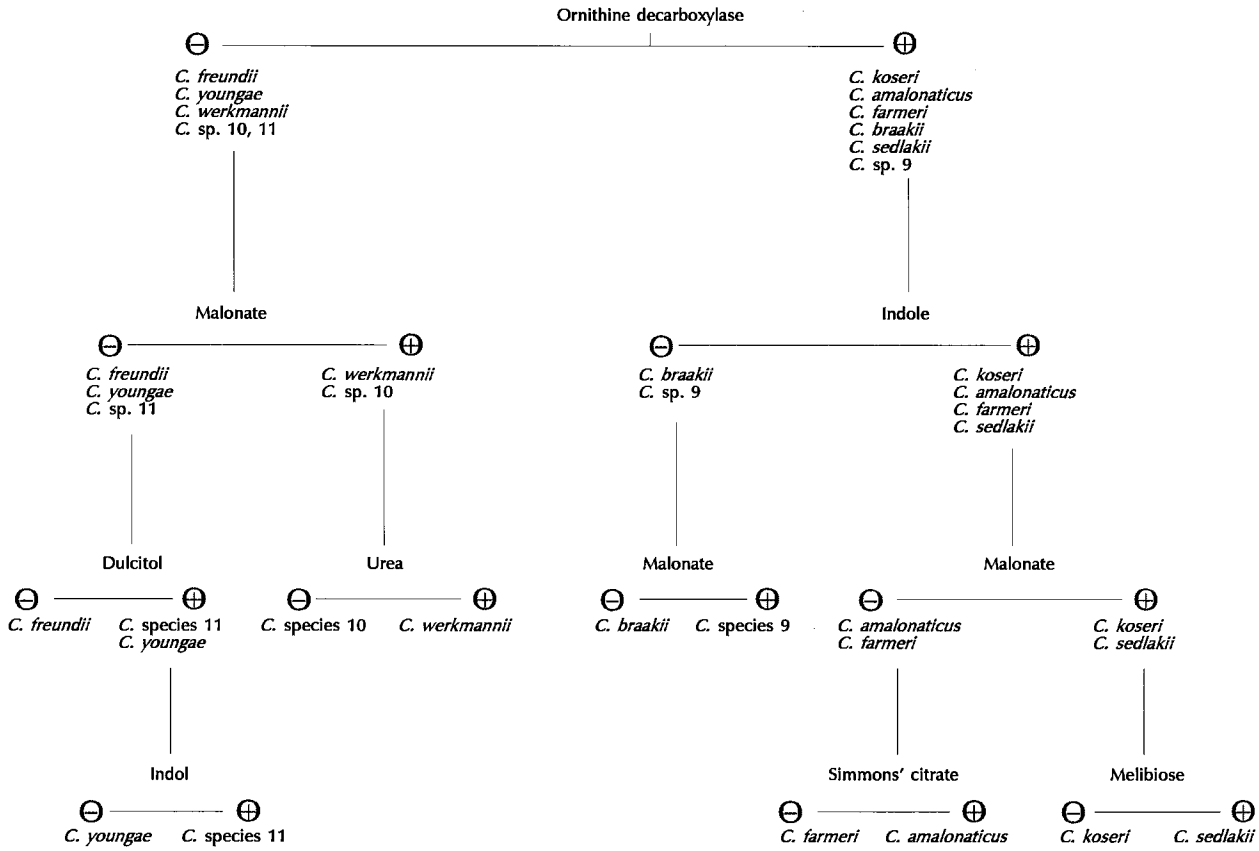

FIG. 1. Identification key for *Citrobacter* spp.

Table 2 also presents the results a laboratory might expect when the newly recognized species and groups of *Citrobacter* are inoculated into these five systems with their current databases which only contain the species *C. freundii*, *C. diversus*, and *C. amalonaticus*. Only the RapID onE was able to identify all nine strains of *C. freundii* accurately. The 16 *C. diversus* strains were renamed *C. koseri*. The RapID onE, Vitek, and Walk/Away were able to identify them all; Biolog

TABLE 1. Biochemical tests useful in differentiating *Citrobacter* genomospecies[a]

Test	% of positive strains by species (no. of hybridized strains)[b]										
	C. freundii (9)	*C. koseri* (16)	*C. amalonaticus* (16)	*C. farmeri* (14)	*C. youngae* (21)	*C. braakii* (15)	*C. werkmanii* (6)	*C. sedlakii* (6)	Species 9 (3)	Species 10 (3)	Species 11 (3)
α-Methyl-D-glucoside	11	40	6	100	0	33	0	0	0	0	0
Arginine dihydrolase	67	94	88	100	50	67	100	100	0	33	67
Dulcitol, acid[c]	11	38	0	0	85	33	0	100	0	0	100
Esculin	0	0	0	0	5	0	0	17	0	0	0
Indole[c]	33	94	100	100	15	33	0	100	0	0	100
Malonate[c]	11	94	0	0	5	0	100	100	100	100	0
Melibiose, acid[c]	100	0	0	100	10	80	0	100	0	67	33
Motility	89	94	94	100	95	87	100	100	0	67	100
Ornithine decarboxylase[c]	0	94	94	100	5	93	0	100	100	0	0
Raffinose, acid	44	0	0	100	10	7	0	0	0	0	33
Salicin, acid	0	6	6	0	10	0	0	17	0	0	33
Simmons' citrate[c]	78	100	100	0	75	87	100	83	0	33	100
Sodium acetate	44	88	100	79	65	53	100	83	0	0	33
Sucrose, acid	89	44	0	100	20	7	0	0	0	33	33
Urease[c]	44	56	88	36	80	47	100	100	100	0	67

[a] Modified and updated from Brenner et al. (1).
[b] Percent positive at 48 h.
[c] This is a reaction used in the key (Fig. 1).

TABLE 2. Identification of hybridized species of *Citrobacter*

Species (no. of strains)	Identification results (no. of strains with result)				
	API 20E	Biolog	RapID onE	Vitek GNI	Walk/Away Rapid Neg ID2
C. freundii (9)	*C. freundii* (8) (excellent [7], very good [1]), unacceptable profile (1)	*C. freundii* (7), *E. coli* (1), no ID (1)	*C. freundii* (9) (implicit [2], satisfactory [2], adequate [3], rare biotype [1], probability overlap [1])	*C. freundii* (8): ≥92%; *Enterobacter agglomerans* (1): 56%	*C. freundii* (8): ≥93.6%; *Klebsiella rhinoscleromatis* (1): 99.9%
C. koseri (16)	*C. koseri* (10) (excellent [1], very good [8], good likelihood [1]); unacceptable profile (1); *C. freundii* (4): very good; *C. amalonaticus* (1): very good	*C. koseri* (15), *C. freundii* (1)	*C. koseri* (16) (implicit [10], satisfactory [5], probability overlap [1])	*C. koseri* (16) (99% [14], 89% [1], 51% [1])	*C. koseri* (16): ≥95.9%
C. amalonaticus (16)	*C. amalonaticus* (16): very good	*C. amalonaticus* (15), *E. agglomerans* (1)	*C. amalonaticus* (15) (implicit [8], satisfactory [3], adequate [4])	*C. amalonaticus* (16) (≥95% [9], ≥85–94% [6], 46% [1])	*C. amalonaticus* (15): ≥92.9%; *E. coli* (1): 99.9%
C. farmeri (14) (formerly *C. amalonaticus* biogroup 1)	*C. amalonaticus* (13): good likelihood; *C. koseri* (1), very good	*C. freundii* (12), *C. amalonaticus* (2)	*C. amalonaticus* (14) (implicit [4], satisfactory [6], adequate [4])	*Escherichia coli* (13): ≥96%; unidentified (1)	*C. amalonaticus*/*C. diversus* (10) (≥96.2% [9], 79.3% [1]); *C. freundii* (1): 99.2%; *Enterobacter amnigenus* (1): 99.9%; no identification (2)
C. youngae (21)	*C. freundii* (21) (excellent [1], very good [13], acceptable [6], very doubtful [1])	*C. freundii* (17), no identification (2), *Salmonella* sp. (1), *Shigella sonnei* (1)	*C. freundii* (19) (satisfactory [10], adequate [8], rare biotype [1]); *C. amalonaticus* [1]: adequate	*C. freundii* (11) (≥98% [7], 59–65% [4]); *E. coli* (9) (≥95% [8]; 58% [1]); unidentified (1)	*C. freundii* (19) (≥89.9% [18], 74.7% [1]); *C. amalonaticus* (1): 88%; *Citrobacter* sp. (1): 94.4%
C. braakii (15)	*C. freundii* (14) (excellent [3], very good [4], acceptable [2], good likelihood [5]); *C. koseri* (1): very good	*C. freundii* (15)	*C. amalonaticus* (7) (adequate [4], questionable [3]); *C. freundii* (5) (adequate [3], satisfactory [1], inadequate [1])	*C. freundii* (9) (99% [6], 80% [2], 61% [1]); *E. coli* (2): 95%; *C. amalonaticus* (2): 46%; *Enterobacter intermedium* (1): 71%; unidentified (1)	*C. freundii* (7) (≥94.9% [6], 82.4% [1]); *Enterobacter cloacae* (1): 52.8%; *Citrobacter* sp. (2): ≥90.4%; *C. amalonaticus* (5) (≥84.1% [4], 58.9% [1])
C. werkmanii (6)	*C. freundii* (6): very good	*C. freundii* (6)	*C. freundii* (3) (satisfactory [2], adequate [1]); *C. amalonaticus* (3): adequate	*C. freundii* (6) (99% [5], 90% [1])	*C. freundii* (6): ≥99.3%
C. sedlakii (6)	*C. amalonaticus* (6) (very good [5], good likelihood [1])	*C. freundii* (6)	*C. amalonaticus* (3) (adequate [1], rare biotype [2]); *C. koseri* (1): adequate; no code (3)	*E. amnigenus* (6) (≥94% [5], 84% [1])	*E. coli* (4): ≥70.1%; *C. amalonaticus* (1): 93.1%; *Klebsiella ornithinolytica* (1): 91.7%
Citrobacter species 9 (3)	*C. freundii* (3): very good	No identification (3)	No code (3)	*E. amnigenus* (3): 97%	*C. freundii* (3): ≥37.3%
Citrobacter species 10 (3)	*C. freundii* (3) (excellent [2], good likelihood [1])	*C. freundii* (3)	*C. freundii* (1): adequate; no code (2)	*C. freundii* (3) (99% [1], ≥80–84% [2])	*C. freundii* (2): 99.9%; *E. agglomerans* (1): 70.4%
Citrobacter species 11 (3)	*C. freundii* (2) (excellent [1], very good [1]); *E. agglomerans* (1): very good	*C. freundii* (3)	*C. freundii* (2): questionable; *C. amalonaticus* (1): questionable	*C. freundii* (2) (99% [1], 44% [1]); *E. coli* (1): 43%	*C. freundii* (3): ≥93.2%

missed only one. The API could correctly identify only 10 of 16 isolates tested.

For certain species of *Citrobacter*, each of the systems used in this study generated consistent results. The API identified all 16 strains of *C. amalonaticus* as *Citrobacter* species at the end of the initial incubation time. With required additional tests, all 16 strains were correctly identified after 48 h as *C. amalonaticus*. However, the API identified 13 of 14 strains of *C. amalonaticus* biogroup 1 (now *C. farmeri*) as *C. amalonaticus*, as one would expect. Biolog identified all but two of the biogroup 1 strains as *C. freundii*, while Vitek identified 13 of them as *Escherichia coli* and reported one as unidentified. The Walk/Away identified 10 of 14 biogroup 1 strains as *C. diversus* or *C. amalonaticus.*

The five new species and three new genomospecies of *Citrobacter* were all previously called *C. freundii*. In the Vitek, 11 of the *C. youngae* strains were identified as *C. freundii*, but 9 were identified as *E. coli*. Of these nine, eight were identified at probabilities of >98%. All of the *C. sedlakii* and group 9 strains tested were identified as *Enterobacter amnigenus* biogroup 2. The Walk/Away identified four of six *C. sedlakii* strains as *E. coli*. In the RapID onE, the group 9 strains were assigned "no code." Table 2 summarizes all identifications for all five systems when tested with the newly named strains.

For reference laboratories, and for those that prefer conventional biochemical methods as backup identification procedures, a flowchart was developed to facilitate recognition of the new genomospecies (Fig. 1). Because some of the reactions used in the diagram do not represent 0% or 100% responses of the organisms, and because few organisms have been evaluated with it, the chart must be used only as a presumptive method of identification to be confirmed by more-extensive testing if deemed necessary.

We believe that the manufacturers of the systems used in this study will be able to incorporate selected strains of the new *Citrobacter* genomospecies with only minor adjustments in their current algorithms. As more data on these species appear in the literature, a clearer picture of their clinical significance should emerge.

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