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Unclassified, Citrate-Positive Member of the Family *Enterobacteriaceae* Resembling *Escherichia coli*

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Three isolates of an unclassified, oxidase-negative and citrate-positive, gram-negative bacillus belonging to the family *Enterobacteriaceae* were studied. With the exception of the citrate reactions, these strains most closely resembled *Escherichia coli* in their biochemical reactivity.

The utilization of citrate by members of the family *Enterobacteriaceae* is common in species of the tribes *Salmonelleae, Klebsielleae, Proteeae*, and *Erwinieae*. The mechanism by which organisms utilize the carbon of citrate to produce the alkaline reaction on Simmons citrate agar remains poorly understood; however, it is known that it is important to inoculate this agar lightly and to minimize carry-over of nutrients in media from which the inoculum is taken (1). The alkaline reaction may be delayed ($\geq$48 h) and may vary considerably in its intensity.

This report was prompted by the isolation in our laboratory of three strains of oxidase-negative, gram-negative bacilli that closely resembled *Escherichia coli* except for the fact that they have repeatedly alkalinized Simmons citrate agar within 3 days of inoculation.

MATERIALS AND METHODS

All three strains were isolated from cultures of clinical material submitted to the Bacteriology Laboratory. Initial screening of gram-negative bacilli in this laboratory is with triple sugar iron agar, lysine iron agar, Simmons citrate agar, Christensen urea agar, ornithine-motility semisolid agar, and indole broth (8). Additional tests were those described by Edwards and Ewing (3). Antimicrobial susceptibilities were determined by use of the agar dilution technique with an expanded dilution scale as described elsewhere (8). No serological studies were performed.

RESULTS

One strain was isolated in 1970 in moderate numbers (15,000 colonies/ml) from the urine of a patient who had undergone 1 year previously a cystectomy and ileal conduit urinary diversion for metastatic carcinoma of the bladder. The second strain was isolated in 1971 in small numbers (1,100 colonies/ml) along with *Proteus mirabilis* (also in small numbers) from the urine of a patient with urethral strictures who had recently treated bacteriuria due to *E. coli* (citrate negative; >100,000 colonies/ml). The third was isolated in 1973 from a pericecal abscess, cultures of which also yielded *Klebsiella pneumoniae, Enterobacter cloacae*, viridans streptococci, *Bacteroides fragilis*, and *Peptococcus*.

The reactions of all three strains and those predicted for *E. coli* according to Edwards and Ewing (3) are listed in Table 1. Two of the three strains produced acid reactions, and the third produced an alkaline reaction, on the slant of triple sugar iron agar; all three acidified the butt of this medium. All three produced an alkaline slant and butt in lysine iron agar. All three strains have been tested repeatedly on different lots of Simmons citrate agar produced by two different manufacturers (BioQuest and GIBCO) with consistently positive results after 2 or 3 days of incubation at 35°C. Care was taken to avoid carry-over of nutrients onto the Simmons citrate agar.

None of the strains were hemolytic on blood agar. Only one strain produced a green metallic sheen on eosin-methylene blue agar.

The antimicrobial susceptibilities of the three strains are listed in Table 2. Except for the fact that all three were resistant to tetracycline, their susceptibilities closely resembled those observed in our laboratory with typical isolates of *E. coli*.

DISCUSSION

The alkalinization of Simmons citrate agar demonstrated by the three isolates in this study is a characteristic associated with the tribes *Salmonelleae, Klebsielleae, Proteeae*, and *Erwinieae*. The reactions produced by these strains, however, are generally incompatible with those reported for these tribes (2, 3). Their decarboxylase, deaminase, urease, and gelatinase reactions, among others, effectively preclude their inclusion in either the *Proteeae* or

TABLE 1. *Comparison of biochemical reactions of three unclassified organisms with predicted[a] reactions of E. coli*

Test or substrate	Positive reactions of unclassified strains in 1-2 days		% Positive predicted for *E. coli* in 1-2 days
	No.	%	
ONPG[b]	2	66	–
Hydrogen sulfide	0	0	0
Urease (Christensen)	0	0	0
Indole	3	100	99
Methyl red	3	100	99
Voges-Proskauer	0	0	0
Citrate (Simmons)	3	100	0
Motility	0	0	69
Gelatin, 22°C	0	0	0
Lysine decarboxylase	3	100	88
Arginine dihydrolase	0	0	17
Ornithine decarboxylase	3	100	63
Phenylalanine deaminase	0	0	0
Malonate	0	0	0
Glucose	3	100	100
Lactose	2	66	90
Sucrose	1	33	51
Mannitol	3	100	96
Dulcitol	2	66	38
Salicin	3	100	37
Adonitol	0	0	5
Inositol	0	0	1
Sorbitol	3	100	93
Arabinose	3	100	100
Raffinose	1	33	54
Rhamnose	3	100	74
Maltose	3	100	86
Xylose	3	100	70
Trehalose	3	100	98
Nitrate to nitrite	3	100	99
Sodium acetate	3	100	84
Oxidase	0	0	0
Esculin	3	100	31

[a] Based on Edwards and Ewing (3).
[b] *o*-Nitrophenyl-β-D-galactopyranoside.

the *Erwinieae* (2). These same reactions, as well as those for indole, methyl red, Voges-Proskauer, malonate, and various carbohydrate fermentations, are also incompatible with those reported for the *Klebsielleae* (3, 5).

There are similarities between the reactions produced by these strains and those reported for H_2S-negative strains of *Citrobacter freundii* and *C. diversus* by Ewing and Davis (4), for *C. koseri* by Frederiksen (6), and for *Levinea* by Young et al. (10). In Table 3 are listed some differential characteristics between the group under investigation and species of *Citrobacter* and *Levinea*. Although there are similarities, one major point of difference is the absence of lysine decarboxylase activity in *Citrobacter* and *Levinea* and its presence in the group under investigation. These strains, therefore, most closely resemble *E. coli* (Table 1).

Although it might be tempting to dismiss the

TABLE 2. *Antimicrobial susceptibilities of unclassified organisms*

Antimicrobial agent	Cumulative no. inhibited at increasing concn (μg/ml)						
	1	5	10	20	50	100	200
Ampicillin	0	2	2	2	–[a]	–	–
Carbenicillin	–	–	–	–	2	2	2
Cephalothin	0	3					
Chloramphenicol	0	1	3				
Gentamicin	3						
Kanamycin	1	3					
Nalidixic acid	–	–	3				
Nitrofurantoin	–	–	–	–	3		
Tetracycline	0	0	0	–	–	–	–
Tobramycin	3						

[a] –, Not tested at this concentration.

TABLE 3. *Some differential characteristics of Citrobacter, Levinea, and the unclassified strains in this investigation[a]*

Test or substrate	*Citrobacter freundii*[b,c]	*Citrobacter diversus*[c]	*Citrobacter koseri*[d]	*Levinea amalonatica*[e]	*Levinea malonatica*[e]	Group under investigation
Urease	+ or –	+, (+), –	+	+	+ or –	–
Indole	+ or –	+	+	+	+	+
Lysine decarboxylase	–	–	–	–	–	+
Arginine dihydrolase	+, (+), –	+ or (+)	+	+	+	–
Malonate	– or +	+ or –	+	–	+	–
Adonitol	–	+	+	–	+	–

[a] Symbols: +, ≥90% positive within 1 to 2 days of incubation; (+), positive after 3 or more days of incubation; +, (+), –, variable reactions; –, ≥90% negative; + or –, majority of reactions positive, some negative; – or +, majority of reactions negative, some positive.
[b] H_2S-negative biogroups.
[c] According to Ewing and Davis (4).
[d] According to Frederiksen (6).
[e] According to Young et al. (10).

positive citrate reactions associated with these strains as being artifactual, their ability to alkalinize citrate within 1 to 3 days has been reproducible on different lots of agar, and the overall pattern of their biochemical reactions has been unique in our experience. Their frequency of occurrence remains unknown because of the characteristically delayed nature of their positive reaction on Simmons citrate agar and because this test is seldom examined beyond 1 day of incubation in our routine screening process. Some perspective as to their rarity can be gained from the fact that our laboratory performs antimicrobial susceptibility tests on nearly 6,000 isolates of *E. coli* annually and that only three citrate-positive strains have been recognized in the past 5 years. In a summary of the biochemical reactions of 1,021 cultures of *E. coli* examined at the Center for Disease Control, Edwards and Ewing reported that 0.6% of these strains were positive on Simmons citrate agar (3); of 210 standard O, K, and H antigen strains, 0.9% gave positive citrate reactions. Certainly, the precise taxonomic position of these organisms remains unclear; however, they appear to represent yet another variant of *E. coli*, in addition to those which have been reported to produce H_2S (7) and urease (9).

LITERATURE CITED

1. **Blazevic, D. J., and G. M. Ederer.** 1975. Citrate utilization, p. 15-18. *In* D. J. Blazevic and G. M. Ederer (ed.), Principles of biochemical tests in diagnostic microbiology. John Wiley and Sons, New York.
2. **Buchanan, R. E., and N. E. Gibbons (ed.).** 1974. Bergey's manual of determinative bacteriology, 8th ed. The Williams & Wilkins Co., Baltimore.
3. **Edwards, P. R., and W. H. Ewing.** 1972. Identification of Enterobacteriaceae, 3rd ed. Burgess Publishing Co., Minneapolis.
4. **Ewing, W. H., and B. R. Davis.** 1972. Biochemical characterization of *Citrobacter diversus* (Burkey) Werkman and Gillen and designation of the neotype strain. Int. J. Syst. Bacteriol. 22:12-18.
5. **Ewing, W. H., B. R. Davis, M. A. Fife, and E. F. Lessel.** 1973. Biochemical characterization of *Serratia liquefaciens* (Grimes and Hennerty) Bascomb et al. (formerly *Enterobacter liquefaciens*) and *Serratia rubidaea* (Stapp) comb. nov. and designation of type and neotype strains. Int. J. Syst. Bacteriol. 23:217-225.
6. **Frederiksen, W.** 1970. *Citrobacter koseri* (n. sp.), a new species within the genus *Citrobacter* with a comment on the taxonomic position of *Citrobacter intermedium* (Werkman and Gilles). Publ. Fac. Sci. Univ. J. E. Purkyne Brno 47:89-93.
7. **Maker, M. D., and J. A. Washington, II.** 1974. Hydrogen sulfide-producing variants of *Escherichia coli*. Appl. Microbiol. 28:303-305.
8. **Washington, J. A., II (ed.).** 1974. Laboratory procedures in clinical microbiology. Little, Brown and Co., Boston.
9. **Washington, J. A., II, and M. D. Maker.** 1975. Unclassified, lactose-fermenting, urease-producing member of the family *Enterobacteriaceae* resembling *Escherichia coli*. J. Clin. Microbiol. 2:70-71.
10. **Young, V. M., D. Kenton, B. J. Hobbs, and M. R. Moody.** 1971. *Levinea*, a new genus of the family *Enterobacteriaceae*. Int. J. Syst. Bacteriol. 21:58-63.