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Biochemical Properties of a Newly Described *Escherichia* Species, *Escherichia albertii*

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Five strains of a newly described *Escherichia* species, *Escherichia albertii*, were extensively characterized by conventional biochemical methods and by commercial identification panels. *E. albertii* is an indole-negative species that ferments D-mannitol but not D-xylose. Because these strains are not included in the databases of commercial systems at present, they were most often identified as *Hafnia*, *Salmonella*, *Escherichia coli*, or, on one system (MicroScan dried overnight panels), *Yersinia ruckeri*.

Until recently, the genus *Escherichia* was composed of five species, including the type species *E. coli* and four less frequently encountered members: *E. blattae*, *E. hermannii*, *E. vulneris*, and *E. fergusonii* (4). Recently, Huys and others (5) described a sixth species, *E. albertii*, associated with diarrheal disease in Bangladeshi children. *E. albertii* was originally isolated and identified by John Albert and collaborators at the International Centre for Diarrhoeal Disease Research, Bangladesh, as *Hafnia alvei* (1). However, subsequent phenotypic and genetic studies conducted by several international groups clearly indicated that these strains do not belong in the genus *Hafnia* (6, 8, 9). Further molecular studies including 16S ribosomal DNA sequencing and DNA-DNA pairing studies have demonstrated that these strains belong in the genus *Escherichia*, and a new species, *E. albertii*, has been proposed (5).

Currently, only five strains of *E. albertii* are known to exist, and it is unclear how common this species is in clinical samples and whether all fecal strains are associated with cases of bacterial gastroenteritis. In the original description of this species (5), the biochemical properties of *E. albertii* were determined primarily by using the API 50 CH system (bioMérieux, Marcy l'Etoile, France), a commercial product not readily available in the United States. Because of this fact and to further characterize these strains as an aid to identification of this species, we have identified these strains in both a conventional and miniaturized commercial test format.

The five strains of *E. albertii* used in this study have been previously described. These strains are Albert 9194 (LMG 20972), Albert 10457 (LMG 20973), Albert 10790 (LMG 20794), Albert 12502 (LMG 20975), and Albert 19982 (LMG 20976^T). All conventional tests were performed by using previously described procedures (7); tests conducted on MicroScan conventional overnight and rapid panels (Dade Behring MicroScan, MicroScan Inc., West Sacramento, Calif.), API 20E strips (bioMérieux, Inc., Durham, N.C.), and Vitek GNI Plus, software version R07.01 (bioMérieux, Inc.), were carried out according to the manufacturer's instructions.

Biochemical test results for all five *E. albertii* strains analyzed by conventional format are listed in Table 1. These results agree with data reported by Huys et al. (5) in that *E. albertii* strains are nonmotile and indole negative, lysine and ornithine-decarboxylase positive, and arginine dihydrolase and Voges-Proskauer negative and produce acid from the fermentation of D-glucose (with gas), L-arabinose, and D-mannitol; sucrose and lactose are not fermented. All five *E. albertii* strains were additionally β-galactosidase positive. *E. albertii* strains also fermented D-arabinose, D-fructose, D-galactose, D-mannose, and ribose but were unable to utilize a wide variety of uncommon sugars, including D-fucose, D-lyxose, palatinose, sedoheptulose anhydride, L-sorbose, D-tagatose, D-turanose, and xylitol. Acid from glycerol was produced by all five strains only after prolonged incubation (3 to 7 days). *E. albertii* strains were phenotypically tight, and very few variable reactions were noted. Only fermentation of lactulose, maltose, and trehalose and esculin hydrolysis produced variable test results. Interestingly, all three strains that fermented maltose also fermented trehalose, suggesting that potential biotypes (biovars) may exist within *E. albertii*. No *E. albertii* strain produced lipase, protease, or pectinase or degraded mucate. Four of five strains produced weak to moderate L-prolineaminopeptidase activity when tested with a commercial assay (Aminopeptidase Wee-Tab; Key Scientific Products, Round Rock, Tex.).

Since *E. albertii* has just been described, we additionally tested these five strains on several commercial systems often used by clinical laboratories to identify gram-negative rods (Table 2). Not surprisingly, four of the five *E. albertii* strains were identified as *H. alvei* with the API 20E strip, findings similar to those originally reported by Albert and colleagues (1). However, all four of these strains were identified at low probabilities (45%). One strain generated a seven-digit code (5104002) that did not yield an identification. Similar to the results with API 20E, Vitek called three of these five strains *H. alvei*, with probability values ranging from 78 to 92%. Vitek called one strain (Albert 10790) a possible *Salmonella* strain

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TABLE 1. Biochemical properties of five strains of *E. albertii*[a]

Test	Cumulative % positive on day:			Reaction for type strain LMG 20976	Test	Cumulative % positive on day:			Reaction for type strain LMG 20976
	1	2	7			1	2	7	
Indole	0	0	0	−	L-Arabinose	100			+
Motility (35°C)	0			−	D-Arabitol	0	0	0	−
Oxidase	0			−	Cellobiose	0	0	0	−
Catalase	100			+	Dulcitol	0	0	0	−
Nitrate reductase	100			+	Erythritol	0	0	0	−
Pigment	0	0	0	−	D-Fucose	0	0	0	−
Urea	0	0		−	D-Fructose	100			+
ONPG	100			+	D-Galactose	100			+
MUG	0			−	Glycerol	0	0	100	−
Citrate	0	0		−	*i*-Inositol	0	0	0	−
Acetate	100			+(w)	Lactose	0	0	0	−
Malonate	0	0		−	Lactulose	0	0	20	−
Growth in KCN broth	0	0		−	D-Lyxose	0	0	0	−
H_2S on:					Maltose	20	60	60	−
TSI	0	0		−	D-Mannitol	100			+
GCF	0	0		−	D-Mannose	100			+
Voges Proskauer (25°C)	0	0		−	Melibiose	0	0	0	−
Voges Proskauer (35°C)	0	0		−	α-Methyl-D-glucoside	0	0	0	−
Lysine decarboxylase (Møeller)	100			+	Palatinose	0	0	0	−
Ornithine decarboxylase (Møeller)	100			+	Raffinose	0	0	0	−
Arginine dihydrolase (Møeller)	0	0		−	L-Rhamnose	0	0	0	−
Glutamate decarboxylase	100			+	Ribose	100			+
PPA	0	0		−	Salicin	0	0	0	−
Degradation of:					Sedoheptulose anhydride	0	0	0	−
Elastin	0	0	0	−	D-Sorbitol	0	0	0	−
Gelatin	0	0	0	−	L-Sorbose	0	0	0	−
Hide powder	0	0	0	−	Sucrose	0	0	0	−
Mucate	0	0	0	−	D-Tagatose	0	0	0	−
Polypectate (25°C)	0	0	0	−	Trehalose	60	60	60	+(w)
L-Tyrosine crystals	0	0	0	−	D-Turanose	0	0	0	−
DNA	0	0	0	−	D-Xylose	0	0	0	−
Corn oil (lipase)	0	0	0	−	Xylitol	0	0	0	−
D-Glucose, acid	100	0	0	−	Hydrolysis of:				
D-Glucose, gas	100	0	0	−	Arbutin	0	0	0	−
Acid from:					Esculin	0	0	20	−
Adonitol	0	0	0	−	L-Prolineaminopeptidase[b]	80	NA	NA	+(w)
Amygdalin	0	0	0	−					
D-Arabinose	60	100		+					

[a] Abbreviations: GCF, hydrogen sulfide formation in gelatin-cysteine-thiosulfate medium; MUG, β-D-glucuronidase; ONPG, *o*-nitrophenyl-β-D-galactopyranoside; NA, not applicable; PPA, phenylpyruvic acid; TSI, triple sugar iron agar; +(w), weakly positive.
[b] 2-h assay.

(top choice), although the probability was low (70%). The type strain of *E. albertii* was identified as an inactive *E. coli* strain. In contrast, the conventional overnight panels of MicroScan called four of these five strains *Yersinia ruckeri* (64 to 65% probability); the remaining strain (type strain of *E. albertii*) was identified as *E. coli* (99% probability), similar to the results on Vitek. On the MicroScan rapid system, results were much more variable, with individual strains being identified as *Salmonella enterica* serovar Choleraesuis (two cases) and as *H. alvei*, *E. coli* O157:H7, and *Citrobacter braakii*/*C. freundii*/*C. sedlakii* in one instance each. Only the *E. coli* O157:H7 identification had high confidence (97% probability).

TABLE 2. Identification of *E. albertii* strains by selected commercial systems

E. albertii strain	Identification (% probability) according to:			
	API 20E	Vitek	MicroScan	
			Dried[a]	Rapid
Albert 19982	*H. alvei* 2 (45)[b]	*E. coli*, inactive (74)	*E. coli* (99)	*Citrobacter* group (44)
Albert 9194	*H. alvei* 2 (45)	*H. alvei* (78)	*Y. ruckeri* (64)	S. *enterica* serovar Cholerasuis (89)
Albert 10457	*H. alvei* 2 (45)	*H. alvei* (92)	*Y. ruckeri* (64)	*H. alvei* (86)
Albert 10790	None	*Salmonella* (70)	*Y. ruckeri* (64)	*S. enterica* serovar Cholerasuis (53)
Albert 12502	*H. alvei* 2 (45)	*H. alvei* (92)	*Y. ruckeri* (65)	*E. coli* O157:H7 (97)

[a] Overnight conventional panel.
[b] *H. alvei* profile 2.

The results of testing five *E. albertii* strains by conventional methods suggest that this species biochemically most closely resembles inactive *E. coli*, although these strains do not resemble the Alkalescens-Dispar group by virtue of their ability to produce gas from D-glucose. Unlike many active and inactive *E. coli* strains, *E. albertii* strains failed to ferment a number of sugars, including lactose, dulcitol, L-rhamnose, and melibiose. As previously noted (5), the ability to ferment D-sorbitol, a trait almost exclusively associated with *E. coli* among *Escherichia* species, clearly helps separate this group (D-sorbitol negative) from the type species of the genus. They can be biochemically distinguished from other *Escherichia* species by a number of tests, including failure to grow in KCN broth, the inability to utilize malonate, and the lack of acid production from D-xylose, D-arabitol, melibiose, and cellobiose. The weak to moderate L-prolineaminopeptidase activity noted in the five *E. albertii* strains is a useful adjunct test to separate this group from *H. alvei*. *H. alvei* strains express very strong L-prolineaminopeptidase activity in only 30 min (3); all five *H. alvei* strains tested produced very strongly positive reactions in 2 h. Additionally, the general pattern demonstrating the inability to ferment other carbohydrates commonly metabolized by several *Escherichia* species (e.g., dulcitol, salicin, raffinose, and adonitol) suggests the possible presence of *E. albertii*.

Because *E. albertii* has only recently been established, no commercial system currently includes this species in its database. Results of testing five *E. albertii* strains on four different systems (20 identifications) suggest that most (16 of 20, or 80%) would generate a final identification with an unacceptable probability (45 to 89%) or no identification at all. Such results should trigger a more in-depth analysis with additional tests and/or identification panels. The fact that in four of five instances *E. albertii* was identified as *Y. ruckeri*, a fish pathogen (2), on dried overnight MicroScan panels should provide an additional clue to the possible presence of *E. albertii*. However, some isolates ($n = 4$) would generate acceptable (92%) to excellent (97 to 99%) identifications as either *H. alvei* or *E. coli*, and in these cases the strains would be clearly misidentified. The single best hint at present to the possible presence of *E. albertii* (excluding overnight MicroScan panels) seems to be an unacceptable first-choice identification of *H. alvei* ($n = 8$; 50%) for an isolate that is both L-rhamnose and D-xylose negative.

The importance of *E. albertii* is currently unknown. Until clinical laboratories are able to identify more strains of this newly described species, the frequency, disease spectrum, and clinical significance will remain in question. A review of our extensive *E. coli* collection containing over 400 strains isolated over a 20-year span revealed no strains with biochemicals compatible with *E. albertii*.

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