

Particular Biochemical Profiles for Enterohemorrhagic *Escherichia coli* O157:H7 Isolates on the ID 32E System

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The ability of the ID 32E system to identify and discriminate 74 *Escherichia coli* O157 isolates among 106 *E. coli* non-O157 isolates was evaluated. The results showed atypical biochemical reactions but accurate identification at the species level and no unique biochemical profile numbers for *E. coli* O157, although these numbers were distinct from those of other serotypes.

Verocytotoxigenic *Escherichia coli* (VTEC), a new public health problem worldwide, is represented by more than 100 serotypes that produce verocytotoxins, with two main serotypes, O157:H7 and O157:H(–). These last two serotypes, classified as enterohemorrhagic *E. coli* (EHEC), are implicated in hemorrhagic colitis, hemolytic-uremic syndrome, and thrombotic thrombocytopenic purpura. EHEC strains have been described as important and emergent food-borne pathogens (4, 18, 31). Other non-O157 VTEC serotypes, not deprived of pathogenicity, have been increasingly implicated in sporadic enteric diseases and outbreaks (18, 31).

Detection of *E. coli* O157:H7 is based on its recovery from samples and the presence of its virulence-associated factors (verocytotoxins) or the detection of its O157 antigens (3, 30, 31). Selective media for isolating O157:H7 strains rely on the fact that most of these strains display characteristic biochemical reactions (31): no β -glucuronidase activity and no D-sorbitol fermentation within 24 h at 37°C, except for some German or U.S. strains (10, 12). However, these last two biochemical features are not commonly used in routine clinical and food laboratories because of the simplicity of well-established commercial identification systems. The use of these systems on presumptive *E. coli* strains offers not only the advantage of confirming strains at the genus and/or species level but also the possibility to detect the presence of O157 EHEC by identifying profiles that are unique to these strains. For the Microscan dried conventional gram-negative identification panel (Dade-MicroScan International, West Sacramento, Calif.), 90% of the tested *E. coli* O157:H7 strains shared two common biochemical profiles (1), whereas in the case of the API 20E (bioMérieux Vitek, Inc., Hazelwood, Mo.), 92% of the tested strains displayed the same profile (8).

A new well-based identification system, ID 32E, was recently developed by bioMérieux Vitek for the identification of *Enterobacteriaceae* and other nonfastidious gram-negative bacteria,

after an incubation period of 22 ± 2 h at $37 \pm 1^\circ\text{C}$ (17, 23). This system is an upgrading of the API 20E gallery and consists of 32 individual conic test wells (13 enzymatic, 3 biochemical, and 16 sugar utilization tests) in polystyrene trays (Table 1).

A total of 180 serotyped *E. coli* strains were tested with the ID 32E system. These included 55 O157:H7 strains, 19 O157:H(–) strains confirmed by PCR on the *rfb*_{O157} locus (3), and 106 reference strains and laboratory isolates of non-O157:H7 or O157:H(–) *E. coli* harboring (76 strains) or not harboring (30 strains) verocytotoxin genes, originating from patients, animals, and food samples. The strains were isolated on casein soymeal peptone agar (Caso medium; Merck, Darmstadt, Germany). Conventional identification of strains was done using the Kligler-Hajna, indole, Voges-Proskauer, Simmon's citrate, β -galactosidase, urease, glutamate decarboxylase, sorbitol fermentation, and β -glucuronidase tests (2, 7, 15, 35). A suspension of each strain, adjusted to a 0.5 McFarland standard (theoretical optical density at 550 nm of 0.125) with a spectrophotometer (Beckman Instruments, Inc., Fullerton, Calif.), was prepared, and the ID 32E strip was inoculated by pipetting 55 μl of this suspension into each well. After an incubation of 22 ± 2 h at $37 \pm 1^\circ\text{C}$, James reagent was added to the indole well, and the variation of color of each well was visually read according to the manufacturer's instructions. The reactions were tabulated into an 11-digit numerical profile and entered into the upgrading APILAB Plus computerized software version 3.3 (bioMérieux Vitek) containing 103 reference bacterial taxa. In the computer report, identifications are classified according to the percentage of identification accuracy (%ID), an estimate of how closely the profile corresponds to the taxon relative to all other taxa in the database, and the T index (T), an estimate of how closely the profile corresponds to the most typical set of reactions for the stated taxon, as follows: excellent (%ID ≥ 99.9 , $T \geq 0.75$), very good (%ID ≥ 99.0 , $T \geq 0.5$), good (%ID ≥ 90.0 , $T \geq 0.25$), and acceptable (%ID ≥ 80.0 , $T \geq 0$). Atypical tests, named "supplemental tests," are listed at the end of the computer report in order to confirm identification by the new classical tests.

E. coli O157:H7 showed significant divergent biochemical activities from classical *E. coli* for ornithine decarboxylase, arginine dihydrolase, urease, 5-ketogluconate, β -glucuroni-

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TABLE 1. *E. coli* biochemical activity profile

Biochemical characteristic	% Activity for <i>E. coli</i> as reported by:			% Activity as determined by ID 32E in:			
	Krieg and Holt (15) (<i>n</i> = ND) ^a	Farmer et al. (7) (<i>n</i> = ND) ^b	Ewing (5) (<i>n</i> = 1,231) ^c	Manufacturer's notice (<i>n</i> = 150) ^d	<i>E. coli</i> non-O157 (<i>n</i> = 106) ^e	<i>E. coli</i> O157:H7 (<i>n</i> = 55) ^e	<i>E. coli</i> O157:H(-) (<i>n</i> = 19) ^e
Ornithine decarboxylase	26-75	65	64.2	69	67	98.2	89.5
Arginine dihydrolase	11-25	17	17.6	3	3.8	0	0
Lysine decarboxylase	76-89	90	88.7	81	91.5	90.9	100
Urease	0-10	1	0	1	0.9	18.2	5.3
L-Arabitol	0-10	5	ND	0	0	0	0
Galacturonate	ND	ND	ND	99	100	100	100
5-Ketogluconate	0-10	ND	ND	44	2.8	0	0
Lipase	0-10	0	0	0	0	0	0
Phenol red	90-100	99	99.9	99	100	100	100
β-Glucosidase	ND	ND	ND	5	17.9	0	0
Mannitol	90-100	98	96.8	98	100	100	100
Maltose	90-100	95	89.9	98	86.8	100	100
Adonitol	0-10	5	5.6	6	2.8	0	0
Palatinose	ND	ND	ND	0	0	0	0
β-Glucuronidase	90-100	ND	ND	92	96.2	0	5.3
Malonate	0-10	0	0	0	0	0	0
Indole	90-100	98	98.6	84	99.1	100	100
N-Acetyl-β-glucosaminidase	ND	ND	ND	0	0.9	0	0
β-Galactosidase	90-100	95	ND	91	94.3	100	100
Glucose	90-100	100	100	100	100	100	100
Saccharose	26-75	50	48.9	31	24.5	45.5	47.4
L-Arabinose	90-100	99	99.4	99	98.1	100	100
D-Arabitol	0-10	5	ND	5	18.9	1.8	0
α-Glucosidase	0-10	0	0	1	0	1.8	5.3
α-Galactosidase	ND	99	ND	99	98.1	100	100
Trehalose	90-100	98	98.8	99	91.5	58.2	52.6
Rhamnose	76-89	80	82.3	95	80.2	63.6	42.1
Inositol	0-10	1	1.1	2	6.6	1.8	0
Cellobiose	0-10	2	2.4	0	0.9	0	0
Sorbitol	90-100	94	93.4	38	31.1	0	5.3
α-Maltosidase	ND	ND	ND	1	5.7	1.8	0
L-Aspartic acid arylamidase	ND	ND	ND	1	1.9	0	0

^a *n*, number of strains; ND, not determined.^b Percentage of strains displaying a positive reaction after 48 h at 36°C. Positive reaction after 48 h are not considered. *n*, number of strains; ND, not determined.^c Percentage of strains displaying a positive reaction after 24 h at 36 ± 1°C. *n*, number of strains; ND, not determined.^d Percentage of strains displaying a positive reaction after 24 h at 36 ± 1°C. *n*, number of strains.^e Percentage of strains displaying a positive reaction after 22 ± 2 h at 37 ± 1°C. *n*, number of strains.

dase, and sorbitol and, to a lesser extent for rhamnose (1), adonitol, D-arabitol, trehalose, and inositol (Table 1). Ten Belgian human and French animal isolates displayed urease-positive reactions. Identification as *E. coli* was excellent or very good. Although no single biochemical profile number could account for all *E. coli* O157:H7 (Table 2) isolates, two-thirds of these isolates were distributed into only five profiles, with one (i.e., 54465743000) representing 25% of the tested strains.

On average, the biochemical activity profiles of O157:H(-) strains were more similar to those of the O157:H7 strain than to those of the non-O157 strain (Table 1). Slight differences between both O157:H serotypes were noted for rhamnose and ornithine decarboxylase activities. One strain, isolated from a Belgian human, was positive for the urease test. The quality of identification as *E. coli* varied from good to very good. Five O157:H(-) biochemical profiles numbers were also shared by O157:H7 strains (Table 2). Interestingly, O157 *E. coli* profile numbers were not encountered with other non-O157 *E. coli* strains, regardless of their verocytotoxin production (data not shown).

Non-O157 *E. coli* isolates displayed significant distinct results compared to the previously reported manufacturer or

published data (Table 1), especially for 5-ketogluconate, β-glucosidase, and D-arabitol. The two main biochemical characteristics of *E. coli* O157:H7, i.e., β-glucuronidase and sorbitol, were fairly frequent (17.9 and 31.1%, respectively) among non-O157 serotypes. No strikingly different biochemical activity profiles were found between verocytotoxigenic and non-verocytotoxigenic non-O157 *E. coli* strains (data not shown). As for O157, the quality of identification of the non-O157 as *E. coli* was mostly excellent, especially with the strains not producing verocytotoxins that displayed less atypical biochemical characters. Sixty-one profiles for VTEC non-O157 strains (main profile number 54465543400) were observed.

The reproducibility of the ID 32E system was determined by performing repeat analyses, at different days and with different batches for 10 O157 and 10 non-O157 strains. The exact same biotype number was generated for each replicate (100% reproducibility).

In the present study, the biochemical characteristics of O157 *E. coli* did not influence accuracy of identification and were responsible for the various biochemical profile numbers shown in Table 2. Although phenotypic variants (5, 6, 15, 20, 21, 23) can cause identification problems for some *E. coli* strains, nu-

TABLE 2. Frequency of biochemical profile numbers of *E. coli* O157:H7 or O157:H(–)

Profile no. ^a	Profile frequencies (%) ^b	
	<i>E. coli</i> O157:H7	<i>E. coli</i> O157:H(–)
54465743000	25	32
54465543000	11	5
54465542000	11	5
54465540000	11	0
54465541000	9	0
54465741000	0	21
54465742000	6	0
55465743000	5	17
55465540000	4	0
54465763000	0	5
54465740000	4	5
54465554000	4	0
44465740000	2	0
44465543420	0	5
44465542000	0	5
15465543000	2	0
15465543040	2	0
15465541000	2	0
15465742000	2	0

^a These numbers represent a tabulation of positive biochemical results of strains based on ID 32E analysis.

^b Each number corresponds to the percentage of strains displaying this profile number. The total numbers of tested strains were 55 and 19 for O157:H7 and O157:H(–) isolates, respectively. Note that none of these profiles were observed among the non-O157 isolates.

merous commercial systems or previous study on ID 32E systems (17, 23) have been shown to identify *E. coli* with a fairly high degree of accuracy (11, 13, 14, 16, 19–22, 25–29, 32, 33, 36, 37).

One strain serotyped as O74:H52 was found to be indole negative without affecting its identification as *E. coli*. These atypical characteristics have been previously described in the literature (29) and especially for the pathogenic class of *E. coli* (10). The O157 *E. coli* characterized in this work seemed to be more often urease positive than the other *E. coli* isolates (15 versus 0.9%, respectively). However, no plasmid detection was investigated (24, 34), and it remains to be seen whether this phenotype offers any ecological advantage to these isolates.

Whereas β -glucuronidase appears to be a confirmed character to differentiate between *E. coli* O157 and non-O157 strains, the sorbitol fermentation is more questionable due to the low number of positive *E. coli* non-O157 strains studied thus far. Interestingly, this observation could be explained, at least partially, by the discrepancy between the classical selective media for O157:H7 *E. coli*, mainly based on sorbitol fermentation, and chromogenic agar, based on β -glucuronidase and β -galactosidase.

Contrary to the data reported for the Microscan (1) or API 20E (8) tests, the results of this study using ID 32E suggested that *E. coli* O157:H7 or O157:H(–) strains do not display a unique profile but instead several particular biochemical profiles. Nevertheless, a single profile (i.e., 54465743000) accounted for 25 and 32% of all O157:H7 and O157:H(–) isolates, respectively. Moreover, eight profiles (i.e., 54465743000, 54465543000, 54465542000, 54465540000, 54465541000, 54465741000, 54465742000, and 55465743000) represented ~ 80% of all

O157:H7 and O157:H(–) strains, and these profiles were not found among the non-O157 *E. coli* strains.

Finally, it is important to note that the current ID 32E database of reference biochemical profiles has been mostly made with classical *E. coli* and does not take into account specific biochemical characteristics of the O157 serotype. Incorporation of these particular biochemical profiles or numbers into the ID 32E database could inform customers of presumptive identification of the *E. coli* serotype O157 and should suggest additional confirmation tests which must be performed.

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