APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Mar. 1981, p. 639-645
0099-2240/81/030639-07$02.00/0
Vol. 41, No. 3

Biochemical Characteristics and Identification of *Enterobacteriaceae* Isolated from Meats

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The isolation and identification of 2,220 *Enterobacteriaceae* from meats indicated that *Escherichia coli* biotype I, *Enterobacter agglomerans*, and *Serratia liquefaciens* were the principal types to be differentiated in meats. *Citrobacter freundii*, *Klebsiella pneumoniae*, *Enterobacter cloacae*, and *Enterobacter hafniae* were also commonly identified. Identification of isolates by the Encise II (Roche Diagnostics Inc., Nutley, N.J.) and Minitek (BBL Microbiology Systems, Cockeysville, Md.) coding systems gave similar results with only 255 (11.5%) discrepancies in identity, but both systems required large numbers of supplementary tests for identification of the isolates. Not only the distribution of *Enterobacteriaceae* types isolated from meats but also some of the biochemical reactions of the isolates differed from those of clinical isolates. The Minitek technique is recommended because of its versatility. However, with the addition of cellobiose and salicin disks and the inclusion of methyl red to the Minitek test and the use of the Voges-Proskauer test and gas production in EC medium at elevated temperature as standard tests, the identification of these *Enterobacteriaceae* from meats would be greatly facilitated. The inclusion of the motility test, for example, using nitrate motility agar, would also be of value to *Enterobacteriaceae* identification.

Considerable interest has been shown by public health officials regarding *Escherichia coli* in foods and water. The implications of *E. coli*, especially *E. coli* biotype I, as an indicator of fecal contamination (1, 22, 26) vary with the food type and the handling that the food has received (18). Some workers have stated that the *Enterobacteriaceae* as a whole, and not just *E. coli*, should be taken into account when considering the sanitary standards and hygiene of food handling (21, 23). In studies on ground beef (24) it was shown that *E. coli* and other *Enterobacteriaceae*, notably *Enterobacter agglomerans* and *Serratia liquefaciens*, were of importance by virtue of their numbers in this product. The *Enterobacteriaceae* can be enumerated as a whole by using violet red bile agar (Difco Laboratories, Detroit, Mich.) with (23) or without (24) the addition of glucose. However, additional information about the types of *Enterobacteriaceae* in a food requires that specific organisms be identified by biochemical tests. A different range of *Enterobacteriaceae* are encountered in meats compared with clinical isolates (5, 24); hence, versatility of biochemical tests might be an important criterion in selecting a rapid test for identification of these organisms isolated from meats.

Many comparisons of commercially available identification kits have been reported to give good agreement with conventional media (2, 11, 14, 19, 20, 27, 28). The API method is generally reported to be superior to the Auxotab, Pathotec, R-B, and Enterotube systems (5, 16, 25). The Minitek system (BBL Microbiology Systems, Cockeysville, Md.) has also been evaluated against conventional media and has been reported to be 96 to 97% accurate (11, 14, 15). Although the API system presents itself as a promising rapid technique, Cox and Mercuri (5) reported that only 82% of 373 isolates from meats were correctly identified by the API system. Hence, the Minitek technique was selected for this study because of its flexibility and because of an initial study in our laboratory which showed that known and unknown cultures were reliably identified (Ng, unpublished data).

This study was designed to determine the biochemical characteristics and identity of *Enterobacteriaceae* isolated from meat packer and retailer levels of meat handling and to allow comparison of the identity of the isolates by using the Minitek and the Encise II (Roche Diagnostics Inc., Nutley, N.J.) coding systems.

MATERIALS AND METHODS

Source of isolates. A total of 442 meat samples were obtained from local supermarkets. These in-

cluded ground beef; frozen and thawed, manufacturer-packaged pork sausage; vacuum-packaged prime cuts of beef and beef trim; pork loin; and retail packages of beef steak and pork chops. Comminuted meat samples were prepared for analysis as described by Ng and Stiles (24). Samples of integral meats were prepared by the spray gun technique described by Clark (3) with 100 ml of sterile, 0.1% peptone water wash collected at five points on the sample surface (approximately 20 ml per sampling point) by using a graduated Erlenmeyer flask. Appropriate dilutions were inoculated onto Difco violet red bile agar, violet red bile agar plus 1% glucose (23), and the most probable number media (18). Colonies were randomly picked from violet red bile agar, violet red bile agar plus 1% glucose, and Difco Levine EMB agar plates to represent each of the colony types on each medium for each sample. Isolates were purified by streaking onto MacConkey agar (Difco) and then onto nutrient agar (Difco).

Identification of isolates. Purified isolates on nutrient agar were screened for gram-negative, glucose-positive, and oxidase-negative (29) reactions. *Enterobacteriaceae* isolates were subjected to the following biochemical tests by using Minitek disks: arabinose, citrate, dulcitol, H_2S-indole, inositol, lysine, malonate, o-nitrophenyl-β-D-galactopyranoside, ornithine, phenylalanine, raffinose, rhamnose, and urea. Isolates were also inoculated into phenyl red glucose and lactose broths, nitrate motility agar (7), triple sugar iron agar, and MR-VP medium (Difco). All tests were incubated at 35°C for 24 h, except MR-VP, which was incubated at 35°C and sampled at 3 days for the Voges-Proskauer test and at 5 days for the methyl red test. Supplementary biochemical tests were carried out as required by the Minitek and Encise II coding systems with conventional media. The ability of cultures to produce gas at 45.5 ± 0.01°C in EC medium (Difco) was also checked (12).

RESULTS

Out of the 442 samples of meats analyzed, *Enterobacteriaceae* were detected in 380 (86.0%) samples. All of the 127 ground beef samples and 65 frozen and 10 thawed, manufacturer-packaged pork sausage samples contained *Enterobacteriaceae*. Furthermore, 76 (89.0%) vacuum packaged beef primal cuts, 26 (92.8%) vacuum packaged beef trim, and 16 (94.1%) pork loin samples also contained *Enterobacteriaceae*. Only 35 (54.7%) beef steak and 25 (54.3%) pork chop samples had detectable levels of *Enterobacteriaceae*.

The biochemical tests selected for use in this study included the basic tests for the Minitek and Enterotube *Enterobacteriaceae* identification systems. As a result, both the Minitek and Encise II coding systems could be used to determine the identity of the isolates. In addition, the complete indole, methyl red, Voges-Proskauer, and citrate (IMViC) reactions for each isolate were obtained, which allowed the subgrouping of *E. coli*. Based on the Minitek coding system, 661 out of 2,220 cultures could be identified without supplementary tests. With the Encise II coding system, with fewer biochemical criteria, 592 cultures could be identified without supplementary tests. The frequency of use of the suggested supplementary tests by the two coding systems has been summarized in Table 1. The most frequently used supplementary tests for both systems for these isolates were the Voges-Proskauer test and utilization of adonitol. Thereafter, sorbitol, dulcitol, motility, and esculin were the most significant supplementary tests used to identify isolates in the Minitek system; whereas, salmonella antisera (not considered by Minitek), arabinose, salicin, arginine dihydrolase, motility, sucrose, raffinose, and cellobiose were the most significant supplementary tests for the Encise II system.

The same biochemical data base, therefore,

TABLE 1. *Frequency of use of supplementary biochemical tests to identify Enterobacteriaceae of meat origin by the Minitek and Encise II coding systems*

Test	No. of isolates requiring supplementary tests	
	Minitek	Encise II
Adonitol	497	698
Alkalescens-Dispar antiserum	–[a]	21
Arabinose	—[b]	448
Arginine dihydrolase	162	343
Capsule stain	–	124
Cellobiose	–	257
Dulcitol	371	—[c]
Deoxyribonuclease	88	54
Esculin	323	–
Gas from glucose	125	—[c]
Inositol	—[b]	19
Jordon's tartrate	–	131
Lactose	85	—[c]
Malonate	–	5
Mannitol	–	3
Motility	365	330
Mucate	–	175
Potassium cyanide	–	111
Raffinose	138	258
Rhamnose	—[b]	161
Salicin	–	397
Salmonella antiserum	–	484
Shigella antiserum	–	71
Sorbitol	458	–
Sucrose	64	278
Voges-Proskauer	582	1,230
Xylose	153	2

[a] –, Supplementary test not used for these isolates.
[b] Used in the Minitek identification coding system.
[c] Used in the Encise II identification coding system.

was used to determine the identity of the isolates. Discrepancies between the two identification systems are summarized in Table 2. A total of 261 (11.8%) cultures out of 2,220 were identified differently by the two systems. Of the 261 cultures with different identities, the principal discrepancies occurred with *Enterobacter hafniae* (20.3%), *S. liquefaciens* (14.9%), *E. agglomerans* (10.7%) and *Klebsiella ozaenae* (10.3%). Only 10 out of 638 *E. coli* I isolates had conflicting identities by the two systems.

For the conflicting identities associated with *Citrobacter freundii*, *Klebsiella pneumoniae*, and *E. agglomerans*, the organism types varied over a wide range of alternatives. However, with *K. ozaenae*, *Enterobacter aerogenes*, *E. hafniae*, and *S. liquefaciens* some important categories of conflicting identification were apparent for example, *K. ozaenae* and anaerogenic *E. agglomerans*, *E. hafniae* and *E. aerogenes*, and *S. liquefaciens* and *E. hafniae*. *Yersinia enterocolitica* differed in 50% of the cases when identified by the two systems, indicating that caution should be exercised in identifying *Y. enterocolitica* from these coding systems.

The most frequently occurring *Enterobacteriaceae* among these isolates were *E. coli* biotype I (638 isolates), *S. liquefaciens* (378 isolates), and *E. agglomerans* (180 aerogenic and 96 anaerogenic isolates). Other organisms of importance included: *C. freundii*, *K. pneumoniae*, *Enterobacter cloacae* and *E. hafniae*. These organisms accounted for 2,003 (90.2%) of the isolates from the meats in this study.

The characteristics of the seven principal *Enterobacteriaceae* isolates from these meat samples are shown in Table 3. The IMViC reaction ++−− was used to define *E. coli* biotype I. A total of 602 (94.4%) of *E. coli* I produced gas at 45.5°C in EC medium. Other *E. coli* included 20 isolates that were identified as biotype II with IMViC reaction −+−− and an additional 16 isolates that were identified as biotype II by the Encise II system; however, 10 of these were identified as *E. aerogenes* by the Minitek system.

The biochemical reactions were considered potential differentiating characteristics of an organism if >80% or <20% of the isolates possessed the specific attribute. Indole, therefore, was a characteristic of *E. coli* I which differentiated it from the other principal *Enterobacteriaceae* in meats, except *K. pneumoniae*. In contrast, ability to utilize arabinose, dextrose, and *o*-nitrophenyl-β-D-galactopyranoside were major characteristics of all of these organisms, but they did not afford any differentiation. Utilization of lactose and arabinose were positive non-differentiating properties of these *Enterobacteriaceae*, whereas dulcitol and phenylalanine were negative non-differentiating properties. The only organism capable of producing H_2S to a significant extent was *C. freundii*. However, only 72.2% of *C. freundii* isolates produced H_2S on triple sugar

TABLE 2. *Relationship between organism identity determined by the BBL Minitek and the Roche Encise II Enterobacteriaceae coding systems using the same biochemical data base*

Minitek identity	Encise II identity[a]																
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	18	19	20
1 *E. coli* I	628	–	–	5	3	–	1	–	–	–	–	–	–	–	–	1	–
2 *E. coli* (−+−−)	–	17	–	–	–	1	–	–	–	–	2	–	–	–	–	–	–
3 Other *E. coli*	–	–	11	–	1	–	–	–	–	–	–	–	–	–	–	–	1
4 *C. freundii*	1	2	1	179	–	1	–	–	–	–	–	3	–	–	–	–	–
5 *K. pneumoniae*	–	–	–	–	179	3	4	1	3	2	–	1	–	–	–	–	1
6 *K. ozaenae*	–	–	–	2	3	19	2	18	–	–	–	–	2	–	–	–	–
7 *E. agglomerans* (aerogenic)	–	1	–	4	–	3	160	–	–	9	–	–	2	–	–	–	1
8 *E. agglomerans* (anaerogenic)	–	–	–	1	–	–	–	88	–	–	–	–	6	1	–	–	–
9 *E. aerogenes*	1	10	–	–	–	–	–	–	43	1	7	–	–	–	–	–	–
10 *E. cloacae*	–	–	–	5	–	2	–	–	1	154	1	4	–	–	–	–	–
11 *E. hafniae*	–	2	–	–	–	–	–	–	35	–	110	7	–	–	9	–	–
12 *S. liquefaciens*	–	–	–	2	–	1	–	1	3	4	25	339	1	–	–	–	2
13 *S. rubidaea*	–	–	–	–	–	1	–	–	5	1	–	–	16	–	–	–	–
14 *Y. enterocolitica*	–	1	–	5	–	–	1	2	–	–	–	2	–	11	–	–	–
15 *A. hinshawii*	–	–	–	2	–	–	–	–	–	–	1	1	–	–	–	–	–
16 *K. rhinoscleromatis*	–	–	–	–	–	–	–	3	–	–	–	–	–	–	–	–	–
17 *Shigella* spp.	–	–	–	–	–	–	2	1	–	1	–	–	–	–	–	–	–
18 *S. marcescens*	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
19 *E. tarda*	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
20 Unidentifiable	–	–	–	4	–	–	1	4	–	–	–	–	–	–	–	–	10

[a] Organism code numbers correspond to identity noted for Minitek identity, e.g., 1 = *E. coli* I, 2 = *E. coli* (−+−−), etc.

TABLE 3. *Percentage of positive biochemical and identifying characteristics of the seven principal Enterobacteriaceae identified from meats based on the Minitek coding system*

Identification test	*E. coli* I	*C. freundii*	*K. pneumoniae*	*E. agglomerans*		*E. cloacae*	*E. hafniae*	*S. liquefaciens*
				Aer[a]	Anaer[b]			
Motility (35°C)	85.6	88.8	4.1	70.6	78.1	89.2	67.5	94.7
IMViC								
Indole	100	5.9	56.2	16.7	9.4	2.4	0.6	0.3
Methyl red	100	94.1	23.7	38.3	34.4	6.6	7.4	6.9
Voges-Proskauer	0	4.3	91.2	56.1	59.8	95.2	93.3	90.2
Citrate	0	66.8	94.8	50.0	72.7	92.2	3.6	98.1
Arabinose	100	100	99.5	100	92.7	100	93.9	99.5
Cellobiose	7.6	80.3	96.0	89.6	67.5	85.7	0.7	6.5
Dulcitol	66.3	37.4	28.4	23.9	4.2	15.6	2.5	1.6
Glucose								
acid	100	100	100	100	100	100	99.9	100
gas	97.8	97.3	98.5	100	0	98.8	96.4	97.7
Inositol	8.2	27.8	95.9	13.3	51.0	38.3	1.8	4.2
Lactose								
acid	98.6	93.6	97.9	63.9	47.9	82.7	36.1	23.0
gas	96.5	91.5	94.3	53.3	12.5	74.3	34.9	21.1
Raffinose	51.6	34.2	96.6	57.8	29.2	93.4	28.8	80.0
Rhamnose	95.3	95.7	97.4	90.6	80.2	91.6	92.6	1.6
Salicin	1.2	12.1	97.0	97.9	88.4	48.8	2.1	96.3
ONPG[c]	98.7	93.0	98.4	81.7	86.5	98.2	98.1	96.6
Malonate	1.1	17.1	84.5	85.6	63.5	82.5	10.4	5.6
H_2S								
From TSI[d]	0.9	72.2	1.0	1.1	0	0	0.6	0
Minitek	3.3	64.2	2.5	1.0	0	3.0	1.2	3.5
Lysine	86.9	1.6	94.8	0.6	0	2.4	97.5	89.7
Ornithine	69.0	66.3	2.6	0.6	2.1	95.2	99.4	99.2
Phenylalanine	0.5	0	1.5	38.3	37.4	1.2	0.6	0.5
Urea	3.6	8.5	83.5	2.8	13.6	19.8	1.8	2.4
Gas in EC (45.5°C)	94.4	0.5	2.1	0.6	0	0	0.7	0
No. of isolates	638	187	194	180	96	167	163	378

[a] Aer, Aerogenic.
[b] Anaer, Anaerogenic.
[c] ONPG, *o*-Nitrophenyl-β-D-galactopyranoside.
[d] TSI, triple sugar iron agar.

iron agar slants, and 64.2% produced H_2S on Minitek H_2S-indole disks.

Important differentiating tests for these seven predominating *Enterobacteriaceae* from meats that are not included in the Enterotube identification method are: motility, methyl red, Voges-Proskauer, cellobiose, inositol, rhamnose, salicin, malonate, and gas in EC medium at 45.5°C. Similar tests missing from the basic Minitek scheme include: motility, methyl red, Voges-

Proskauer, cellobiose, salicin, and gas in EC medium at 45.5°C. As a result, the versatility of the Minitek test for identification of these meat isolates becomes an important factor. Use of a test such as Enterotube requires too many supplementary or additional conventional tests. The principal characteristics differentiating the three main *Enterobacteriaceae* isolates from meats are shown in Table 4.

DISCUSSION

Enterobacteriaceae are commonly found as part of the flora of ground meats, but they occur less frequently on meat cuts. This study indicated that *E. coli* I, *E. agglomerans*, and *S. liquefaciens* were the principal *Enterobacteriaceae* occurring in these meat cuts. However, the frequency of *E. coli* and other lactose-fermenting types might be over-represented because of the selectivity of the most probable number technique. These data confirmed the observation by Cox and Mercuri (5) that identification tests for *Enterobacteriaceae* isolated from foods would be more efficient if they differentiated *E. agglomerans* and *S. liquefaciens* more readily. Although *E. agglomerans* and *S. liquefaciens* are included in both the Minitek and Encise II identification systems, neither of them was included as a species in the family *Enterobacteriaceae* in the eighth edition of *Bergey's Manual of Determinative Bacteriology* (4). *E. agglomerans* is included in the Herbicola group of the genus *Erwinia*, despite its proposed taxonomic classification as *E. agglomerans* by Ewing and Fife (10) in 1972. *S. liquefaciens* is even less visible, and is suggested as a nonpigmented species of *Serratia*, but it is not included as such in the classification. This organism was originally classified as *Aerobacter liquefaciens* (13) and was placed in the genus *Enterobacter* (8). Subsequently, it was proposed that this organism be included as a species in the genus *Serratia* (9). The relative importance of these organisms in meats indicates that clarification of their taxonomy and nomenclature is desirable to avoid future confusion.

TABLE 4. *Principal tests enabling differentiation of E. coli biotype I, E. agglomerans, and S. liquefaciens isolates from meats*

Test	*E. coli* I	*E. agglomerans*	*S. liquefaciens*
IMViC			
Indole	+	−	−
Methyl red	+	±	−
Voges-Proskauer	−	±	+
Citrate	−	±	+
Cellobiose	−	+	−
Rhamnose	+	+	−
Salicin	−	+	+
Malonate	−	+	−
Lysine	+	−	+
Ornithine	±	−	+

The identity of the isolates determined by Minitek and Encise II coding systems was similar, with only 255 (11.5%) discrepancies between the two systems. The main discrepancies between the two systems were between minor rather than major *Enterobacteriaceae* in meats; for example, only 10 out of 638 *E. coli* were differently identified by the two systems, but 53 out of 163 *E. hafniae* were differently identified. The biochemical reactions for the principal *Enterobacteriaceae* isolates in this study were compared to the reactions reported for these organisms by Edwards and Ewing (6) and Ewing and Fife (10). The strains of *E. coli* biotype I differed from those reported by Edwards and Ewing (6) by having a markedly higher percentage of strains that were motile and produced acid from dulcitol and rhamnose, whereas fewer strains fermented raffinose and salicin. Salicin represented a marked difference in that only 1.2% of these *E. coli* isolates fermented salicin, compared with 37.1% reported by Edwards and Ewing (6).

E. agglomerans isolates in this study also showed differences in biochemical reactions compared with the results of Ewing and Fife (10). For aerogenic strains a greater percentage fermented dulcitol, used malonate as a carbon source, and produced phenylalanine deaminase and urease, whereas fewer strains produced indole and fermented lactose. For anaerogenic strains, a greater percentage produced acid from cellobiose, inositol, and salicin, and fewer produced indole and acid from lactose. With fewer strains producing indole, anaerogenic strains would be easier to distinguish from indole-positive *E. coli*.

S. liquefaciens isolates included a greater percentage of strains that were motile and a greater proportion with IMViC tests −−++ than would be expected from the biochemical tests for this organism reported by Edwards and Ewing (6). Fewer strains fermented cellobiose and inositol. They were readily distinguished from the *E. coli* strains because of the low percentage of indole-positive isolates. However, the fact that inositol was not fermented made it more difficult to distinguish *S. liquefaciens* from *E. cloacae* and *E. hafniae*. Rhamnose and raffinose became im-

portant tests in differentiating these inositol-negative organisms.

The difference in inositol fermentation could account for some of the discrepancies between the Minitek and Encise II typing, yet the 25 strains identified as *S. liquefaciens* by Minitek, but as *E. hafniae* by Encise II, were able to ferment inositol. These inositol-positive strains were raffinose negative, and raffinose is the only test that differentiates *S. liquefaciens* and *E. hafniae* in the supplementary tests for the Encise II coding system.

C. freundii and *E. hafniae* also differed quite markedly in several of their biochemical tests compared to the results reported by Edwards and Ewing (6). This might be attributable to specific characteristics of these organisms isolated from meats. However, *E. cloacae* and *K. pneumoniae* only differed appreciably in one or two of the biochemical characteristics tested. As a result, rapid identification kits with a limited range of biochemical tests oriented to identification of clinical isolates might not be adequate for identifying the spectrum of isolates from meats.

To screen or monitor the three principal *Enterobacteriaceae* in meats, *E. coli* I, *E. agglomerans*, and *S. liquefaciens*, only a limited range of biochemical tests appear necessary. These include: the IMViC test, cellobiose, rhamnose and salicin fermentation, utilization of malonate, and lysine and ornithine decarboxylation. This could be done by using conventional media (6) or by a versatile system such as that used in the Minitek technique. If the full range of tests for the Minitek technique is to be used in an effort to identify *Enterobacteriaceae* isolates from meats, the Voges-Proskauer test, cellobiose, and salicin are recommended as standard inclusions in the test. In addition, the motility test would be a valuable inclusion.

The Voges-Proskauer reaction was determined by using MR-VP medium, which also allowed the methyl red test to be carried out and, hence, the completion of the IMViC test. The Voges-Proskauer test is available as a Minitek disk test, but the loss of information from not knowing the IMViC reaction would have to be assessed. In addition, the test for gas production at elevated temperature was carried out routinely in this study. It served as a valuable tool for identifying *E. coli* type I of suspected fecal origin (17, 22). A total of 603 (94.4%) *E. coli* I isolates produced gas in EC medium at 45.5°C, but only 11 out of 1,582 other *Enterobacteriaceae* (0.7%) were gas positive. This confirms the production of gas in EC medium at 45.5°C as a valuable screening test.

ACKNOWLEDGMENTS

This work was supported by a research contract with Agriculture Canada.

M.E.S. is an Honorary Professor, Department of Microbiology, The University of Alberta.

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