APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Sept. 1984, p. 601–605
0099-2240/84/090601-05$02.00/0

Vol. 48, No. 3

Escherichia coli Variants for Gas and Indole Production at Elevated Incubation Temperatures

D. H. BUESCHKENS AND M. E. STILES*

Departments of Microbiology and Foods and Nutrition, The University of Alberta, Edmonton, Alberta, Canada T6G 2M8

Received 29 May 1984/Accepted 5 June 1984

Two strains of *Escherichia coli* were subjected to heat and cold-storage treatments to determine the stability of the fecal *E. coli* characteristics of gas production from lactose and indole production at elevated incubation temperatures. No variants were detected with repeated sublethal heat treatment. A high incidence of variants was observed with extended cold storage of the organisms in liquid and semisolid media, especially with poor nutrient composition, and in the absence of cryoprotective agents. The indole characteristic at elevated temperature was more stable than the production of gas from lactose. The critical temperature at which both gas production from lactose and the indole characteristic were lost was 44.5°C. It appeared that the variants resulted from increased temperature sensitivity of the formic hydrogen lyase and tryptophanase enzymes, respectively.

Historically, in the fecal coliform test, *Escherichia coli* has been considered to produce gas from lactose at elevated incubation temperatures (10, 17). However, only 90% of *E. coli* isolates produce acid and gas from lactose within 48 h (11), and many researchers have reported the loss of gas production by *E. coli* (2, 18, 19). Another distinguishing characteristic of fecal *E. coli* cells is their ability to produce indole at elevated temperatures. This has been suggested as a specific test for *E. coli*, because 95% of organisms that were indole positive within 24 h at 44°C were *E. coli* biotype I and 3.4% were other fecal coliforms (3).

Cells that survive heat treatment and frozen or refrigerated storage in foods may sustain damage to their cell membranes, RNA, or DNA, causing enzyme functions to be weakened or altered (1, 21, 23). Injured cells reinoculated into suitable recovery media regain their normal growth characteristics (8, 22, 27–29). Long-term mild thermal stress has been cited as a cause of altered biochemical reactions in *E. coli*, notably loss of ability to produce gas from lactose at elevated incubation temperatures (14). Injury of cells stored at 1 and 4°C has also been reported (22), and considerable loss of viability has been reported in cells stored at −20°C (1, 16). Availability of nutrients and viscosity can protect bacteria in foods during low-temperature storage (25).

The object of this study was to determine the stability of gas production from lactose compared with the stability of indole production by *E. coli* at elevated incubation temperature. The strains of *E. coli* used in this study were exposed to conditions of sublethal heat treatment and cold-temperature storage that simulated conditions in food handling.

MATERIALS AND METHODS

Bacterial cultures. *E. coli* 11775 from the American Type Culture Collection (ATCC) and *E. coli* 1840, a strain isolated from meat, were used. These bacteria were subcultured weekly onto nutrient agar plates and slants, incubated at 35°C for 24 h, and stored at 4°C. The biochemical characteristics of the strains were checked for each subculture with the Minitek enteric identification system (BBL Microbiology Systems, Mississauga, Canada), incubated at 35°C for 24 h, and tubes of triple sugar iron agar, tryptic soy broth (TSB), EC broth, and lactose broth (Difco Laboratories, Detroit, Mich.), incubated at 35°C for 24 h, to determine H_2S, indole, glucose, and lactose reactions.

Plating media and screening of variants. Nutrient agar (NA) and violet red bile agar (VRBA) were prepared from Difco dehydrated media. Tryptone bile agar (TBA) was prepared by the method of Anderson and Baird-Parker (3). For the TBA medium, cellulose acetate membranes were not used (26), and a system of replicate plating with a sterile filter paper (Whatman no. 1) was used before staining, so that viable colonies would be available after staining with *p*-dimethylaminobenzaldehyde stain.

Morphological variants on VRBA (reduced colony size or altered bile precipitation) and TBA (reduced colony size or altered indole staining reaction) were isolated for further study. Smaller size or random colonies, up to a total of three colonies per plate, were picked from NA plates. All isolates were inoculated into triple sugar iron agar, TSB, EC broth, and lactose broth and incubated at 35 and 45°C for 48 h. Any variant cultures were stored on NA slants at 4°C.

Experimental treatments. The test organisms were subjected to a range of treatments.

(i) Heat treatment. Stock cultures were grown in 9 ml of TSB at 35°C for 24 h for 7 successive days before sublethal heat treatment. A 24-h culture was inoculated into 200 ml of sterile TSB tempered to 35°C and was allowed to grow (ca. 3 h) to an optical density at 600 nm of 1.0. A 1.0-ml portion was added to 99 ml of sterile TSB tempered to 52°C. After an equilibration period of 30 s, the culture was heated for 15 min. The heating flask was placed in an ice bath to reduce the temperature to about 23°C, and then the culture was incubated at 35°C for 20 h for heat treatment the next day. Once a week, 1-ml samples of unheated and heated (52°C for 15 min) cultures were diluted in 0.1% peptone water blanks, plated onto NA, TBA, and VRBA, and incubated at 35°C for 48 h. This procedure was repeated for 18 successive weeks.

(ii) Extended storage. Stock cultures were inoculated into three 100-ml amounts of TSB and incubated at 35°C for 24 h. One flask of each culture was stored at 4, 35, and 45°C and sampled daily. Samples were serially diluted in 0.1% peptone water and plated onto NA, TBA, and VRBA. This

* Corresponding author.

procedure was continued until the organism died out or the broth was used up.

(iii) Cold storage. Stock cultures were inoculated into 100 ml of TSB and incubated at 35°C for 24 h; 2.5-ml portions were dispensed in sterile glass vials and stored in a freezer at −16°C. Cultures were sampled weekly and plated onto NA, TBA, and VRBA.

(iv) Restricted nutrients. Stock cultures were inoculated onto NA plates and incubated at 35°C for 24 h. Isolated colonies were inoculated into sloppy agar (3.0 g of Trypticase [BBL], 7.5 g of agar [Difco] in 1 liter of deionized water), incubated at 35°C for 24 h, and stored at 4 and −16°C. Cultures were plated initially after 1 day of storage and then weekly for 20 weeks. Frozen cultures were thawed and tempered to 23°C before being plated onto NA, TBA, and VRBA.

(v) Nutrition studies. A range of media based on TSB was prepared, including TSB at full strength (30 g/liter) and with 20, 10, and 3 g of TSB per liter. TSB at 30, 15, and 3 g/liter was also prepared without soytone (Difco), without dextrose, without dextrose or soytone, or without salts (NaCl and dipotassium phosphate). In addition, media containing 30, 20, 10, and 3 g of tryptone (Difco) per liter were also prepared. Each medium was prepared as a broth and as a semisolid agar (7.5 g of agar per liter), dispensed in 2.5-ml portions in sterile glass vials, inoculated with the stock cultures, and treated as described for the restricted nutrient study.

(vi) UV studies. Stock cultures were grown in TSB at 35°C for 24 h, and a 1.0-ml portion was subcultured daily into 9 ml of TSB for the 93-day study period. A 1.0-ml portion of each subculture was placed in a sterile petri dish and irradiated for 5 min with a broad-beam germicidal UV lamp (Canadian Laboratory Supplies Ltd., UV Products line, Toronto). Appropriate dilutions were plated onto NA, TBA, and VRBA. Test plating was done on a daily basis for 60 days and then on a weekly basis until the termination of the experiment.

Screening and verification of variant strains. All colonies that were picked as possible variants were subcultured onto NA and checked for biochemical variations by inoculation into triple sugar iron agar, TSB, EC broth, and lactose broth and incubated at 45.0°C for 24 h. Confirmed variants were subcultured for 8 successive weeks to determine the stability of the new characteristic. The variants were also checked for their relationship to the parent culture by biochemical tests, antibiotic sensitivity testing, and measurement of growth rates of the stock and variant cultures. Biochemical tests were incubated at 35°C for 24 h, and the identities of the isolates were determined by using the Minitek enteric identification technique. The Kirby-Bauer antibiotic sensitivity test was used with *E. coli* ATCC 25992 as the reference strain by the procedure described by Barry (4) and with the following antibiotic sensitivity disks: ampicillin (10 μg), carbenicillin (100 μg), cephalothin (30 μg), chloramphenicol (30 μg), clindamycin (2 μg), erythromycin (15 μg), gentamicin (10 μg), kanamycin (30 μg), oxacillin (1 μg), penicillin (10 U), tetracycline (30 μg), and tobramycin (10 μg). Growth rates were determined in TSB at 22, 35, and 44.5°C.

Enzyme assays. The following assays were conducted to determine the nature of the lactose and indole variants.

(i) Formic hydrogen lyase assay. Stock and variant cultures were inoculated into sterile broth containing 1% glucose and 1% yeast extract and incubated at 35°C for 12 h. Cells were harvested and the enzyme was extracted by the procedure of Bovarnick (6). Gas production assays were conducted with a 0.1 ml portion of the enzyme extract in 9 ml of sterile 0.1 M formate solution containing inverted Durham tubes and adjusted to pH 6.2 with a 0.5 M sodium and potassium phosphate buffer (6). Assays were conducted at 43, 44, 45, 46, and 47°C, and gas production was recorded after 48 h of incubation. The thiobarbituric acid assay for formic acid was used (9).

(ii) Tryptophanase assay. Tryptophanase activity was determined by a modification of the assay of Newton and Snell (20). Stock and variant cultures were grown in nutrient broth, and a 0.2-ml portion of the nutrient broth culture was added to 0.2 ml of an assay mixture containing 0.2 M sodium phosphate buffer (pH 7.8), 0.025 M tryptophan, and distilled water (1:1:6 [vol/vol]). Assay mixtures were then incubated at 35 and 44.5°C for 24 and 48 h. After incubation, 0.12 ml of a 25% trichloroacetic acid solution was added to each tube and mixed well; indole was extracted by addition of 0.75 ml of toluene to each tube. After thorough mixing for 30 s, a 0.25-ml portion of the clear toluene was removed and placed in a spectrophotometer tube, and 0.5 ml of Erlich reagent and 4.25 ml of acid alcohol reagent were added to each tube. The reagents were mixed well and held at room temperature for 15 min, and absorbance at 540 nm was determined with a spectrophotometer (Spectronic 21).

(iii) Tryptophan pyrrolase assay. The tryptophan pyrrolase assay, based on kynurenine production (5, 15) was modified for use in this study by excluding hematin and ascorbic acid from the assay mixture. Stock and variant cultures were grown on NA plates at 35°C for 24 h and inoculated into TSB for growth at 35, 44, and 44.5°C for 48 h. The enzyme reaction mixture consisted of 4.0 ml of the TSB cell supernatant, 2.0 ml of 0.2 M phosphate buffer (pH 7.0), 0.6 ml of 0.03 M L-tryptophan, and 1.4 ml of distilled water. The enzyme reaction mixtures were held at 37°C for 10 h and chilled on ice, and 4.0 ml of 5% trichloroacetic acid at 0°C was added to each tube. Absorbance at 360 nm was determined with a Gilford spectrophotometer.

RESULTS

The biochemical and growth characteristics of the stock *E. coli* cultures remained constant throughout the study (300 days). Both cultures produced gas in EC (EC^+) and lactose broth [$Lac(g)^+$] and were indole positive (Ind^+) at 35 and 44.5°C. The test organisms were subjected to the various treatments, plated onto nonselective (NA) and selective (TBA and VRBA) growth media, and screened for Lac(g) and Ind variants.

No variants were detected when the stock cultures were stored on NA at 4°C. Similarly, no variants were detected when the stock cultures were stored on NA slants at 4°C for 166 days. The numbers of colonies recovered on VRBA and TBA were 25% and 50 to 66%, respectively, of the number recovered on NA. Heating at 52.0°C for 15 min resulted in a 1,000-fold decrease in the number of colonies recovered per milliliter on NA and TBA and a 10,000-fold decrease in the number recovered on VRBA. However, no variants were detected among the isolates for the 124-day duration of the heat treatment study.

During extended storage, the culture stored at 45°C died out after 52 days, the culture stored at 35°C died out after 75 days, and the culture stored at 4°C was tested for 100 days. A summary of the variants detected as a result of the extended storage and the other treatments is shown in Table 1. After 6 days of storage at 45°C, a colony was detected that failed to precipitate bile on VRBA at 45°C. This variant was $Lac(g)^-$ Ind^- at 45°C. After three subcultures, the variant recovered the ability to develop sufficient acidity to precipitate bile in

TABLE 1. Summary of *E. coli* variants for gas production from lactose and indole production at elevated temperature (44.5°C) and the treatments from which they were obtained

Treatment	Variants found[a]	Time required (days)	Type of 44.5°C variant
Extended storage			
45°C	ATCC	6	Ind^- EC^- $Lac(g)^-$
35°C	ATCC	75	Ind^+ EC^- $Lac(g)^-$
35°C	Meat	68, 75	Ind^+ EC $Lac(g)^-$
4°C	ATCC	75, 76	Ind^+ EC $Lac(g)^-$
4°C	Meat	68, 75, 76	Ind^+ EC^- $Lac(g)^-$
Frozen storage			
−16°C	ATCC	59	Ind^- EC^- Lac(g)
−16°C	ATCC	70	Ind^+ EC $Lac(g)^-$
Nutrient studies			
Sloppy agar (4 and −16°C)	ATCC and meat	5[b]	Ind^+ EC^- $Lac(g)^-$
Nutritional semi-solid media (4°C)[c]	ATCC and meat	11[b]	Ind^+ EC^- $Lac(g)^-$
Nutritional broths (−16°C)[d]	ATCC and meat	11[b]	Ind^+ EC^- Lac(g)
UV treatment	ATCC	45	Ind^- EC^- $Lac(g)^-$

[a] ATCC, *E. coli* ATCC 11775; Meat, *E. coli* 1840 (isolated from meat).
[b] Time at which the altered characteristic was first noted.
[c] Variants were observed in all media except TSB (30 g/liter).
[d] Variants were observed in all media except those with greater than 3 g of TSB per liter which did not exclude soytone.

VRBA at 45°C, but it remained EC^- $Lac(g)^-$ Ind^-. This was the only variant detected during storage at 45°C, but EC^- $Lac(g)^-$ Ind^+ variants were detected in the cultures stored at 35 and 4°C (Table 1).

Frozen storage of the *E. coli* cultures at −16°C for 24 h resulted in a 100-fold decrease in the number of cells recovered on NA, VRBA, and TBA. With continued frozen storage, the loss of viability became 4 log cycles or more. Variants were detected on two occasions during the 204-day duration of this experiment (Table 1). Cultures were also inoculated into sloppy agar and stored at 4 and −16°C. *E. coli* variants were detected after 5 days of storage at both temperatures, and unlike that for other storage treatments, the incidence of variants was high. In later samples during the 142-day duration of this experiment, 20 colonies were picked and inoculated into a series of tubes, and all gave the same reactions. All of the variants were EC^- $Lac(g)^-$ Ind^+.

A greater frequency of occurrence of variants was observed in media with poor nutrient levels, such as the semisolid (sloppy) agar, compared with that observed in TSB. This prompted the study of different levels of nutrition on the production of *E. coli* variants. The cultures stored at 4°C in semisolid agar produced variants in all media except full-strength TSB (30 g of TSB per liter). Variants were first detected at 11 days and then at a high frequency at subsequent sampling times. All variants were EC^- $Lac(g)^-$ Ind^+. The cultures stored at −16°C in broth produced variants except in those broths which contained greater than 3 g of TSB per liter and those which included soytone.

Daily irradiation for 5 min with a germicidal UV lamp resulted in a 10,000-fold decrease in the number of cells recovered on NA, VRBA, and TBA. After repeated UV treatment on 45 successive days, three *E. coli* colonies were observed that grew on VRBA but failed to precipitate bile at 45°C. The variants precipitated bile in VRBA after four subcultures, but they remained EC^- $Lac(g)^-$ Ind^-. No other variants were detected during the 93 days of this study.

A sample of 13 *E. coli* variants, 8 from *E. coli* ATCC 11775 and 5 from *E. coli* 1840 (the meat isolate), was selected. These variants were representative of the different variant types and the treatments. The sample was selected to confirm the relationship of the variants to the stock cultures. The stock cultures and their variant strains had identical biochemical reactions at 35°C. The antibiograms for the two *E. coli* strains differed only in their susceptibilities to erythromycin and cephalothin. Both strains were susceptible to

TABLE 2. Gas production in 0.1 M formate solution by formic hydrogen lyase extracts of *E. coli* ATCC 11775 and *E. coli* 1840 (meat isolate) stock and variant cultures

Culture	Gas production in assay at the following temperature (°C):[a]				
	43	44	45	46	47
E. coli ATCC 11775					
Stock	+	+	+	w	−
Variant					
1	+	w	ww	−	−
2	+	w	ww	−	−
3	+	w	−	−	−
4	+	w	ww	−	−
5	+	w	−	−	−
6	+	w	ww	−	−
7	+	w	−	−	−
8	+	w	−	−	−
E. coli 1840 (meat isolate)					
Stock	+	+	+	w	−
Variant					
9	+	w	ww	−	−
10	+	w	−	−	−
11	+	w	−	−	−
12	+	w	−	−	−
13	+	w	−	−	−
Uninoculated broth	−	−	−	−	−

[a] Symbols: +, reaction with 0.1 ml of enzyme extract; w, weak reaction; ww, very weak reaction with 1.0 ml of enzyme extract; and −, no reaction.

TABLE 3. Summary of the data for 24-h tryptophanase assays at 35.0 and 44.5°C on *E. coli* ATCC 11775 stock and variant cultures grown at 35.0 and 44.5°C in nutrient broth

Culture	Absorbance at 540 nm			
	35.0°C culture assayed at:		44.5°C culture assayed at:	
	35.0°C	44.5°C	35.0°C	44.5°C
E. coli ATCC 11775				
Stock	0.349	0.170	0.297	0.153
Variant				
1	0.339	0.168	0.247	0.157
2	0.273	0.117	0.185	0.098
3	0.247	0.147	0.270	0.134
4	0.278	0.143	0.272	0.136
5	0.263	0.133	0.241	0.117
6	0.246	0.000	0.038	0.000
7	0.281	0.000	0.123	0.000
8	0.280	0.000	0.096	0.000
Uninoculated broth	0.000	0.000	0.000	0.000

ampicillin (30 μg), carbenicillin (100 μg), chloramphenicol (30 μg), gentamicin (10 μg), kanamycin (30 μg), tetracycline (30 μg), and tobramycin (10 μg) and resistant to clindamycin (2 μg) and oxacillin (1 μg). *E. coli* ATCC 11775 was resistant to erythromycin (15 μg) and susceptible to cephalothin (30 μg), whereas *E. coli* 1840 was susceptible to erythromycin (15 μg) and had an intermediate reaction to cephalothin. Antibiograms for the stock cultures and their variant strains were identical. Similarly, the stock cultures and variant strains had approximately the same growth rates when grown in TSB at each of the specific incubation temperatures, 22, 35, and 45°C. *E. coli* 1840 had slower growth rates at each temperature compared with *E. coli* ATCC 11775.

The temperature limits for growth, gas production from lactose and indole production were determined at 0.5°C intervals. The stock and variant strains of both *E. coli* cultures did not grow in TSB, EC broth, and lactose broth at 48.5°C. Gas was produced in EC and lactose broths by both *E. coli* stock cultures up to 47°C, whereas the Lac(g)$^-$ variants lost the ability to produce gas at 44.5°C. Similarly, the stock cultures stopped producing indole at 48°C, whereas the Ind$^+$ and Ind$^-$ variants stopped producing indole at 47.5 and 44.5°C, respectively.

Analyses to determine the cause of the loss of gas production from lactose by the Lac(g)$^-$ variants indicated that all stock and variant cultures were positive when tested for *o*-nitrophenyl-β-D-galactopyranoside at 35 and 44.5°C. The final pH values for the stock and variant *E. coli* strains grown in lactose broth at 35, 44, and 44.5°C for 48 h were similar. The mean pH after incubation at 35°C was 4.9, compared with mean pH values of 5.3 and 5.5 at the elevated incubation temperatures. Temperature limits for gas production in a formate solution with formic hydrogen lyase extracts from 12-h cultures of the *E. coli* stock and variant strains are shown in Table 2. The data indicate greater temperature sensitivity of the formic hydrogen lyase system in the variant strains than in the stock cultures.

Studies of the Ind$^-$ variants to determine the nature of the loss of indole production involved tryptophan pyrrolase and tryptophanase assays. The tryptophan pyrrolase activity was low for stock cultures as well as the Ind$^+$ and Ind$^-$ *E. coli* ATCC 11775 variant cultures. Detectable levels of enzyme activity could only be obtained with extended assay times of up to 10 h. In contrast, the tryptophanase activity data (Table 3) indicate not only that the synthesis of tryptophanase might be inhibited at elevated incubation temperature, but also that the tryptophanase produced by the Ind$^-$ variants is more temperature sensitive.

DISCUSSION

Considerable emphasis is placed on the production of gas from lactose at elevated incubation temperature to determine fecal origin of *E. coli*, or *E. coli* biotype I (13). In North America, incubation temperatures of 44.5 to 45.5°C are used to reduce the incidence of false-positive results and because gas production from lactose has been shown to be more specific for fecal *E. coli* at these temperatures (12). In Europe, it is believed that specificity for *E. coli* as an indicator organism is obtained with an elevated incubation temperature of 44°C for gas production from lactose and indole production (10). In this study, the critical temperature for Lac(g)$^-$ and Ind$^-$ of the *E. coli* variants was 44.5°C, indicating that the maximum incubation temperature for detection of these variant strains as fecal *E. coli* was 44°C.

The production of gas from lactose at elevated incubation temperature is widely used as a criterion of fecal coliform bacteria. The use of indole production at elevated incubation temperature is gaining acceptance (24). Based on the lower incidence of Ind$^-$ variants compared to Lac(g)$^-$ variants in this study, indole production is considered to be a more reliable characteristic for measuring *E. coli* association, especially if elevated incubation temperatures of 44°C and above are preferred. This observation supported the report that the indole characteristic is more stable than lactose fermentation in *E. coli* (2).

This study was undertaken with the expectation that repeated exposure of *E. coli* to sublethal heat treatment might produce lactose variants. However, no variants were detected in the heat treatment study, whereas large numbers of *E. coli* variants were produced in the extended-storage studies. This was most marked in media with poor nutrient composition, in media lacking cryoprotective agents, and in broths and semisolid agars as opposed to standard-strength agar. This suggests that the phenomenon of lactose variability of *E. coli* in thermally stressed reactor effluent waters reported by Kasweck and Fliermans (14) might have been a nutrient effect. The role of nutrients and cryoprotective agents in cold storage damage has been reported (7, 25). Variants were obtained in TSB with extended storage. However, in less protective media, variants were produced at an earlier time and in predominating concentration. This emphasizes the need for carefully selected conditions for storage of *E. coli* in the laboratory.

The permanent nature of the variants and the fact that similar variants could be induced by UV irradiation suggests that the variations were due to genetic damage or alteration rather than a temporary cellular injury. The changes in gas production from lactose and indole production at temperatures of 44.5°C and above appeared to result from the action of a more temperature-sensitive formic hydrogen lyase and a decrease in tryptophanase production as well as an increase in the temperature sensitivity of the tryptophanase in indole production.

ACKNOWLEDGMENT

This research was supported by Natural Sciences and Engineering Council of Canada grant A7203.

LITERATURE CITED

1. **Alur, M. D., and N. Grecz.** 1975. Mechanism of injury of *Escherichia coli* by freezing and thawing. Biochem. Biophys. Res. Comm. **62**:308–312.
2. **Anderson, J. G., P. S. Meadows, B. W. Mullins, and K. Patel.** 1980. Gas production by *Escherichia coli* in selective lactose fermentation media. FEMS Microbiol. Lett. **8**:17–21.
3. **Anderson, J. M., and A. C. Baird-Parker.** 1975. A rapid and direct plate method for enumerating *Escherichia coli* biotype I in food. J. Appl. Bacteriol. **39**:111–117.
4. **Barry, A. L.** 1977. Standardized disc diffusion methods of antimicrobic susceptibility testing of common rapid-growing bacterial pathogens, p. 9–10. *In* A. Bondi, J. T. Bartola, and J. E. Prier (ed.), The clinical laboratory as an aid in chemotherapy of infectious disease. University Park Press, Baltimore.
5. **Blake, R. L., and E. Kun.** 1971. Methods of enzyme induction by nicotinamide. Methods Enzymol. **18b**:113–122.
6. **Bovarnick, M.** 1965. Formic hydrogenlyase from *Escherichia coli*. Methods Enzymol. **1**:539–542.
7. **Calcott, P. H.** 1978. Freezing and thawing microbes, p. 67. Meadowfield Press, Ltd., Bushey, England.
8. **Clark, C. W., and Z. J. Ordal.** 1969. Thermal injury and recovery of *Salmonella typhimurium* and its effect on enumeration procedures. Appl. Microbiol. **18**:332–336.
9. **Dawes, E. A., D. J. McGill, and M. Midgley.** 1971. Analysis of fermentative products, p. 53–216. *In* Methods in microbiology, vol. 6A. J. R. Norris and D. W. Ribbons, (ed.), Academic Press,

Inc., New York.
10. **Dufour, A. P.** 1977. *Escherichia coli*: the fecal coliform, p. 48–58. *In* W. W. Hoadley and B. J. Dutka (ed.), Bacterial indicators/health hazards associated with water. American Society for Testing and Materials, Philadelphia.
11. **Ewing, W. H.** 1972. The nomenclature of *Salmonella*, its usage, and definitions for the three species. Can. J. Microbiol. **18**:1629–1637.
12. **Fishbein, M., and B. F. Surkiewicz.** 1964. Comparison of the recovery of *Escherichia coli* from frozen foods and nutmeats by confirmatory incubation in EC medium at 44.5 and 45.5°C. Appl. Microbiol. **12**:127–131.
13. **International Commission on Microbial Specifications for Foods.** 1978. Microorganisms in foods: their significance and methods of enumeration, vol. 1, 2nd ed., p. 434. University of Toronto Press, Toronto.
14. **Kasweck, K. L., and C. B. Fliermans.** 1978. Lactose variability of *Escherichia coli* in thermally stressed reactor effluent waters. Appl. Environ. Microbiol. **36**:739–746.
15. **Knox, W. E., M. M. Piras, and K. Tokuyama.** 1966. Tryptophan pyrrolase of liver. J. Biol. Chem. **241**:297–303.
16. **Lapage, S. P., J. E. Shelton, T. G. Mitchell, and A. R. Mackenzie.** 1970. Culture collections and the preservation of bacteria, p. 135–228. *In* J. R. Norris and D. W. Ribbons (ed.), Methods in microbiology, vol. 3A. Academic Press, Inc., New York.
17. **Leclerc, H., D. A. Mossel, P. A. Trinel, and F. Gavini.** 1977. Microbiological monitoring a new test for fecal contamination, p. 21–31. *In* W. W. Hoadley and B. J. Dutka (ed.), Bacterial indicators/health hazards associated with water. American Society for Testing and Materials, Philadelphia.
18. **Mehlman, I. J., and A. Romero.** 1982. Enteropathogenic *Escherichia coli*: methods for recovery from foods. Food Technol. **72**:73–79.
19. **Mehlman, I. J., N. T. Simon, A. C. Sanders, and J. C. Olson, Jr.** 1974. Problems in the recovery and identification of enteropathogenic *Escherichia coli* from foods. J. Milk Food Technol. **37**:350–356.
20. **Newton, W. A., and E. E. Snell.** 1962. Inducible tryptophan synthesis in tryptophan auxotrophs of *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A. **48**:1431–1438.
21. **Patterson, T. E., and H. Jackson.** 1979. Effect of storage at 1° and 4°C on viability and injury of *Staphylococcus aureus*, *Escherichia coli* and *Streptococcus faecalis*. J. Appl. Bacteriol. **46**:161–167.
22. **Pellon, J. R., and R. F. Gomez.** 1981. Repair of thermal damage to the *Escherichia coli* nucleoid. J. Bacteriol. **145**:1456–1458.
23. **Pellon, J. R., K. M. Ulmer, and R. F. Gomez.** 1980. Heat damage to the folded chromosome of *Escherichia coli* K-12. Appl. Environ. Microbiol. **40**:358–364.
24. **Rayman, M. K., G. A. Jarvis, C. M. Davidson, S. Long, J. M. Allen, T. Tong, P. Dodsworth, S. McLaughlin, S. Greensburg, B. G. Shaw, H. J. Beckers, S. Qvist, P. M. Nottingham, and B. J. Stewart.** 1979. ICMSF methods studies. XIII. An international comparative study of the MPN procedure and the Anderson-Baird-Parker direct plating method for the enumeration of *Escherichia coli* biotype I in raw meats. Can. J. Microbiol. **25**:1321–1327.
25. **Speck, M. L., and B. Ray.** 1977. Effects of freezing and storage on microorganisms in frozen foods: a review. J. Food Prot. **40**:333–336.
26. **Stiles, M. E., and Ng, L.-K.** 1980. Estimation of *Escherichia coli* in raw ground beef. Appl. Environ. Microbiol. **40**:346–351.
27. **Stiles, M. E., and L. D. Witter.** 1965. Thermal inactivation, heat injury, and recovery of *Staphylococcus aureus*. J. Dairy Sci. **48**:677–681.
28. **Stiles, M. E., L. A. Roth, and L. F. L. Clegg.** 1973. Heat injury and resuscitation of *Escherichia coli*. Can. Inst. Food Sci. Technol. J. **6**:226–229.
29. **Tomlins, R. I., M. Yoh, Y. Takeda, and T. Miwatant.** 1971. Effect of thermal injury on the TCA cycle enzymes of *Staphylococcus aureus* MF 31 and *Salmonella typhimurium* 7136. Can. J. Microbiol. **17**:759–765.