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Loss of Plasmids During Enrichment for *Escherichia coli*

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Enrichment with sodium lauryl sulfate and incubation at 44.5°C resulted in a loss of plasmids and decreased efficiency in the recovery of pathogenic *Escherichia coli* strains from foods.

When foods are tested for contamination with *Escherichia coli*, the contaminant is often overgrown on the culture medium by the microflora indigenous to the food. Rigorous enrichment conditions are commonly used to prevent this overgrowth and thus enhance the recovery of *E. coli* (8, 9). A standard enrichment procedure now in use involves two steps: inclusion of a surface-active agent in the medium (the detergent sodium lauryl sulfate) to prevent the multiplication of the many gram-positive species and a subsequent incubation at 44.5°C to inhibit the growth of most gram-negative bacteria other than *E. coli*. However, detergents and high temperatures could also favor the recovery of cells which have lost plasmids (1, 6, 12). Because the genetic determinants for several virulence factors of *E. coli* are located on plasmids (4, 5, 13, 14), the effect of this enrichment procedure on the maintenance of plasmids in several strains of *E. coli* was examined.

Four strains of *E. coli* isolated from human cases of gastroenteritis were used in this study. Three of these isolates (35897, TD412C1, and

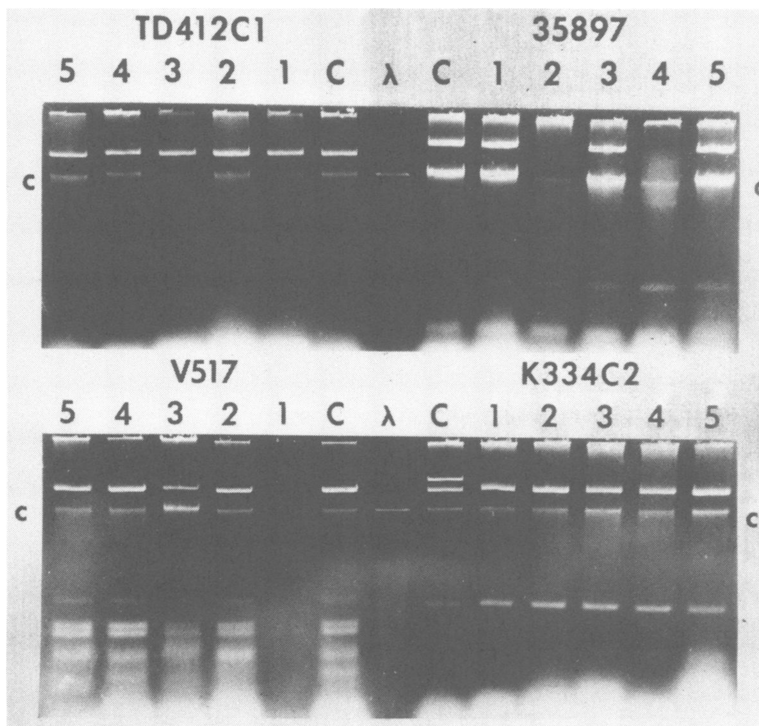

FIG. 1. *Agarose gel electrophoresis of E. coli clones after two-step enrichment procedure. Clones in lanes 1 to 5 were derived from the enrichment procedure. C, Untreated control clones; λ, linear bacteriophage lambda DNA as a marker; c, location of residual chromosomal deoxyribonucleic acid.*

K334C2) were donated by R. B. Sack (11). Strain V517 was provided by D. J. Kopecko (7). Assay for the heat-labile toxin (LT) was done with Y-1 mouse adrenal cells (8, 9); the heat-stable toxin (ST) was determined by the suckling mouse method of Dean et al. (3). Strain TD412C1 produced both ST and LT; strain 35897 produced only ST; strain V517 produced only LT; strain K334C2 did not product LT or ST.

During these experiments only pure cultures were used. A loopful from each frozen stock (thawed from −70°C storage) was inoculated into 5 ml of tryptic soy broth (Difco Laboratories, Detroit, Mich.), which was then incubated overnight at 37°C. Each culture was streaked onto Levine eosin methylene blue plates (Difco). An isolated colony was transferred into 5 ml of tryptic soy broth, incubated overnight at 37°C, and frozen at −70°C in tryptic soy broth-10% glycerol. These samples served as controls. A loopful of the sample was then subcultured in 5 ml of lauryl sulfate-tryptose broth (8) and incubated for 48 h at 37°C. After incubation, a loopful of each culture was transferred into 5 ml of EC broth (8) and incubated for 48 h at 44.5°C. When the resulting cultures had been streaked onto Levine eosin methylene blue plates, five isolated colonies were picked and treated as described above for the controls.

To examine plasmids, 0.1 ml of frozen culture was inoculated into 5 ml of tryptic soy broth and incubated overnight at 37°C; lysates were prepared by the procedure of Birnboim and Doly (2). A 25-μl sample of lysate was mixed with 5 μl of Endo R Stop Solution (Bethesda Research Laboratories, Rockville, Md.) and applied to a 0.7% agarose gel. Electrophoresis of the lysates continued for 3 h at 70 V in a buffer containing 89 mM tris(hydroxymethyl)aminomethane, 8.9 mM boric acid, and 2.5 mM ethylenediaminetetraacetic acid at pH 8.2 (10).

After the strains had been subjected to the lauryl sulfate-tryptose broth and the 44.5°C enrichment procedure, five clones of each *E. coli* strain were examined for the presence of plasmids (Fig. 1) and the ability to produce the two toxins, LT and ST (Table 1). Results, as compared with the controls, showed that the large plasmid in strain TD412C1 was maintained during the enrichment and that all clones from the enrichment still produced both LT and ST. After enrichment, isolates 2 and 4 of strain 35897 lost both the large plasmid and the ability to produce ST, suggesting that some function required for the expression of this toxin is carried by this plasmid. All isolates of V517 lost the ability to produce detectable amounts of LT after enrichment; however, it is difficult to associate this loss with that of a particular plasmid.

TABLE 1. *Toxin tests on E. coli strains after enrichment in sodium lauryl sulfate and incubation at 44.5°C*

Strain	Isolate	LT[a]	ST[b]
TD412C1	Control	+	+
	1	+	+
	2	+	+
	3	+	+
	4	+	+
	5	+	+
35897	Control	−	+
	1	−	+
	2	−	−
	3	−	+
	4	−	−
	5	−	+
V517	Control	+	−
	1	−	−
	2	−	−
	3	−	−
	4	−	−
	5	−	−
K334C2	Control	−	−
	1	−	−
	2	−	−
	3	−	−
	4	−	−
	5	−	−

[a] More than 50% of cells exhibit rounding characteristic of the LT effect (8).

[b] Gut-to-carcass ratio > 0.083 (3).

A drastic reduction in plasmid copy number could be responsible for this apparent loss of LT production. Although strain K334C2 is not a toxin producer, all five isolates no longer contained the larger of the two plasmids which migrate slowly compared with residual chromosomal deoxyribonucleic acid.

From these results we conclude that an enrichment procedure for *E. coli* based on a surface-active agent (the detergent sodium lauryl sulfate) and a high incubation temperature (44.5°C) can decrease the probability of recovering strains which harbor their original complements of plasmids. Since the genetic determinants of some virulence factors may reside on these extrachromosomal deoxyribonucleic acid molecules (4, 5, 13, 14), such an enrichment can significantly reduce our ability to detect pathogenic strains of *E. coli* in foods. Experiments are being conducted to evaluate the relative roles of detergent and high temperature in the loss of plasmids by *E. coli*. New enrichment and detection schemes that will ensure efficient recovery from foods of bacterial strains carrying pathogenic determinants are being sought.

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