R-Factor Transfer in Selenite and Tetrathionate Broths

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After overnight incubation of R+ Escherichia coli with R− Salmonella typhimurium in selenite and tetrathionate with Brilliant Green (TBG) broths, R-factor transfer was demonstrated in 10 of 12 experiments. R-factor transfer in these enrichment broths occurred at a markedly reduced frequency in comparison to that in Trypticase soy broth, apparently due to an adverse effect either on the viability of the donor E. coli or the conjugation process itself. Transfer of R factors in commonly used enrichment broths may give rise to falsely resistant antibiotic patterns in Salmonella. However, the frequency of R-factor transfer is so low that it is unlikely to affect significantly the interpretation of R-factor studies.

The evaluation of the public health significance of resistance (R) factors has been complicated by conflicting results regarding the frequency of in vivo transfer of R factors in the intestinal tract of man and animals and also by marked variation in the reported incidence of R factor-containing Enterobacteriaceae, especially Salmonella and Shigella, from clinical sources (1, 2, 5, 8, 10). Since the frequency of R-factor transfer is affected by a number of environmental factors such as temperature, acidity, bile salts, and anaerobic conditions (10), the differing methodologies employed in studies of R factors may be important variables to consider. Jarolmen and Kemp (5), in describing an animal model for the study of R-factor transfer in vivo, found that, although little transfer from R+ Escherichia coli to R− Salmonella could be demonstrated when feces were directly cultured on selective agar plates, overnight incubation of fecal specimens in nutrient broth allowed significant R-factor transfer with resultant R+ Salmonella. Studies of the efficiency of R-factor transfer in selective media commonly used in diagnostic laboratories have been few (2, 9). In addition to primary plating of stool specimens, it is standard practice in diagnostic bacteriology laboratories to incubate stool specimens overnight in tetrathionate or selenite broth to enrich for Salmonella prior to culturing on agar plates (3). Interspecies transfer of R factors during this enrichment incubation would result in the expression of antibiotic resistance in organisms which were initially antibiotic-sensitive. The current investigation was carried out to evaluate if, and to what extent, R-factor transfer can occur in tetrathionate and selenite broths.

MATERIALS AND METHODS

Bacteria. Twelve strains of E. coli isolated from clinical specimens and carrying R factors mediating multiple resistances, including tetracycline, were used as the donors of the R factors. The recipient strain was Salmonella typhimurium LT 2-1, a spontaneous mutant selected for resistance to > 100 µg of nalidixic acid per ml.

Media. Trypticase soy (BBL), tetrathionate (BBL) with Brilliant Green (TBG; 3), and selenite (Difco) were the broths used; the solid media were MacConkey (factor), and Levin eosin-methylene blue-lactose-agar. When added to the latter media, antibiotic agents were at concentrations used previously (7).

Procedures. The mixed cultures for bacterial mating were initiated by adding 0.3 ml of an overnight Trypticase soy broth culture of R+ E. coli (approximately 106 bacteria) and 0.1 ml of a Trypticase soy broth culture of S. typhimurium LT 2-1 in midexponential growth (approximately 2 × 108 bacteria) to 5 ml of each of the test media in test tubes (18 by 150 mm). The mixed cultures were incubated stationary at 37 C. Samples were removed at 0 and 19 hr, diluted where necessary, and plated for total numbers of viable E. coli on MacConkey agar containing 25 µg of tetracycline (Tet) per ml, for total S. typhimurium on MacConkey agar containing 50 µg of nalidixic acid (Nal) per ml, and for R+ S. typhimurium on MacConkey agar containing 50 µg of Nal per ml and 25 µg of Tet per ml. Colonies were counted after a 19-hr incubation period at 37 C. Random colonies on the MacConkey-Nal-Tet medium were

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tested for patterns of drug resistance as described (7). The ratio of E. coli to S. typhimurium used to initiate the mating mixture was selected after preliminary results with one of the E. coli test strains indicated that the rates of R-factor transfer under these conditions were equal to, or greater than, those observed with each of several other tested ratios.

Spontaneous loss of R factors was assessed by incubating bacteria in the test media overnight, diluting in buffer, plating approximately 100 clones on MacConkey agar, and, after an overnight incubation, replicating the colonies to drug-containing MacConkey agar (7).

RESULTS

Attempts to transfer R-factors from 12 E. coli strains to S. typhimurium during overnight incubation in Trypticase, selenite, and TBG broths are compared in Table 1. In Trypticase soy broth, R-factor transfer occurred with all mating mixtures, although the number of R+ Salmonella present (5 \( \times \) 10^4 to > 10^9/ml) after 19 to 24 hr of incubation varied widely with the E. coli used as the R-factor donor (10). Small differences were noted when the same experiment was repeated. R-factor transfer could be demonstrated in either selenite or TBG broths in 10 of the 12 mating mixtures. The number of R+ Salmonella recovered from selenite or TBG broth was 10^6 to 10^8 less than in Trypticase soy broth. The same mating mixtures showed considerable variation in results, especially when infrequent transfer was demonstrated. When R-factor transfer occurred in one enrichment broth, it generally could be demonstrated in the other. However, for two mating mixtures, R-factor transfer was demonstrated in selenite but not in TBG broth, and for two other mating mixtures the converse was true.

The observed decrease in recovery of R+ Salmonella after incubation of the mating mixtures in selenite or TBG broth might be explained by any of the following possible effects of the broths: (i) increased rate of segregation of R factors from S. typhimurium; (ii) selective inhibi-

| Escherichia coli donors | Expt | Trypticase soy | Selenite | TBG^a |
|------------------------|------|---------------|----------|--------|
| CI 72                  | 1    | 2 \( \times \) 10^4-3 \( \times \) 10^6 | 10^3     | <10^4  |
| CI 92                  | 1    | 1.7 \( \times \) 10^6 | 8 \( \times \) 10^3 | 2 \( \times \) 10^1 |
| CI 93                  | 3    | 2.7 \( \times \) 10^6 | 10^1     | <10^4  |
| CI 94                  | 1    | 10^-<10^4 | 1.5 \( \times \) 10^4 | 2.7 \( \times \) 10^4 |
| CI 98                  | 2    | 1.3 \( \times \) 10^4 | 4.3 \( \times \) 10^3 | 6 \( \times \) 10^3 |
| CI 100                 | 3    | 1.1 \( \times \) 10^4 | <10^4    | <10^4  |
| CI 112                 | 3    | 6.9 \( \times \) 10^4 | 10^1     | 10^4   |
| CI 126                 | 3    | 10^4-10^5 | 2.7 \( \times \) 10^4 | 5.2 \( \times \) 10^3 |
| CI 134                 | 3    | 3.6 \( \times \) 10^4 | 2.8 \( \times \) 10^3 | 1.8 \( \times \) 10^3 |
| CI 158                 | 3    | 1.3 \( \times \) 10^4 | <10^4    | 10^1   |
| CI 163                 | 3    | 1.4 \( \times \) 10^4 | <10^4    | 4 \( \times \) 10^4 |
| CI 164                 | 3    | 2.3 \( \times \) 10^4 | <10^4    | <10^4  |
| CI 181                 | 3    | 7.5 \( \times \) 10^4 | <10^4    | <10^4  |
| CI 192                 | 3    | 8 \( \times \) 10^4 | <10^4    | <10^4  |
| CI 193                 | 3    | 4.3 \( \times \) 10^4 | <10^4    | <10^4  |
| CI 194                 | 3    | 3.7 \( \times \) 10^4 | 10^1     | 7 \( \times \) 10^1 |
| CI 195                 | 3    | 10^4     | <10^4    | <10^4  |
| CI 196                 | 3    | 1.4 \( \times \) 10^4 | 10^1     | <10^4  |
| CI 197                 | 3    | 2 \( \times \) 10^4 | <10^4    | <10^4  |
| CI 198                 | 3    | 1.2 \( \times \) 10^4 | <10^4    | <10^4  |
| CI 199                 | 3    | 2.0 \( \times \) 10^4 | <10^4    | <10^4  |
| CI 200                 | 3    | 2.1 \( \times \) 10^5 | 1.2 \( \times \) 10^4 | 3.2 \( \times \) 10^3 |

* Number of R+ S. typhimurium per ml in mating mixture after 19 to 24 hr of incubation.
* TBG, tetrathionate with Brilliant Green.

| Strains          | No. of colonies in^a |
|------------------|----------------------|
|                  | Trypticase soy | Selenite | TBG^b |
| Escherichia coli | CI-98............ | 434/434 | 343/343 | 278/278 |
| Salmonella typhi- | 862/864 | 826/828 | 725/727 |
| murium R-98...... | 826/828 | 725/727 |

* Number of colonies retaining resistance/number tested.
* TBG, tetrathionate with Brilliant Green.

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TABLE 1. Relation of R-factor transfer to media R+ Salmonella typhimurium after conjugation

TABLE 2. Relation of R-factor segregation to culture media
tion of the growth of R\(^{+}\) *Salmonella*; or (iii) inhibition of the transfer of R factors.

The first possibility was excluded as a significant variable by the following experiment. R\(^{+}\) *S. typhimurium* cells, isolated during the first series of experiments, were recultured in the Trypticase soy, selenite, or TBG broth and restested for antibiotic resistance by replica plating. Low rates (< 1\%) of spontaneous R-factor loss were observed in all three media (Table 2). When large inocula of bacteria were incubated in TBG or selenite broths, the viable count of R\(^{+}\) and R\(^{-}\) *S. typhimurium* remained at approximately the same level for 48 hr, whereas those of E. coli gradually decreased (Table 3). Therefore, inhibition of the transfer of R factors in enrichment broth appears the most likely explanation for the observed results.

**DISCUSSION**

Although selenite and TBG broths suppress R-factor transfer, it is apparent that R-factor transfer does occur, albeit at markedly reduced rates, in these media. These experiments have not delineated the mechanism by which these media suppress R-factor transfer, but an adverse effect, either on the viability of the donor *E. coli*, the conjugation process itself, or both, seems probable. The possibility that these observations were due to R-factor segregation was excluded since R\(^{+}\) *E. coli* and R\(^{+}\) *S. typhimurium* retained their resistance patterns when grown in selenite and TBG broths. Selenite and TBG broths did not selectively inhibit growth of R\(^{+}\) *Salmonella*.

Incubation of stool specimens in enrichment broth is reported to increase the yield of *Salmonella* isolates by as much as 164 to 500\% (4, 6). Therefore, most of the antibiotic-sensitivity patterns of *Salmonella* isolated from clinical specimens are determined on isolates recovered after overnight incubation in enrichment broth. The acquisition of an R factor by previously sensitive *Salmonella* might result in "false resistance" when antibiotic sensitivities are determined. However, the low number of recipient R\(^{+}\) *Salmonella* (10\(^{6}\) to 10\(^{7}\)) compared to the high number of R\(^{-}\) *Salmonella* (10\(^{9}\) to 10\(^{10}\)) in enrichment media after 19 to 24 hr indicates that the chances of selecting a falsely resistant colony for sensitivity testing are very small. Therefore, although investigators attempting to demonstrate in vivo transfer of R factors in the intestinal tracts of man or animals must be aware of possible in vitro transfer of R factors in the processing of individual specimens, it is unlikely that the low frequency of R-factor transfer observed in these experiments will affect significantly the overall interpretation of R-factor studies.

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**TABLE 3. Effect of culture media on the viability of R\(^{+}\) and R\(^{-}\) *Salmonella typhimurium* and *R*\(^{+}\) *Escherichia coli***

| Strains               | Incubation time (hr) | Trypticase soy | Selenite | TBG* |
|-----------------------|----------------------|----------------|----------|------|
| *S. typhimurium* LT 2-1 | 0                    | 2 × 10^7       | 1.8 × 10^7 | 1.5 × 10^7 |
|                       | 24                   | 2 × 10^7       | 4.4 × 10^7 | 5.1 × 10^7 |
| *S. typhimurium* LT 2-1/R-98 | 0                  | 4.2 × 10^7     | 2.7 × 10^7 | 3.2 × 10^7 |
|                       | 24                   | 3.1 × 10^7     | 7.0 × 10^7 | 1.5 × 10^8 |
| *E. coli* CI-98       | 0                    | 1.5 × 10^7     | 1.5 × 10^7 | 10^7  |
|                       | 24                   | 8.1 × 10^8     | 4.1 × 10^4 | 2.1 × 10^4 |

* TBG, tetrathionate with Brilliant Green.