SHORT COMMUNICATIONS

Anaerobic R Factor Transfer in *Escherichia coli*

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**INTRODUCTION**

R factor mediated transfer of antibiotic resistance between Enterobacteriaceae has been reported to occur in the mammalian gastrointestinal tract (Kasuya, 1964; Reed, Sieckmann & Georgi, 1969; Guinée, 1970; Wiedemann, Knothe & Doll, 1970) where conditions are mainly anaerobic as demonstrated by the abundance of obligate anaerobic bacteria such as Bacteroides species (Moore, Cato & Holdeman, 1969) and direct studies of intestinal gas composition (Askevold, 1956). However, most laboratory investigations of the incidence of R factors and their transfer frequencies have been performed under aerobic conditions using faecal facultative strains. The only investigation of resistance transfer under anaerobic conditions in vitro is that of Mitsuhashi (1965), who reported complete inhibition of transfer of an R factor from a *Shigella flexneri* donor to an *Escherichia coli* recipient. On the basis of this result, it may be questioned whether in vivo R factor transfer is in fact possible (Chabbert, Baudens & Bouanchaud, 1969).

Fisher (1957) reported restriction of chromosomal transfer by an *E. coli* Hfr strain under anaerobic conditions in various minimal media. However, it has recently been shown by Stallions & Curtiss (1972) that anaerobic chromosomal transfer by an *E. coli* Hfr strain can occur at a high frequency provided that the mating medium is nutritionally rich. This gives rise to a contradiction, since Mitsuhashi (1965) used brain heart infusion broth, a rich medium, yet found inhibition of R factor transfer. This puzzling situation prompted a re-examination of R factor transfer under anaerobic conditions by facultative faecal *E. coli* strains in a rich medium.

Fisher (1957) and Mitsuhashi (1965) obtained anaerobic conditions by evacuation. Stallions & Curtiss (1972) flushed with N₂ for the duration of the mating. In this investigation, both mating and selection of recombinants were performed under stringent anaerobic conditions using methods developed for the isolation of obligate anaerobes (Hungate, 1969) to obtain a degree of anaerobiosis similar to that found in vivo.

**METHODS**

*Bacteria.* The following abbreviations are used for genetic markers and drugs: amp (ampicillin), cml (chloramphenicol) and nal (nalidixic acid).

Three *Escherichia coli* strains were isolated from human faecal specimens: two R factor-containing strains A₁ (amp<sup>+</sup>, cml<sup>-</sup>), A₂ (amp<sup>-</sup>, cml<sup>+</sup>) and a recipient strain B (nal<sup>-</sup>). An *E. coli* ps–r (amp<sup>+</sup>) strain contained an R factor originally derived from a faecal *Pseudomonas aeruginosa* strain.
Table 1. *R* factor transfer frequencies 6 h after mixing donor and recipient strains

Frequencies were calculated as the number of *nal*<sup>+</sup> *amp*<sup>+</sup> recombinants/donor cell added.

<table>
<thead>
<tr>
<th>Cross</th>
<th>Aerobic mating</th>
<th>Stringent</th>
<th>Evacuated</th>
</tr>
</thead>
<tbody>
<tr>
<td>(A_1 \times B)</td>
<td>3.5 (\times 10^{-4})</td>
<td>2.4 (\times 10^{-5})</td>
<td>1.4 (\times 10^{-5})</td>
</tr>
<tr>
<td>(A_2 \times B)</td>
<td>7.0 (\times 10^{-4})</td>
<td>1.1 (\times 10^{-6})</td>
<td>7.2 (\times 10^{-7})</td>
</tr>
<tr>
<td>(PS-R \times B)</td>
<td>3.9 (\times 10^{-5})</td>
<td>3.6 (\times 10^{-6})</td>
<td>8.2 (\times 10^{-5})</td>
</tr>
</tbody>
</table>

Donor and recipient strains were in exponential phase prior to mating in crosses \(A_1 \times B\) and \(A_2 \times B\) and in stationary phase in cross \(PS-R \times B\). Selection of *nal*<sup>+</sup> *cml*<sup>+</sup> recombinants in crosses \(A_1 \times B\) and \(A_2 \times B\) yielded results similar to those above.

*Media.* The medium used for selection of drug-resistant recombinants was nutrient agar (Difco) containing (\(\mu\)g/ml): *amp*, 25; *cml*, 30; or *nal*, 30. Anaerobic nutrient agar was prepared by the methods of Hungate (1969). The medium contained 2 \(\mu\)g methylene blue/ml as a redox indicator, 1.1 mg sodium thioglycollate/ml as a reducing agent and 10 \(\mu\)g Silicollapse 5001 (I.C.I.)/ml as an antifoam agent. The medium was steamed until homogeneous and in the reduced state. Samples (4 ml) were dispensed into modified Astell roll tubes (Toerien & Siebert, 1967) and perfused for 7 min with \(O_2\)-free gas containing 98% \(N_2\) and 2% \(H_2\). After autoclaving, the tubes were maintained at 50°C and drug solutions previously perfused with the \(N_2-H_2\) gas mixture were added anaerobically to give the above concentrations.

*Aerobic mating experiments.* Exponential phase cultures were obtained by diluting 1 ml of a 12 h stationary phase culture with 9 ml nutrient broth (Difco) and incubating at 37°C for 90 min. Samples of donor (4 ml) and recipient (0.2 ml) cultures were mixed in 2 ml double strength nutrient broth. As methylene blue and sodium thioglycollate were present in the anaerobic mating mixture, these substances were also added to the unreduced aerobic medium. However, this medium was maintained in the oxidized state by aeration and the methylene blue remained unreduced (\(E_h > 5\) mV). After static incubation at 37°C for 6 h samples were withdrawn, diluted and plated on selective media.

*Anaerobic mating experiments.* The techniques of Hungate (1969) were used for all manipulations involving mating of donor and recipient strains under stringent anaerobic conditions. Samples were added to or withdrawn from Astell roll tubes by injecting through the rubber stoppers with 1 ml or 10 ml all-glass Luer Lock syringes fitted with 1 inch 21-gauge needles. Exponential phase cultures were obtained by diluting 1 ml of a 24 h stationary phase culture with 9 ml broth and incubating at 37°C for 7 h. Samples of donor (4 ml) and recipient (0.2 ml) cultures were mixed in 2 ml double strength reduced nutrient broth containing methylene blue and sodium thioglycollate. After incubation at 37°C for 6 h, samples were withdrawn and recombinants were selected anaerobically in Astell roll tubes. Tubes were discarded if the methylene blue was in the oxidized state.

*Evacuated mating experiments.* As a second method of obtaining anaerobic conditions, aerobic resting phase donor and recipient broth cultures were mixed as above and placed in anaerobic jars (Baird & Tatlock Ltd, London), which were immediately evacuated. After incubation at 37°C for 6 h, the jars were opened and the cultures were immediately diluted and plated on selective media.
RESULTS AND DISCUSSION

The values obtained for R factor transfer frequencies in the three aerobic matings (Table 1) are similar to those for repressed R factors in previous reports (Meynell & Datta, 1967; Watson, 1967; Yokota, Kasuga, Kaneko & Kuwahara, 1972). Transfer also occurred in each of these crosses under stringent anaerobic conditions and with evacuation of the mating mixture (Table 1). The result of cross P8-R × B shows that an R factor can be transferred at a similar frequency under aerobic and stringent anaerobic conditions. Anaerobiosis resulted in reduced transfer frequencies in crosses A1 × B (approximately tenfold lower) and A2 × B (approximately 1000-fold lower). The reason for these differences in response to anaerobic conditions is not known but is probably dependent in some way on the nature of the donor strains, since the same recipient was used in all matings.

This result differs from that of Mitsuhashi (1965) and provides support for previous reports of in vivo R factor transfer (Kasuya, 1964; Reed et al. 1969; Guinée, 1970; Wiedemann et al. 1970). We have also confirmed the results of Stallions & Curtiss (1972) by demonstrating that chromosomal transfer from Escherichia coli Hfr and F’ donors to an E. coli F− recipient can occur at similar frequencies under aerobic and our stringent anaerobic conditions (unpublished results). Our findings demonstrate that the anaerobic conditions prevailing in the intestinal tract are not a limiting factor for in vivo R factor transfer.

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REFERENCES


Short communication

