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 *Escherichia 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^r*, *cml^r*), A₂ (*amp^r*, *cml^r*) and a recipient strain B (*nal^r*). An *E. coli* PS-R (*amp^r*) 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 strainsFrequencies were calculated as the number of *nal^r amp^r* recombinants/donor cell added.

Cross	Transfer frequency		
	Aerobic mating	Anaerobic mating	
		Stringent	Evacuated
$A_1 \times B$	3.5×10^{-4}	2.4×10^{-5}	1.4×10^{-5}
$A_2 \times B$	7.0×10^{-4}	1.1×10^{-6}	7.2×10^{-7}
$PS-R \times B$	3.9×10^{-5}	3.6×10^{-5}	8.2×10^{-5}

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^r cml^r* 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 ($\mu\text{g/ml}$): amp, 25; cml, 30; or nal, 30. Anaerobic nutrient agar was prepared by the methods of Hungate (1969). The medium contained 2 μg methylene blue/ml as a redox indicator, 1.1 mg sodium thioglycollate/ml as a reducing agent and 10 μg Silicolapse 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 ($E_h > 5 \text{ mV}$). 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 $PS-R \times B$ 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 $A_1 \times B$ (approximately tenfold lower) and $A_2 \times B$ (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

- ASKEVOLD, F. (1956). Investigations on the influence of diet on the quantity and composition of intestinal gas in humans. *Scandinavian Journal of Clinical and Laboratory Investigation* **8**, 87-94.
- CHABBERT, Y. A., BAUDENS, J. G. & BOUANCHAUD, D. M. (1969). Medical aspects of transferable drug resistance. In *Bacterial Episomes and Plasmids*, p. 240. Edited by M. O'Connor and G. E. W. Wolstenholme. London: J. A. Churchill.
- FISHER, K. W. (1957). The role of the Krebs cycle in conjugation in *Escherichia coli* K-12. *Journal of General Microbiology* **16**, 120-135.
- GUINÉE, P. A. M. (1970). Resistance transfer to the resident intestinal *Escherichia coli* of rats. *Journal of Bacteriology* **102**, 291-292.
- HUNGATE, R. E. (1969). A roll tube method for cultivation of strict anaerobes. In *Methods in Microbiology*, vol. 3B, pp. 117-132. Edited by J. R. Norris and D. W. Ribbons. London: Academic Press.
- KASUYA, M. (1964). Transfer of drug resistance between enteric bacteria induced in the mouse intestine. *Journal of Bacteriology* **88**, 322-328.
- MEYNELL, E. & DATTA, N. (1967). Mutant drug resistant factors of high transmissibility. *Nature, London* **214**, 885-887.
- MITSUHASHI, S. (1965). Transmissible drug-resistance factor R. *Gunma Journal of Medical Science* **14**, 169-209.
- MOORE, W. E. C., CATO, E. P. & HOLDEMAN, L. V. (1969). Anaerobic bacteria of the gastrointestinal flora and their occurrence in clinical infections. *Journal of Infectious Diseases* **119**, 641-649.
- REED, N. D., SIECKMANN, D. G. & GEORGI, C. E. (1969). Transfer of infectious drug resistance in microbially defined mice. *Journal of Bacteriology* **100**, 22-26.
- STALLIONS, D. R. & CURTISS, R. (1972). Bacterial conjugation under anaerobic conditions. *Journal of Bacteriology* **111**, 294-295.
- TOERIEN, D. F. & SIEBERT, M. L. (1967). Modification of the Astell roll tube apparatus for the enumeration and cultivation of anaerobic bacteria. *Laboratory Practice* **16**, 320-322.

- WATSON, C. E. (1967). Infectious drug resistance in *Shigellae* in Cape Town. *South African Medical Journal* **41**, 728–731.
- WIEDEMANN, B., KNOTHE, H. & DOLL, E. (1970). Übertragung von R-Faktoren in der Darmflora des Menschen. *Zentralblatt für Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene* (Abteilung I. Originale) **213**, 183–193.
- YOKOTA, T., KASUGA, T., KANEKO, M. & KUWAHARA, S. (1972). Genetic behavior of R factors in *Vibrio cholerae*. *Journal of Bacteriology* **109**, 440–442.