

other oxidizable fuels from the anode. Actually, the presence of multiple fuels (with non-interfering reactions) would enhance the ultimate objective. The presence of a combination of the carbonaceous compounds of Table 2 and/or others would not serve as a limitation of application.

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Isolation from Faeces of Cells capable of Transferring R-factors at a Derepressed Rate

MUTANT strains of enteric bacteria that carry a derepressed R-factor have been isolated during laboratory experiments. These strains are characterized by the production of sex-pili by the majority of the cells in the culture and by the cells' ability to transfer their R-factors to R-recipients at high frequency. Transfer rates per donor cell of 10^{-1} to 10^{-2} often occur, as compared with a rate of transfer from a repressed strain of about 10^{-4} to 10^{-5} (refs. 1-3).

During a recent survey of antibiotic sensitivity patterns and incidence of R-factors among the bacteria of the gut flora of healthy children, we isolated a strain of *Escherichia coli* that was capable of transferring its R-factor to an R-recipient at a frequency about 1,000-fold higher than is normally encountered among natural isolates of R⁺ bacteria. This observation suggested that derepressed R⁺ strains might be encountered in natural conditions. Further, their high transfer potential might make them an important reservoir of transferable antibiotic resistance determinants in the gut.

Faeces from healthy children in the Bristol area were examined by estimating the total viable count in aerobic conditions in MacConkey's agar, and also the viable count of the organisms resistant to antibiotics incorporated into plates of MacConkey agar at a concentration of 25 µg/ml. The antibiotics were tetracycline, ampicillin and streptomycin. The resistant clones were then examined for their ability to act as donors of R-factors by a plate method which tests the transfer of R-factors quantitatively⁴.

So far, fifty-four faecal samples have been examined by these techniques and twenty-seven were found to contain resistant organisms. From these twenty-seven samples, thirty-seven resistant strains were isolated. Twenty-eight of these contained R-factors capable of transferring resistance to one or more of the antibiotics used in the original culture of organisms from the faeces. In all but one of the samples the resistant organisms seemed to be typical repressed R⁺ strains; that is, they acted as a source for transfer of the R-factor with a probability of less than 10^{-4} . This is a typical rate of transfer of a repressed

R-factor (see R-factor R1 in Table 1). But in one case, a tetracycline resistant strain was found to transfer its R-factor at high frequency. In this case the faecal sample contained 6×10^6 aerobic bacteria (capable of growing on MacConkey's agar) per g of faeces and, of these, 3×10^4 organisms/g were resistant to tetracycline. Further examination of three tetracycline resistant colonies from this sample showed that they were capable of transferring the tetracycline resistance gene to the recipient with an approximate frequency per donor cell of between 10^{-1} and 2×10^{-2} . Stock repressed and derepressed R-factors gave values of 5×10^{-5} and 10^{-1} respectively in the same conditions (see Table 1).

Table 1. STATISTICS OF RESISTANCE TRANSFER

Donor	Number of resistant cells tested (potential donors)	Number of recipients that received resistance	Frequency of transfer per donor cell
Stock culture containing factor R1 (repressed)	20,000	1	5×10^{-5}
Stock culture containing factor R1-19 (derepressed)	2,000	195	$\approx 1 \times 10^{-1}$
Culture C491 from faeces:			
Colony A	2,000	67	$\approx 3.5 \times 10^{-2}$
Colony B	2,000	46	2.3×10^{-2}
Colony C	2,000	170	8.5×10^{-2}

When cells of the derepressed isolate were plated on non-selective media, they gave rise to colonies of two sizes. Both types of colony still carried the R-factor, as could be shown by transfer of tetracycline resistance. Cells from the small colonies transferred with a frequency of about 5×10^{-2} and were still derepressed. Cells from the large colonies, however, transferred at a frequency per donor cell of about 10^{-5} and were therefore now carrying the R-factor in the repressed state. Recloning of the cells in the large and small types of colony showed that the small derepressed colonies always contained a large proportion of repressed cells which gave rise to large repressed colonies; on the other hand, colonies that were initially large could not be shown to contain any derepressed cells giving rise to small derepressed colonies. This behaviour meant that cultures of this naturally occurring derepressed strain tended to revert to the repressed state unless steps were taken continually to re-isolate derepressed variants.

All the experimental evidence available suggests that it is only the derepressed cells of an R⁺ culture that are capable of acting as donors in an R-factor transfer. The presence of a naturally occurring derepressed strain therefore provides a particularly potent reservoir of R-factors for transfer to any superinfecting pathogens. It is even possible that, in practice, only strains of organisms that are in this derepressed state are of clinical importance in infectious drug resistance.

We were unable to obtain more specimens from the child who produced this interesting strain, or specimens from other members of her family. Information from the child's general practitioner showed that she had recently been given a course of sulphonamides but had received no tetracycline.

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