HOST SPECIFICITY AND FERTILITY IN SALMONELLA TYPHIMURIUM LT7

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The relatively high frequency of recombinants observed when Escherichia coli Hfr is mated with Salmonella typhimurium LT7 mut (harboring a mutator gene) has been shown to depend upon the presence of fertile mutants, able to act as good recipients, among a majority of unfertile cells. The hybrids obtained from such crosses show a greatly improved fertility when re-crossed with E. coli (Miyake, I962). Using the replica plating technique, Miyake isolated fertility mutants (fer) which had never been hybridized.

Lederberg (I966) suggested that the fertility mutation could be the loss of a restriction normally exercised by S.typhimurium LT7 on the DNA of E.coli. This was based on the observation of several investigators that host-controlled restriction is not limited to phage DNA but also affects the DNA of the bacterial chromosome and episomes when transferred by conjugation or transduction (Arber, I964 ; Boyer, I965 ; Arber and Morse, I965).

" A priori," there is no reason to postulate that such a restriction would control the acceptance of Salmonella phage P22 as well as that of E.coli DNA, but the following observations stimulated the investigation reported here. Zinder (I960) observed that most lac^+ hybrids obtained from crosses between Hfr Cavalli and LT7 mut produce a P22 phage that undergoes host-controlled restriction when plated on LT7. Miyake (I962) noticed that P22 grown on fer mutant has a reduced e.o.p. on LT7 mut and LT7 mut^+. Both lac^+ hybrids (which must be fer mutants as well) and fer mutants isolated as such appear to have lost the ability to confer upon P22 a host-specificity that is normally conferred by the unfertile LT7, and LT7 appears to exercise an otherwise undetected restriction.

Genetical investigations in several systems of host-specificity have shown that mutants with impaired restriction capacity (r^-), frequently have impaired ability to confer host-specificity as well (m^-), (Glover, Schell, Symonds, Stacey, I963 ; Colson, Glover, Symonds, Stacey, I965 ; Wood, I966). These observations reported by Zinder (I960) and those by Miyake (I962) on the fer mutants of LT7 mut might thus reflect the presence of an r^-m^- mutation, the increased fertility of these strains being solely due to their inability to restrict E.coli DNA.

The experiments presented in this paper show that this hypothesis is valid for the LT7 mut fertility mutation, while results obtained with LT7 mut^+ suggest that others factors may be involved.

MATERIALS AND METHODS

Strains. Salmonella typhimurium LT7 mut^+ and LT7 mut, prototrophs, were obtained from Dr G. Meynell.
Escherichia coli K-12 donors were Hfr Cavalli met (order of transfer : lac - pro - leu) and W1655 met lac F-lac^+.
Phage P22, wild type, was obtained from Prof. P. Fredericq.
Bacterial crosses were performed at 37° by mixing 1-ml. samples of log. broth cultures of donor and recipient in large bottles, to allow good aeration without shaking. When the level of fertility was measured, the donor was diluted 1 : 10 before mixing , contact was limited to 45 min. and the mixture was exposed to a high multiplicity of phage T6 for 10 min. to kill the donor before plating on selective media.
Mutagenic treatment with nitrosoguanidine (N.T.G.) was performed by adding 30 μg./ml. of the drug to log cultures for 25 min. at 37°. The cells were then washed and diluted in broth.

RESULTS AND DISCUSSION

Fer mutants have been isolated in LT7 mut by replica plating, as described by Miyake (I962). Since Eisenstark (I965) obtained mutagen-induced hybridization between E.coli and S.typhimurium LT2, attempts were made to isolate fer mutants from N.T.G.-treated LT7 mut^+.
Such mutants were obtained only when an F-lac^+ donor was used instead of Hfr Cavalli.

On the other hand, non restricting (r^-) mutants were obtained by the method of sib-selection described by Glover and Colson,

(I965). A hybrid akin to those of Zinder (I960) was first isolated to provide a stock of P22 devoid of the normal host-specificity, in order to test for the presence of r^- mutants in populations. The e.o.p. of this phage was found to vary between $2x10^{-2}$ and $4x10^{-4}$ among twenty single cell-grown cultures of LT7 *mut* whereas its e.o.p. remained constant ($\pm$ $5x10^{-5}$) on LT7 *mut*$^+$. This variation was caused by the presence of r^- mutants induced by the *mut* gene : selecting the best-accepting cultures as starting points for sib-selection lines, a set of pure r^- mutants were isolated. Only one mutant clone was kept from each line. In LT7 *mut*$^+$, r^- mutants were isolated by the same method, except that the cultures were first subjected to N.T.G. treatment, which usually increased the acceptance of non-specific P22 to about $1x10^{-3}$.

Table 1

Host-specificity and fertility of r^- and *fer* mutants.

Mutant isolated	Host-specificity for P22	Fraction per male imput of inheritance of *lac*$^+$ from	
		Hfr Cav.	W1655 F-*lac*$^+$
LT7 *mut* r^-1	r^-m^-	$1.9x10^{-4}$	$5.9x10^{-4}$
LT7 *mut* r^-7	r^-m^-	$2.5x10^{-4}$	$6.4x10^{-4}$
LT7 *mut* r^-16	r^-m^-	$2.1x10^{-4}$	$1.6x10^{-3}$
LT7 *mut* r^-81	r^-m^-	$1.3x10^{-4}$	$7.7x10^{-4}$
LT7 *mut* r^-82	r^-m^+	$4.7x10^{-5}$	$1.1x10^{-4}$
LT7 *mut fer* 72	r^-m^-	$9.9x10^{-5}$	$6.4x10^{-4}$
LT7 *mut fer* 82	r^-m^-	$1.0x10^{-4}$	$8.2x10^{-4}$
LT7 *mut*$^+$$r^-$72	r^-m^-	$< 1x10^{-6}$	$6.4x10^{-4}$
LT7 *mut*$^+$$r^-$ I7I	r^-m^-	$< 1x10^{-6}$	$4.2x10^{-4}$
LT7 *mut*$^+$$r^-$ 603	r^-m^-	$< 1x10^{-6}$	$2.2x10^{-3}$
LT7 *mut*$^+$ *fer* 2	r^-m^-	$< 1x10^{-6}$	$1.0x10^{-3}$
Control strains			
LT7 *mut*	r^+m^+	$7x10^{-6}$	$4x10^{-5}$
LT7 *mut*$^+$	r^+m^+	$< 1x10^{-6}$	$< 1x10^{-6}$

The r^- and *fer* mutants were analysed for ability to accept non-specific P22 (restriction), specificity conferred to P22 (modification), acceptance of an F-*lac*$^+$ episome from *E.coli*, and capacity to yield *lac*$^+$ recombinants when crossed with a suitable *E.coli* Hfr. The results of these experiments are summarized in table 1. The three *fer* mutants isolated were

found to behave as r^-. Conversely, all r^- mutants isolated in LT7 *mut* showed the same fertility as the *fer* mutants. In all but one case (LT7 *mut* r^- 82), the mutants had lost both restriction and modification properties. These facts confirm the prediction that the fertility mutation in LT7 *mut* reflects the loss of a restriction function which normally acts on foreign DNA.

A surprising result is that r^- mutants of LT7 mut^+ showed no increase of fertility when crossed with *E.coli* Hfr, in spite of the evidence that they are able to conjugate and express the lac^+ phenotype as frequently as LT7 *mut* r^- does, when mixed with an F-lac^+ donor. This means that there is a fertility barrier in LT7 mut^+ for lac^+ recombinants, in addition to restriction, and that this barrier operates at a late stage in the process of recombinant formation.

REFERENCES

Arber, W., Virology, 23, 173 (1964).
Arber, W., and Morse, M.L., Genetics, 51, 137 (1965).
Boyer, H., J. Bacteriol., 88, 1652 (1964).
Colson, C., Glover, S.W., Symonds, N., and Stacey, K.A., Genetics, 52, 1043 (1965).
Eisenstark, A., Proc. Natl. Acad. Sci.U.S., 54, 117 (1965).
Glover, S.W., and Colson, C., Genet. Res. Camb., 7, 223 (1966).
Glover, S.W., Schell, J., Symonds, N., and Stacey, K.A., Genet. Res. Camb., 4, 480 (1963).
Lederberg, S., J. Bacteriol., 91, 1029 (1966).
Miyake, T., Genetics, 47, 1043 (1962).
Wood, W.B., J.Mol. Biol., 16, 118 (1966).
Zinder, N.D., Science, 131, 813 (1960).