Conjugation in *Escherichia coli*

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ABSTRACT

BOYER, HERBERT (Yale University, New Haven, Conn.). Conjugation in *Escherichia coli*. J. Bacteriol. 91:1767-1772. 1966.—The sex factor of *Escherichia coli* K-12 was introduced into an *E. coli* B/r strain by circumventing the host-controlled modification and restriction incompatibilities known to exist between these closely related strains. The sexual properties of the constructed F^+ B strain and its Hfr derivatives were examined. These studies showed that the *E. coli* strain B/r F^+ and Hfr derivatives are similar to the *E. coli* strain K-12 F^+ and Hfr derivatives. However, the site of sex factor integration was found to be dependent on the host genome.

Bacterial conjugation occurs in several genera, but most experimentation has involved *Escherichia coli* strain K-12 and several species of *Salmonella*. The establishment of sexuality in these organisms is brought about by the *E. coli* sex factor, F_1 . There are other *E. coli* strains, such as B, C, W, 15, and others, which are popular organisms for investigative purposes, and it is desirable to have independent conjugal systems for these organisms. It would seem a simple matter to transfer the sex factor to these strains and obtain the desired male derivatives. However, in some cases, host-controlled modification and restriction present some difficulty in achieving this (2, 3). This problem can be eliminated, and this report is concerned with the transfer of the *E. coli* strain K-12 sex factor (F_1) to *E. coli* strain B/r and the sexual properties of this strain and its Hfr derivatives.

MATERIALS AND METHODS

The *E. coli* strain B/r and the derivatives described here originated from a culture obtained from E. Englesberg, and will be referred to throughout the paper as *E. coli* B. The K-12 strains of *E. coli* were obtained from the culture collection of E. A. Adelberg. The MS2 male specific bacteriophage was originally obtained from John Clark. A complete list of the bacterial strains used here is presented in Table 1.

The media and culture methods are the same as those described or referred to in a previous publication (3). The interrupted mating experiments are adapted from the experiments of DeHaan and Gross (8). The matings between F^+ and F^- cultures consisted of mixing 5×10^8 F^+ cells from a log-phase culture and 2×10^9 F^- cells in a total volume of 5 ml of nutrient broth and incubating the cells for 1 hr at 37 C. The mating mixture was centrifuged, resus-

pended in an equal volume of minimal medium, and dilutions were made for plating.

The procedure for isolating Hfr derivatives from an F^+ population was described earlier (20).

Mutant strains were obtained by treatment of cultures with 2-aminopurine or 1-methyl-3-nitro-N-nitrosoguanidine (NG), plating survivors on enriched nutrient agar medium, and then replicating these clones to minimal medium. Mutagenesis by NG was carried out according to the procedure outlined by Adelberg, Mandel, and Chen (1). Those clones failing to grow on the minimal medium which could be identified with a response to a single growth factor were purified for further study.

The curing of sex factor by acridine orange treatment was described by Hirota (13).

Terminology for phenotypes and genotypes is that used by Eggertsson and Adelberg (10). That is, genotypes are given by three italicized lower case letters without a superscript designation. Phenotypes are given by three lower case letters, and a "+" superscript designates wild type and a "-" superscript designates the mutant configuration.

RESULTS

Transfer of the E. coli strain K-12 sex factor to E. coli strain B. The transfer of sex factor from an F^+ K-12 strain to an F^- K-12 (P1) strain, i.e., lysogenic for the P1 bacteriophage (2), or to *E. coli* strain B (3) is restricted. It is thought that in each case the sex factor is transferred to the F^- recipient but degraded in a manner analogous to the host-controlled degradation of the DNA of the bacteriophage λ (9).

DeHaan (5) first reported the transfer of the K-12 sex factor to *E. coli* strain B. However, the conditions used to obtain the F^+ derivative of strain B were rather undefined, requiring several

TABLE 1. *Bacterial strains**

Derivative no.	Restriction and modification properties	Sexual type	Phenotype												
			<i>try</i>	<i>gal</i>	<i>lac</i>	<i>pro</i>	<i>thr</i>	<i>leu</i>	<i>met</i>	<i>ade</i>	<i>val</i>	<i>arg</i>	<i>his</i>	<i>str</i>	<i>thi</i>
AB264	K-12	F ⁺	+	+	+	+	+	+	+	+	+	+	+	S	—
AB2101	K-12	F [—]	—	+	+	—	—	—	+	+	+	+	—	R	—
AB266	K-12	F [—]	+	—	—	—	—	—	+	+	+	+	+	R	—
AB2604	B	F ⁺	+	—	—	—	+	—	+	+	+	+	+	R	—
AC2516	B	F- <i>lac</i>	+	+	+	+	+	+	+	+	+	+	+	S	+
AC2511	B	F [—]	+	+	+	+	+	+	+	+	+	+	+	S	+
AC2519	B	F [—]	—	—	—	—	—	—	+	+	+	—	—	R	+
AC2520	B	F ⁺	+	+	+	+	+	+	+	+	+	+	+	S	+
AC2522	B	HfrB1	+	+	+	+	+	+	+	+	+	+	+	S	+
AC2524	B	HfrB3	+	+	+	+	+	+	+	+	+	+	+	S	+
AC2523	B	HfrB2	+	+	+	+	—	+	—	+	+	+	+	S	+
AC2521	K-12	F [—]	—	—	—	—	+	—	—	+	+	+	+	R	+
AC2525	B	F [—]	+	—	—	—	—	—	+	—	+	+	—	R	+
AC2526	B	F [—]	+	—	—	—	—	—	+	+	—	+	—	R	+

* Strain numbers prefixed with AB are K-12 strains; strain numbers prefixed with AC are non-K-12 strains. All AC strain numbers here are B, r strains. The following abbreviations are used in the table and the text: *try*, tryptophan; *gal*, galactose; *lac*, lactose; *pro*, proline; *thr*, threonine; *leu*, leucine; *met*, methionine; *ade*, adenine; *val*, valine; *arg*, arginine; *his*, histidine; *str*, streptomycin.

serial transfers of K-12 F⁺ and B F[—] mixtures; transfer of F⁺ was less efficient than in K-12 cultures and was obtained with a mutant derivative of strain B. The F⁺ derivative of *E. coli* B was competent as a male donor, but it was reported to be unstable (6). In addition, the results of conjugation experiments with this F⁺ strain were variable (7). In retrospect, many of these aberrations can be explained by the mechanisms mentioned above.

With the role of host-controlled restriction and modification on transfer of sex factor between strains of *E. coli* defined, and with the mapping of the genetic region responsible for host controlled modification and restriction in *E. coli* strains K-12 and B (3), the sex factor of *E. coli* strain K-12 was transferred to *E. coli* strain B with considerable ease.

The *E. coli* strain K-12 sex factor, F₁, was introduced into a multiply auxotrophic K-12 strain, AB266, by mating it with an F⁺ culture of K-12, AB264. An F[—] phenocopy of the F⁺ AB266 strain was then crossed to a B strain carrying F-*lac* (AC2516). This B strain will act as a genetic donor and transfers chromosome to female strains (3). Selection was made for *thr*⁺ from the B strain. The *thr*⁺ *leu*[—] *pro*[—] *lac*[—] *gal*[—] *thi*[—] *str*-r K-12 recombinants were tested for the presence of sex factor by spotting a few hundred MS2 phage particles on soft-agar layers containing an inoculum of the recombinant. Those recombinants that were still F⁺ were tested for their host-controlled restriction and modification properties by determining efficiencies of plating of

a λ lysate prepared from a K-12 strain that contained the K-12 restriction and modification properties and a λ lysate prepared from a K-12 strain constructed to have the B restriction and modification properties. About 30% of the *thr*⁺ recombinants had the *E. coli* strain B modification and restriction properties. One of these recombinants (AB2604) was then used to introduce the F₁ episome, now modified with the strain B specificities, into a prototrophic B strain. The transfer was accomplished by mixing equal volumes of the AB2604 and AC2511 cultures at a final titer of 5×10^8 cells per milliliter and incubating for 1 hr at 37 C. The mating was diluted and about 200 to 300 cells were deposited on an agar medium that would only support growth of the AC2511 strain. Several hundred clones were then picked to nutrient agar plates (50 per plate) and after 6 hr of incubation at 37 C were replicated to a minimal agar medium spread with a lawn of *E. coli* B (AC2519) which is multiply auxotrophic and *str*-r. Selection was made for *thr*⁺ and *leu*⁺ from the suspected male donors. Most of the positive tests consisted of only several recombinant colonies per replicated patch. One of these was purified and used as an F⁺ B strain (AC2520).

Fertility of the F⁺ B population. The F⁺ B strain was used as a male donor for a multiply marked B F[—] strain (AC2519). The relative positions of the loci on the B genome are presented in Fig. 1. No major deviations from the K-12 map have been detected. The results of these crosses are presented in Table 2. The F⁺ B male is just as

FIG. 1. Diagrammatic representation of the relative position of the markers used in this investigation. The order of chromosome transfer by the three Hfr derivatives is indicated.

TABLE 2. Conjugation frequencies with F^+ B cultures*

Recombinant class	Recombinants per ml/ F^+ cells per ml $\times 100\%$		
	AC2520/AC2519	AB264/AC2521	AB264/AB2102
<i>leu</i> ⁺	3×10^{-4}	40×10^{-4}	70×10^{-4}
<i>pro</i> ⁺	5×10^{-4}	20×10^{-4}	70×10^{-4}
<i>gal</i> ⁺	4×10^{-4}	1×10^{-4}	—
<i>try</i> ⁺	50×10^{-4}	1×10^{-4}	4×10^{-4}
<i>his</i> ⁺	2×10^{-4}	4×10^{-4}	5×10^{-4}
<i>arg</i> ⁺	3×10^{-4}	5×10^{-4}	—
<i>met</i> ⁺	3×10^{-4}	10×10^{-4}	—

* Comparison of F^+ B and F^+ K-12 crosses. Procedures for these crosses are described in Materials and Methods. These data represent the results of several experiments and are numerically averaged.

fertile as the F^+ K-12 male strains; however, there was a higher frequency for the *try*⁺ recombinant class. The K-12 strain carrying the same sex factor, AB264, was then used as a donor for two female strains. AB2102 is a K-12 F^- derivative and AC2521 is a B F^- derivative, isogenic to AC2519 but containing the K-12 restriction and modification region linked to *thr*. Both strains mate well with a K-12 male. These crosses were performed to see whether the *try*⁺ recombinant class was also much higher than other recombinant classes with this F^+ population as well as

with the B F^+ population. But, as seen from the data presented in Table 2, in both of these crosses the *leu*⁺ and *pro*⁺ recombinant classes occur more frequently than the others.

When the recombinants of the F^+ B $\times$ F^- B conjugation experiment were back-crossed to an appropriately marked F^- strain, 10 to 40% of the recombinants acted as low-frequency males and are presumed to carry the F_1 episome. So, as in K-12 crosses involving F^+ males, the sex factor is transferred to F^- cells during mating.

The sex factor can be eliminated by treatment of the F^+ culture of *E. coli* B with acridine orange.

Isolation of Hfr derivatives of the F^+ B strain. Hfr derivatives of the AC2520 strain were obtained after ultraviolet irradiation of the AC2520 strain to about 0.01% survival. The irradiated cells were plated on nutrient agar to give about 150 to 200 colonies per plate. After 16 hr of incubation, these plates were replicated to various selective media containing a lawn of about 10^9 cells of the AC2519 strain. It was not difficult to obtain Hfr males with this procedure.

Three distinct types of Hfr derivatives (Hfr B1, Hfr B2, and Hfr B3) have been isolated from the AC2520 F^+ B strain. Their order of chromosome transfer is indicated in Fig. 1. In the first attempt to obtain an Hfr derivative, selection was made for *thr*⁺ *leu*⁺, and the Hfr B1 type was obtained. This Hfr type was obtained at least once in each of six independent screening procedures with the use of several different selections, such as *thr*⁺ *leu*⁺, *ade*⁺, *his*⁻, *try*⁺, and *pro*⁺. The second type of Hfr, Hfr B2, was isolated by selecting for *ade*⁺ recombinants. This Hfr has two additional properties. (i) It has a requirement for L-methionine and L-threonine which can be transduced to

TABLE 3. Linkage analysis of unselected markers to selected markers obtained from a cross between AC2520 and AC2519*

Unselected phenotype	Selected phenotype						
	<i>try</i> ⁺	<i>gal</i> ⁺	<i>pro</i> ⁺	<i>leu</i> ⁺	<i>met</i> ⁺	<i>arg</i> ⁺	<i>his</i> ⁺
<i>try</i> ⁺	—	60	43	44	17	15	20
<i>gal</i> ⁺	28	—	54	41	15	8	1
<i>pro</i> ⁺	23	28	—	55	18	2	1
<i>leu</i> ⁺	24	18	50	—	19	3	1
<i>met</i> ⁺	6	6	10	16	—	5	1
<i>arg</i> ⁺	2	1	1	2	5	—	1
<i>his</i> ⁺	5	2	1	3	4	20	—

* In each case, 200 recombinant clones were picked to homologous medium, incubated, and then replicated to various selective media and scored for the unselected phenotype. Results are given as per cent.

the wild-type configuration by phage P1; however, the transductants are never males (D. Shepard, *personal communication*). (ii) This Hfr strain is not as stable as the others that have been isolated and must be checked routinely for male-ness. The third type of Hfr was predicted after examination of the linkage of markers in recombinants from the $F^+ B \times F^- B$ cross. Chromosome transfer in F^+ cultures is thought to occur by the rare Hfr derivatives that arise spontaneously in the F^+ population. Since a large percent-

age of his^+ recombinants were also try^+ (Table 3), it appeared that in contrast to the Hfr B1 type, which inserts his as a terminal marker, there was another type of Hfr in the F^+ population that inserted the chromosome in an order so that try and his were closely linked on the donor chromosome. By selecting for his^+ recombinants in the screening procedure, an Hfr of type 3, Hfr B3, was recovered.

The results of interrupted mating experiments with these Hfr derivatives and various $F^- B$

FIG. 2. Interrupted mating experiments with the Hfr derivatives of *Escherichia coli* B. (A) AC2522 with AC2519. (B) AC2523 with AC2519 for met^+ recombinants; AC2523 with AC2525 for ade^+ recombinants; AC2523 with AC2526 for val^+ recombinants. (C) AC2524 with AC2519.

derivatives are shown in Fig. 2. The recombinant frequencies for early recombinant classes in crosses with Hfr B1, Hfr B2, and Hfr B3 are similar to those of K-12 Hfr derivatives; i.e., up to 50% of the input donor cells can be recovered as recombinants.

In crosses with all three Hfr derivatives, none of the recombinants for early markers was a male donor. However, when terminal markers were selected from the Hfr B1 derivative (*his*⁺) of the Hfr B3 derivative (*gal*⁺), 40 to 60% of the recombinants were male donors of the Hfr type.

The Hfr derivatives are not curable of the F₁ episome by treatment with acridine orange.

It is worthy of mention that the MS2 bacteriophage does not form plaques on the F⁺, Hfr, and F-*lac* derivatives of the B/r strain. However, they are adsorbed from the supernatant fluid by those strains and appear to propagate in spite of the failure to form plaques. It is known that the F-*lac* derivative of the B strain has pili controlled by the sex factor, and other male-specific phages are known to adsorb to this appendage (4). These results are quite similar to those described with a *Proteus mirabilis* derivative carrying an F-merogenote originating in a K-12 strain (14).

Linkage analyses. The linkage analyses of unselected markers to selected markers from F⁺ crosses were carried out after the Hfr B1 male was found to occur frequently in the F⁺ population. If the spontaneous appearance of Hfr derivatives in the F⁺ culture is responsible for chromosome transfer, then one might expect that the Hfr B1 male would be the most predominant one present in the F⁺ culture. This is supported by high numbers of *try*⁺ recombinants obtained from F⁺ B × F⁻ B crosses and repeated isolation of this Hfr from the F⁺ culture. Secondly, one would expect that the linkage of unselected markers to selected markers would resemble that obtained from an Hfr B1 × F⁻ B mating. These data are compared in Tables 3 and 5. These linkage relationships are similar but not as similar as expected. One notable discrepancy, mentioned earlier, successfully predicted the occurrence of the Hfr B3 type of male in the F⁺ culture. These two types of Hfr derivatives may contribute the majority of recombinants in a cross between the F⁺ population and a recipient.

The linkage analysis was then carried out with recombinants from an F⁺ K-12 × F⁻ K-12 cross. A unique linkage pattern was found which, together with the recombination frequencies obtained from F⁺ K-12 × F⁻ K-12 crosses, supports the idea that, in this case, an Hfr similar to the Hfr P4X type (i.e., *lac* is the terminal marker) is occurring most frequently in the F⁺ population (Table 4). No attempt was made to determine the

TABLE 4. Linkage analysis of unselected markers to selected markers obtained from a cross between AB264 and two F⁻ derivatives*

Unselected phenotype	Selected phenotype					
	<i>try</i> ⁺	<i>pro</i> ⁺	<i>leu</i> ⁻	<i>met</i> ⁺	<i>arg</i> ⁺	<i>his</i> ⁺
<i>try</i> ⁺	—	<0.5	<0.5	<0.5	2	<0.5
<i>gal</i> ⁺	<0.5	<0.5	5	<0.5	<0.5	<0.5
<i>pro</i> ⁺	2	—	34, 59	6	<0.5	<0.5
<i>leu</i> ⁺	2, 2	59, 50	—	22	<0.5	<0.5
<i>met</i> ⁺	<0.5	14	21	—	<0.5	<0.5
<i>arg</i> ⁺	<0.5	2	<0.5	<0.5	—	<0.5
<i>his</i> ⁺	4, 4	<0.5	<0.5	<0.5	12	—

* The italicized data are from a cross between AB264 and AB2102; the other data are from a cross between AB264 and AC2521. Results are given as per cent.

TABLE 5. Linkage analysis of recombinants obtained from a cross between AC2522 and AC2519*

Unselected phenotype	Selected phenotype						
	<i>try</i> ⁺	<i>gal</i> ⁺	<i>pro</i> ⁺	<i>leu</i> ⁺	<i>met</i> ⁺	<i>arg</i> ⁺	<i>his</i> ⁺
<i>try</i> ⁺	—	54	45	44	37	26	9
<i>gal</i> ⁺	3	—	49	46	51	22	3
<i>pro</i> ⁺	<1	19	—	53	54	32	1
<i>leu</i> ⁺	<1	6	22	—	53	26	1
<i>met</i> ⁺	<1	<1	5	12	—	36	1
<i>arg</i> ⁺	<1	<1	<1	<1	2	—	4
<i>his</i> ⁺	<1	<1	<1	<1	<1	<1	—

* One hundred recombinants of each class were tested for unlinked markers by replica plating. Results are given as per cent.

Hfr types that could be obtained from this F⁺ culture.

DISCUSSION

Conjugation in various bacterial genera has been reported and is by no means limited to *E. coli*. Conjugation experiments in other enteric bacteria, namely, *Salmonella* and *Shigella*, are quite extensive (11, 15, 18, 19). The fertility of these strains relies on the sexual properties introduced with the *E. coli* K-12 sex factor. The sexual properties of the male derivatives of these genera are quite similar. It is therefore no surprise to find that the presence of the K-12 sex factor in the B strain of *E. coli* results in that derivative being very much like the K-12 male donors.

What is interesting is that the site where the sex factor integrates into the host genome varies with the host genome. Falkow and Citarella (12) showed that one-half of the sex factor is homolo-

gous to the *E. coli* K-12 genome. This homology is thought to be a determinant in the integration process. In *E. coli* K-12, some 17 sites of sex factor integration are known (16), but, as pointed out by Falkow and Citarella (12), one or two types of Hfr derivatives are the predominant isolates from an F⁺ population. The K-12 F⁺ strain (AB264) used here exemplifies this statement. The *E. coli* K-12 and B strains harboring the same sex factor behave as if the sex factor integrates at two different regions of the host genome. In both cases, one site of integration is predominant. In a *Salmonella* species, the K-12 sex factor integrates most frequently at still another region of the host genome (19) so that the *ilv* region is transferred as an early marker.

Integration of the sex factor with the bacterial chromosome is pictured as a recombinational event (17) which assumes homologous regions of DNA on both structures. The probability of integration at any one genomic site depends on the extent of homology that site has with the sex factor. The numerous sites of sex factor integration known in the *E. coli* K-12 genome may represent rare integration events obtained under rigorous selective pressures. The *E. coli* strain K-12 F⁺ male used here seems to spontaneously integrate its sex factor at one site on the chromosome.

These data suggest that the F₁ sex factor integrates at different sites in each of three hosts, *E. coli* strains K-12 and B and *Salmonella typhimurium*. The reason for this discrimination can only be the subject of speculation here, but there are two simple possibilities. (i) The preferred sites of integration in the host genomes are qualitatively different from one another, implying that an equal number of different sites are present on the sex factor. (ii) The sites are qualitatively identical but are located in different regions of the genome in the three organisms used here.

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