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Genetic Exchange Between *Escherichia coli* Strains in the Mouse Intestine

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Germ-free mice contaminated with selected *Escherichia coli* strains were used for experiments designed to demonstrate gene transfer and recombinant formation in vivo. The well-characterized conjugation system of *E. coli* K-12 was examined in these experiments. Contamination of germ-free mice with a polyauxotrophic F^- strain followed by the addition of isogenic Hfr, F′, or F^+ strains resulted in the appearance of all recombinant classes at frequencies that would be expected from an in vitro mating experiment. Inheritance of unselected donor markers occurred at frequencies that were dependent on linkage relationships established in experiments in vitro. The presence of *Lactobacillus* had no influence on gene transfer and recombinant formation in an F′ × F^- in vivo mating. The R factor ROR-1 was transferred from *E. coli* strain M7-18 to an *E. coli* F^- strain in the mouse intestine.

The question of how much gene flow takes place between bacterial species in nature and what significance it might have in the evolution of bacterial species is largely unanswered. In recent years, it has been shown that certain bacterial genera possess mechanisms for genetic exchange and recombinant formation (14). Conjugal transfer of genetic material in *Escherichia coli* K-12 and some related genera has been studied in great detail in vitro (6, 14, 15, 19, 21). The list of plasmids harbored by bacteria isolated from natural environments continues to increase (24). Some of these recently described plasmids include the genetic determinants for colicin production (25), multiple-drug resistance in enteric species (39) and staphylococci (26), suppressor and mutagenic factors (10), surface antigens (K88 factor; 27), production of enterotoxin (Ent factor; 33), and production of hemolysin (Hly factor; 33). Many of these plasmids can be conjugally transferred to recipient strains, and some act as fertility factors in being able to mediate chromosomal transfer at a low frequency (3, 35). If these plasmids are readily transferred in natural environments, they may be of great importance in the evolution of bacterial species. Aside from the more theoretical aspects of bacterial evolution, the dissemination of R factors among bacterial populations in nature may assume an important role in public health and clinical medicine.

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One difficulty in attempting to study gene transfer in nature is the microbial complexity of the ecological niches in which such transfer is likely to occur. One such niche is the mammalian intestinal tract. Many microorganisms representing diverse genera and species live in close association within the intestinal tract, providing the opportunity for genetic exchange. Certain bacterial genera, particularly members of the *Enterobacteriaceae*, which are common inhabitants of this niche are known to possess mechanisms for gene transfer (16).

Genetic transfer in vivo has been demonstrated by a number of workers (1, 11-13, 16-18, 28, 30, 32, 38), and our findings will be discussed in light of these previous studies. In the present investigation, genetically compatible *E. coli* K-12 strains were used to demonstrate gene transfer and recombinant formation in vivo. To minimize possible variables caused by other members of the resident microflora, specifically contaminated germ-free mice were used.

MATERIALS AND METHODS

Media. The formulas for the minimal media used in these experiments have been described (4). Minimal agar was prepared by adding double-strength liquid minimal medium to an equal volume of 3.0% melted agar. A carbon source, at 0.5% final concentration, and desired growth factor supplements were added to the minimal medium.

The EMB agar used contained: tryptone (Difco), 0.8%; yeast extract, 0.1%; NaCl, 0.5%; eosin Y,

0.04%; methylene blue, 0.0065%; and agar, 1.5%. Just before plates were poured, the desired sugar was added to give a 1.0% final concentration, and K_2HPO_4 was added to give a 0.2% final concentration (5).

The complex media used were L broth and L agar of Lennox (22), Penassay agar (Difco) containing 0.8% NaCl, Brain Heart Infusion Agar (Difco), and Rogosa SL agar (Difco).

The diluent used in most experiments was buffered saline (BSG) containing: NaCl, 0.85%; KH_2PO_4, 0.03%; Na_2HPO_4, 0.06%; and gelatin, 100 μg/ml (4).

Nutritional supplements were purchased from Calbiochem (Los Angeles, Calif.), and were used in the following concentrations (μg/ml): L-arginine HCl, 22; L-histidine HCl, 22; L-proline, 30; DL-threonine, 80; adenine, 40; uracil, 40; and thiamine HCl, 2. Streptomycin sulfate (E. R. Squibb and Sons, New York, N.Y.) and oxytetracycline (Chas. Pfizer & Co., Inc., Brooklyn, N.Y.) were used at 200 and 50 μg/ml, respectively.

Bacterial strains. The following strains were used: χ894 (Hfr OR74) *prototroph*, λ⁻, T6ˢ, *strˢ*; χ209 F⁺ *prototroph*, T6ˢ, λ⁻, *strˢ*; χ859 (ORF-210) F′ *prototroph*, T6ˢ, λ⁻, *strˢ*, *cycˢ*/F-*purE*⁺; and χ820 F⁻ *purE*⁻, T6ʳ, *proC*⁻, *thr*⁻, *cycʳ*, *xyl*⁻, *strʳ*, *his*⁻, *pyrC*⁻, or *pyrD*⁻ λ⁻. The above strains were all derivatives of *E. coli* K-12. Strain M-718 was an *arg*⁻, *mal*⁻ mutant of *E.coli* strain M7 which was isolated from a mouse colony at Jackson Laboratory, Bar Harbor, Me., by R. C. Allen. This strain carried an R factor (ROR-1) which conferred resistance to >200 μg of oxytetracycline per ml. Stock cultures of these strains were maintained on Penassay agar slants at 4 C. The *Lactobacillus* strain used was isolated from conventional mice at Oak Ridge. Titers of this strain were determined on Rogosa SL agar after incubation in torbal anaerobic jars (The Torsion Balance Co., Clifton, N.J.) with an atmosphere of 95% hydrogen and 5% CO_2.

Mice. The germ-free mice used for these experiments were from stocks (RFM/Wg) whose ancestors were Caesarean-derived and foster-nursed in a sterile environment, as described by Walburg (37). Germ-free mice were housed in the gnotobiotics facilities at Oak Ridge; the operation and maintenance of this facility have been described by Robie et al. (29).

Mating procedures. Matings in liquid medium for 90 min were used to determine gradients of donor chromosome transfer and to recover rare recombinant clones (8). Donor strains were grown in L broth without aeration to achieve maximal numbers and length of F pili per cell (7). Recipient strains were grown in L broth with aeration at 37 C. The donor-to-recipient ratio was usually between 1:10 and 1:20. After incubation, the mating mixture was diluted in BSG [containing 10% (v/v) L broth] and agitated on a vortex mixer for 30 sec to separate mating partners. Samples were then plated on selective media for the isolation of recombinant clones. Recombinant frequencies were calculated by dividing the recombinant titers by the titer of the donor parent at the commencement of mating. Plate matings as described by Berg and Curtiss (2) were used for rapid differentiation of Hfr and F⁺ cultures. The genotypes of the recombinants were determined after purification on the medium used to select the recombinant class. Unselected markers for nutritional requirements, fermentation, and antibiotic resistance were scored by cross-streaking or replica-plating (20) to appropriate selective media.

Contamination of germ-free mice. Overnight broth cultures (approximately 10^9 cells/ml) of the bacterial strains to be used for contamination were aseptically transferred to sterile vials. The vials were passed into polyvinyl chloride (PVC) plastic film isolators by use of the standard peracetic acid transfer technique (36). The mice in the isolator to be contaminated were placed in cages without bedding. Sterile food pellets placed in the cages were saturated with the contents of the vial. When most of the food had been consumed (2 to 3 hr), the mice were transferred to cages containing sterile food, water, and bedding, where they were held until ready for sampling.

Fecal samples. Mice were transferred to sterile empty cages (without food or water) and allowed to defecate. Fecal pellets were collected in sterile screw-capped vials. The vials were passed out of the isolators by the peracetic acid transfer technique. The fecal samples were immediately weighed, and an initial dilution of 1:10 (w/v) was made with BSG. The mixture was agitated on a vortex mixer (Lab-Line Instruments, Inc., Melrose Park, Ill.) for 2 to 3 min to disperse the fecal material. The samples were then serially diluted in BSG, and appropriate dilutions were plated in 0.05-ml amounts on various selective and nonselective media. Viable counts were determined after incubation of the plates at 37 C for 24 to 48 hr.

To rule out the possibility of significant mating between donor and recipient cells during the assay procedure, the following control was included: fecal samples obtained from mice monoassociated with either the donor or recipient strain were mixed; the mixture was then assayed as described above, and dilutions were plated for recombinant clones. Occasional recombinants for proximally transferred markers were noted in this control, but at levels much lower than those of samples from mice fed the donor and recipient strains. Recombinants for distally transferred markers were not detected. These essentially negative results may be attributed to the short period of contact between donor and recipient cells before agitation and dilution, and to the fact that BSG at room temperature is a suboptimal medium for conjugal transfer. It is concluded from these results that the majority of the recombinant clones originated in the mouse intestinal tract.

Bacteriophage. The donor-specific ribonucleic acid phage MS-2 and the donor-specific deoxyribonucleic acid phage f1 were used to test potential donor strains (7).

RESULTS

Selection and characterization of E. coli strains to be used for in vivo studies. The selection of strains to be used for these in vivo matings was based on certain genetic criteria. To minimize the possibility of genetic incompatibilities, only

isogenic strains having a common ancestry were used. The F^- recipient strain χ820 was chosen because it possessed fermentation and auxotropic markers which were well distributed on the circular genome of *E. coli*. Too, the mutations in strain χ820 were stable and reverted only at low frequency. This genetic stability was tested in vitro and also after growth in the mouse. The F^+ donor strain used in these studies was χ209. The Hfr strain selected was a Cavalli type with its origin of chromosome transfer near the *purE* locus and chromosome transfer being in the counterclockwise direction. The selection of this strain (Hfr OR74) was based on its stability of F integration (9); the Hfr state was quite stable over many generations when transferred repeatedly in vitro. The F′ strain used was ORF-210, which carries the *purE* locus on the F Factor. When this F′ is integrated into the χ859 chromosome, gene transfer proceeds with the same direction and orientation as it does in Hfr strain OR74.

Figure 1 shows the linkage map of *E. coli* K-12. The arrows indicate the origin, direction, and gradient of chromosome transfer of Hfr strain OR74 and the F′ strain ORF-210. The relevant fermentation and auxotrophic markers of the F^- strain χ820 are shown.

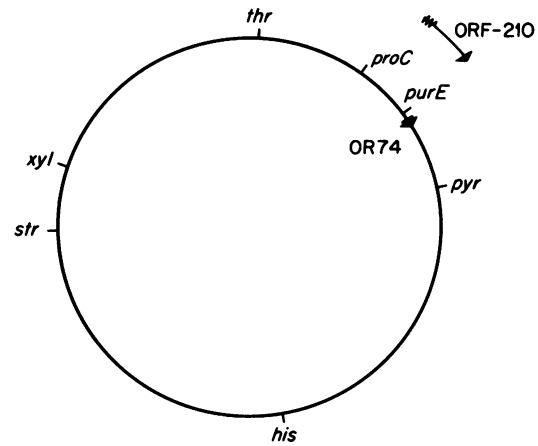

FIG. 1. *Linkage map of E. coli K-12. The arrow indicates the origin, direction, and gradient of chromosome transfer of Hfr strain OR74. The F′ strain ORF-210 used in these studies had the same origin and direction of chromosome transfer as Hfr OR74 when the F′ was integrated into the chromosome. The relevant fermentation and auxotrophic markers of the F^- recipient strain χ820 are shown. Strain χ820 is str^r.*

In an early experiment, Hfr strain OR74 was used to monocontaminate germ-free mice to determine the stability of F integration in an in vivo environment. Fecal samples were collected over a 40-day period, and representative clones from each sample were tested for donor ability and sensitivity to donor-specific phages. Figure 2 presents data on the in vivo stability of Hfr strain OR74. After 24 hr in vivo, 85% of the population were Hfr donors and 15% were F^+. No F^- clones were detected at that time. It can be seen that after 40 days in vivo the Hfr donors in the population had decreased to about 2.5% and about 15% of the population had become F^+. Almost 80% of the population had lost the F factor and become F^-.

Table 1 gives the recombination frequencies obtained from in vitro liquid matings between the three donor strains selected for in vivo studies and the F^- recipient χ820. The $F^+ \times F^-$ cross gave recombination frequencies of about 5×10^{-5} for any marker. The recombinant frequencies obtained from the Hfr $\times$ F^- cross were high for proximally transferred markers and decreased for markers situated at increasing distance from the origin of chromosome transfer. The recombination frequency for the *purE* marker (1.2×10^{-1}) is slightly lower than that for the more distally transferred *proC* marker (2.4×10^{-1}). It has previously been shown that the probability of integrating markers located very near the origin of chromosome transfer (e.g., the *purE* locus in Hfr strain OR74) is lower than for more distally transferred markers (23). In the F′ $\times$ F^- cross, the *purE* locus carried on the ORF-210F′ factor was transferred at the same high frequency as the F factor. The frequency 3.5 reflects the rapid transmission of the F′ element through the recipient population and the fact that the recipient cells undergo about 3 to 4 generations of growth during a 90-min mating. The frequency of recombinants inheriting chromosomal markers was similar to that found in matings with Hfr strain OR74. Proximally transferred markers showed high frequencies of inheritance, which decreased as the distance from the origin of chromosome transfer increased. These data show that the donor and recipient strains chosen for in vivo experiments behaved in a predictable manner in standard in vitro matings.

Genetic exchange between an Hfr and F^- strain in the mouse intestine. Figure 3 gives the titers of Hfr and F^- parental strains and also of the various recombinant classes over a 12-day period following contamination of germ-free mice. The recipient strain χ820 was established 3 days before the addition of the Hfr strain OR74. All genetic markers of the F^- strain were checked for reversion before the addition of the Hfr strain and were found to be nonreverting in vivo. The first fecal sample was taken 6 hr after the addition of the Hfr strain. It can be seen, from the data presented in Fig. 3, that the Hfr strain was

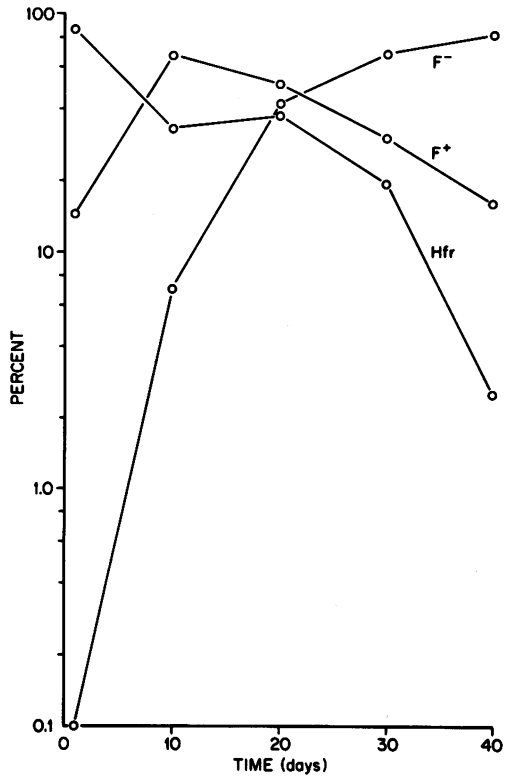

FIG. 2. *In vivo stability of Hfr strain OR74. Germ-free mice were monocontaminated with Hfr strain OR74 as described in Materials and Methods. The first fecal sample was taken 24 hr after contamination. One hundred clones isolated from each sample were tested for donor ability by plate mating with the F⁻ strain χ820, selecting for proximally transferred markers. All clones were tested for the presence of F by examining them for sensitivity to the donor-specific phages f1 and MS-2. The percentage of Hfr, F⁺, and F⁻ clones in each sample was determined.*

established at a titer of 10^8 cells/g of feces after contamination. All selected recombinant classes were also present after 6 hr. The recombinant classes inheriting proximally transferred markers (*purE*$^+$, *pro*$^+$, and *thr*$^+$) appeared in highest titer during the 12-day period, while recombinants inheriting distally transferred markers (*xyl*$^+$, *his*$^+$, and *pyr*$^+$) were detected in low titer. This result appears to reflect the frequency of transfer of the various markers. We would expect proximally transferred donor markers to be transferred at high frequency, which would increase the probability of recombinant formation for those markers. With increasing distance from the origin of chromosome transfer, random breaks in the deoxyribonucleic acid would tend to exclude from the recipient those portions of the donor chromosome needed to produce recombinants for distally transferred markers (prezygotic exclusion; 14). If conjugation proceeds in vivo as it does in vitro, we would expect recombinants for distally transferred donor markers to be much less frequent than those for proximally transferred donor markers.

In standard Hfr × F⁻ crosses, the inheritance of unselected markers among the various recombinant classes is determined by linkage distances between markers (15). If unselected markers are transferred distal to a selected marker, they show a gradient of inheritance among the recombinants which is proportional to their distance from the selected class. This results from chromosome breakage and prezygotic exclusion. If the unselected markers are transferred proximal to the selected ones, it can be assumed that they had entered the recipient and would be inherited among the selected markers with a probability of about 0.5 (15). Closely linked markers would tend to be inherited together. Table 2 compares the percentage of unselected markers inherited among *purE*$^+$, *pro*$^+$, *thr*$^+$ recombinants isolated from in vitro liquid matings and recombinants formed in the mouse intestine. These results show that, with regard to unselected markers, recombinants produced in an in vivo environment do not differ from those produced in vitro.

TABLE 1. *Recombination frequencies obtained from in vitro liquid matings between the Hfr, F′, and F⁺ strains chosen for in vivo studies and the F⁻ recipient strain χ820*[a]

Cross	Recombinant class selected	Recombination frequenices
χ209 F⁺ × χ820 F⁻	*purE*$^+$, *str*^{r}	6.0×10^{-5}
	proC$^+$, *str*^{r}	3.0×10^{-5}
	thr$^+$, *str*^{r}	6.0×10^{-5}
	his$^+$, *str*^{r}	3.5×10^{-5}
Hfr OR74 × χ820 F⁻	*purE*$^+$, *str*^{r}	1.2×10^{-1}
	proC$^+$, *str*^{r}	2.4×10^{-1}
	thr$^+$, *str*^{r}	5.0×10^{-2}
	his$^+$, *str*^{r}	3.0×10^{-5}
F′ ORF-210 × χ820 F⁻	*purE*$^+$, *str*^{r}	3.5
	proC$^+$, str^r	8.0×10^{-2}
	thr$^+$, *str*^{r}	2.4×10^{-2}
	his$^+$, *str*^{r}	6.4×10^{-5}

[a] The bacteria were grown and mated in L broth. The matings were interrupted after 90 min by diluting the mating mixtures into BSG containing 10% L broth (v/v) and immediately agitating the diluted mixtures for 30 sec on a vortex mixer. The initial cell densities in the mating mixture for χ209 and χ820 were 1.2×10^8/ml and 3.5×10^8/ml, respectively. For Hfr OR74 and χ820, the titers were 7.0×10^7/ml and 5.2×10^8/ml, respectively. For F′ ORF-210 and χ820, the titers at the commencement of mating were 5.1×10^7/ml and 1.9×10^8/ml, respectively.

Genetic exchange between an F+ and F− strain in the mouse intestine. Figure 4 gives the parental and recombinant titers following contamination of germ-free mice with the F+ strain χ209 and the F− strain χ820. The order of contamination and method of sampling were the same as described in Fig. 3. It can be seen that χ209 had established a titer of about 10^7 cells/g of feces 6 hr after contamination. No recombinants were detected at that time. At 24 hr, recombinants for *purE*, *pro*, *thr*, and *his* were detected in low titer. Recombinants for *his* were not detected after 24 hr. The other recombinants were detected at low levels for the 12-day period. The F+ donor strain was finally established at a titer of 10^9 cells/g of feces. The F− recipient decreased from an initial titer of 10^9 cells/g of feces to about 10^5 cells/g of feces. A much lower frequency of all recombinant classes in an F+ × F− cross would

TABLE 2. *Percentage of unselected markers inherited among purE+, pro+, and thr+ recombinants*[a]

Recombinants isolated from mice				Recombinants isolated from in vitro matings			
Selected	Unselected			Selected	Unselected		
	purE	*pro*	*thr*		*purE*	*pro*	*thr*
purE	100	40.3	15.4	*purE*	100	52	7.7
pro	7.2	100	13.5	*pro*	6.7	100	10.6
thr	7.7	55.8	100	*thr*	12.7	61.5	100

[a] Recombinant colonies representative of each class were transferred to fresh single omission plates with sterile toothpicks (52 per plate) and incubated for 2 days at 37 C. After incubation, the recombinant colonies were replica-plated to double-omission media in all combinations. After incubation, the inheritance of unselected markers among 104 recombinants of each class was determined.

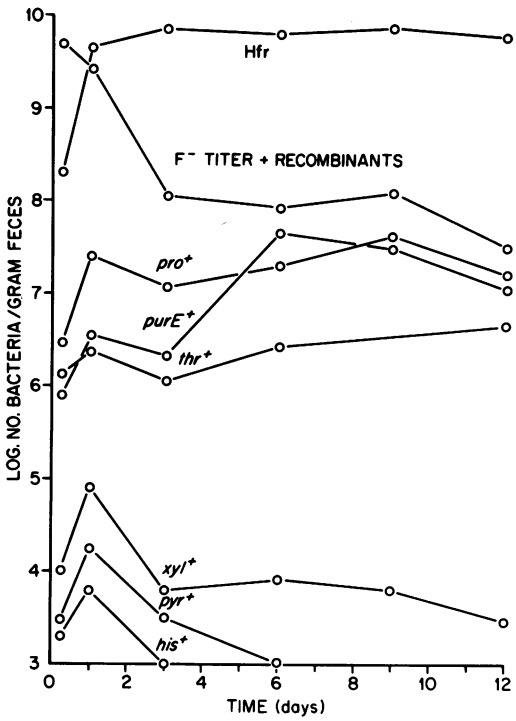

FIG. 3. *Parental and recombinant titers after contamination of germ-free mice with Hfr strain OR74 and the F− strain χ820. The recipient strain was established 3 days before the addition of the Hfr strain. All genetic markers of the F− strain χ820 were checked for reversion before the addition of the Hfr strain and were found to be nonreverting. The first fecal sample was taken 6 hr after the addition of Hfr strain OR74. The total titer was determined on Brain Heart Infusion (BHI) Agar plates. Since the majority of the clones appearing at high dilutions on BHI plates were shown to be high-frequency donors in mating with F− strain χ820, the Hfr titer was equated to the total titer. The titer of the recipient population was determined on minimal medium containing all the growth factors required by strain χ820 plus streptomycin. The titers of the recombinant classes were determined on single omission minimal media containing streptomycin.*

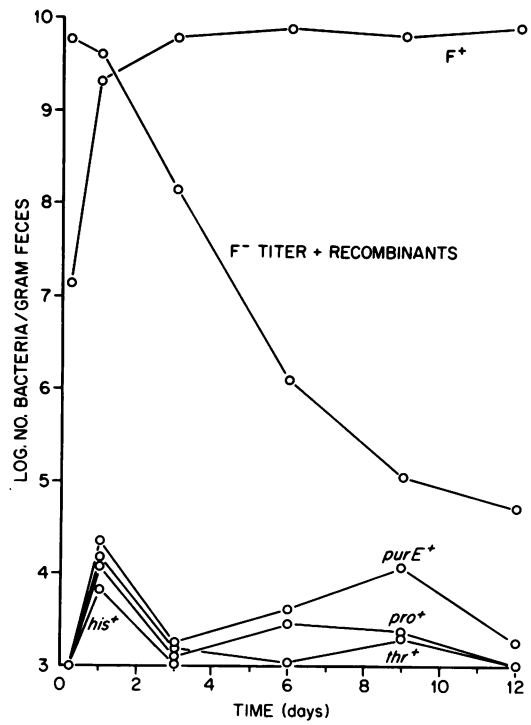

FIG. 4. *Parental and recombinant titers after contamination of germ-free mice with F+ strain χ209 and F− strain χ820. The order of contamination and method of sampling were as described in Fig. 3.*

be expected as compared with an Hfr × F⁻ cross. These results show that genetic transfer with subsequent recombinant formation can occur at a low frequency in an in vivo environment. Most recombinants formed in this F⁺ × F⁻ in vivo cross were also F⁺, indicating that the F factor was also efficiently transferred to the recipient strain.

Genetic exchange between an F′ and F⁻ strain in the mouse intestine. Figure 5 gives the parental and recombinant titers following contamination of germ-free mice with F′ strain ORF-210 and F⁻ strain χ820. The order of contamination and method of sampling were the same as described in Fig. 3. Since the *purE* locus in F′ strain ORF-210 is associated with the fertility factor F, we assume that transfer of this locus is a reflection of the efficiency of F transfer. In Fig. 5, it can be seen that almost the entire F⁻ population had received the *purE* locus, as determined by recombinant formation, 6 hr after the addition of the F′ strain. We take this as evidence that F′ transfer under the proper conditions in vivo is a very efficient process. In F′ × F⁻ matings in vitro, chromosomal markers are transferred at a much higher frequency than in F⁺ × F⁻ matings in vitro, because the chromosomal markers on the F factor have a high probability for reintegration at their site of homology on the host chromosome (31). When this happens, chromosome transfer proceeds in a manner similar to that of the Hfr strain from which the F′ originated, but with a reduced frequency. Recombinants inheriting the proximally transferred donor markers *pro* and *thr* were detected in reasonable titers following addition of the F′ strain, but dropped in titer during the 12-day period. Recombinants inheriting distally transferred donor markers were initially present in low titer, but were not detected after 3 days. These results suggest that F transfer is very efficient in vivo and also that F′ strains can mediate chromosome transfer in vivo as they do in matings in vitro.

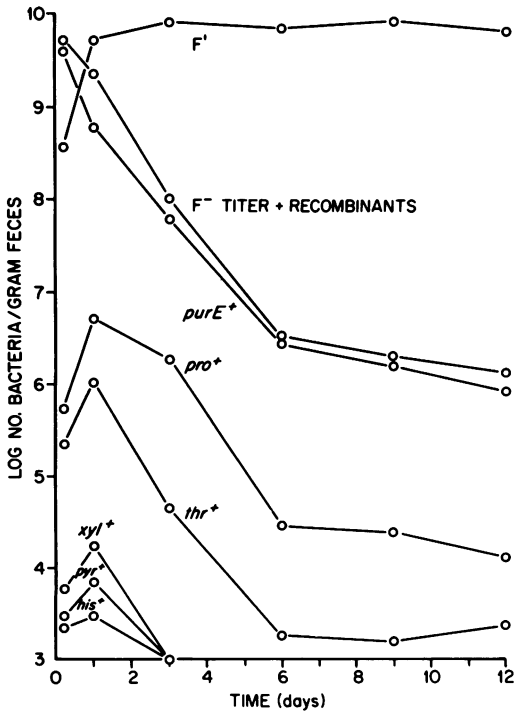

FIG. 5. *Parental and recombinant titers after contamination of germ-free mice with F′ strain ORF-210 and F⁻ strain χ820. The order of contamination and method of sampling were as described in Fig. 3.*

In vivo genetic exchange between an F′ and F⁻ strain in the presence of Lactobacillus. Since F′ strains mediate both F transfer and chromosome transfer, inhibition of either or both processes should be detectable in such strains. If gene transfer in nature is an important mechanism in bacterial evolution, we must assume that it takes place in a microbially complex environment. These experiments with germ-free mice contaminated with selected genetically compatible strains represent simple systems from an ecological standpoint. One must ask whether genetic exchange in vivo is affected by other microbial species which inhabit the same ecological niche. To test the effect of another bacterial genus on the F′ × F⁻ in vivo cross described above, a *Lactobacillus* species of mouse origin was established with the F⁻ recipient χ820 in germ-free mice. After 3 days, the F′ strain ORF-210 was added, and recombinant titers were determined as in the previous experiment. From the results of this experiment (Fig. 6), it can be seen that the *Lactobacillis* strain established at a titer of about 10^8 cells/g of feces and maintained this level throughout the experiment. The F′ strain was established in high titer 6 hr after its addition. Recombinants inheriting the donor markers *purE*, *pro*, and *thr* were detected at 6 hr, but in lower titer than observed in the F′ × F⁻ cross without *Lactobacillus* (Fig. 5). The titers of all recombinant classes reached a peak at 24 hr and then dropped. The results, however, are basically the same as those observed in the previous F′ × F⁻ mating in the absence of *Lactobacillus*. We can therefore conclude that the presence of *Lactobacillus* did not alter the ecological environment in such a way as to interfere with gene transfer and recombinant production. However, the influence of other genera in various combinations will have to be tested before any general conclusions can be made.

Transfer of R factor ROR-1 to F⁻ strain χ820 in the mouse intestine. The R factor ROR-1 carried by *E. coli* strain M7 and its derivatives was transferred to F⁻ strains of *E. coli* K-12 at a frequency of about 10^{-5} during a 60-min mating in vitro. Figure 7 shows the development of Tc^r in a strain χ820 F⁻ population after the addition of the R factor-containing strain M7-18. The recipient strain χ820 was established in germ-free mice 3 days before the addition of strain M7-18. The first fecal sample was taken 6 hr after the addition of strain M7-18. Tc^r χ820 clones were detectable in low titer (10^5 cells/g of feces) 6 hr after addition of strain M7-18. During the 12-day period, the χ820 population was slowly converted to Tc^r (about 25%) as a result of transfer of the R factor ROR-1 from strain M7-18. Strain M7-18 was established in a titer of 10^{10} cells/g of feces. Strain χ820 dropped in titer and finally established at about 5×10^8 cells/g of feces. These results are similar to those reported by Reed et al. (28) and Salzman and Klemm (30). The transfer of ROR-1 may be considered "natural" in that no antibiotics were present in the environment to select for resistant clones.

DISCUSSION

The relevance of gene transfer between microbial species in vivo is difficult to study because the ecosystems involved are quite complex. By contaminating germ-free mice with genetically well-characterized bacterial strains, we have been able to develop a simplified ecosystem in which gene transfer can be studied. Although this system is much simpler than those found in nature, it is felt that studies at this level are necessary before interactions within more complex ecosystems are considered.

In our first experiment, an attempt was made to determine the stability of an Hfr strain (stability of F integration) in the mouse intestinal tract. Hfr strain OR74 was chosen because of its in vitro stability. Curtiss (9) carried this strain for more than 80 generations in vitro without detecting transition to the F⁺ or F⁻ states. After growth in the mouse intestine, the percentage of Hfr cells in the population decreased. F⁺ and F⁻ clones were detected among the Hfr cells and comprised the majority of the population after 40 days in vivo. Clones isolated from the in vivo environment were classified as F⁻ if they were insensitive to donor-specific phage and if no recombinants were detected in matings with the recipient strain χ820. It has been reported that many strains isolated from natural sources and harboring fertility factors other than F (e.g., col factors, R factors) are in a state of repression with regard to donor ability (24). In the above

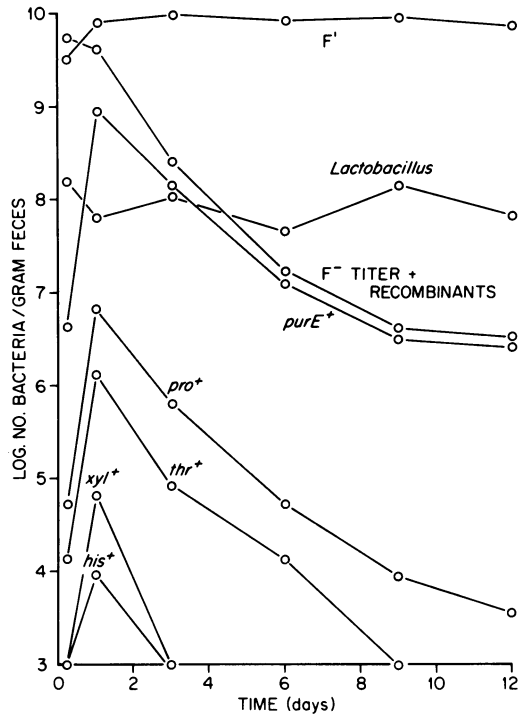

FIG. 6. *Parental and recombinant titers after contamination of germ-free mice with F' strain ORF-210 and F⁻ strain χ820 in the presencd of Lactobacillus. The order of contamination and method of sampling are the same as described in Fig. 3, except that Lactobacillus was established with the F⁻ strain χ820 before the addition of the F' strain. Lactobacillus titers were determined on Rogosa SL agar.*

experiment, it is possible that F function was inhibited in the in vivo environment. The apparent F⁻ clones which were isolated, however, did not revert to donor phage sensitivity after in vitro growth and were not able to produce recombinants in mating with the F⁻ recipient strain; seemingly, therefore, the F factor was actually lost from these cells. If the F factor was no longer present in these cells, one might wonder why they were not quickly reinfected with F from F⁺ cells. Further experiments are needed to determine whether the F⁻ clones isolated from the in vivo environment are for some reason resistant to reinfection with the fertility factor F.

It is difficult to compare results of in vivo experiments with those performed in vitro. The number of generation cycles which the Hfr strain underwent in the mouse during the 40-day period is not known. Also the factors (selection, chemical environment, etc.) which might influence the transition Hfr $\rightleftarrows$ F⁺ $\rightarrow$ F⁻ in vivo or in vitro have not been studied. The in vivo conditions

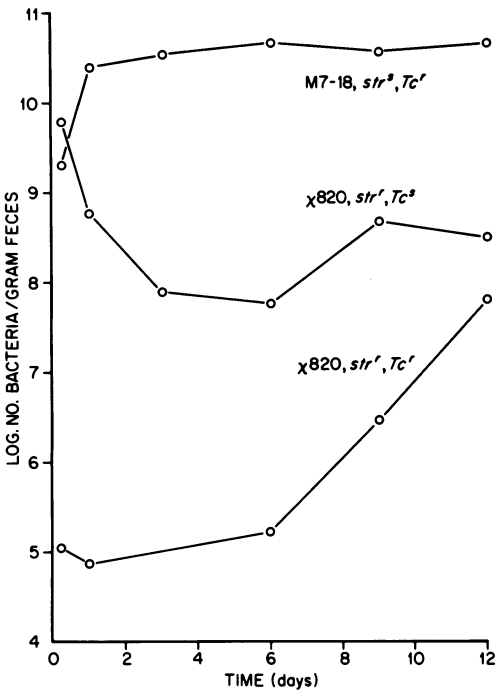

FIG. 7. *In vivo transfer of ROR-1 (Tc^r) to F^- strain χ820 from strain M7-18. The recipient strain χ820 was established in germ-free mice 3 days before the addition of strain M7-18. The first fecal sample was taken 6 hr after the addition of strain M7-18. Strain M7-18 titers were determined on minimal medium containing arginine and oxytetracycline. The total χ820 population was determined on minimal medium containing all the growth factors required by χ820 plus streptomycin. The titers of χ820 receiving ROR-1 (Tc^r) from strain M7-18 were determined on minimal medium containing all the growth factors required by χ820 plus streptomycin and oxytetracycline.*

which influence the expression of donor ability of strains harboring fertility factors must be assessed before the importance of gene transfer between members of a mixed flora can be determined.

The titers of the various recombinant classes in the Hfr × F^- in vivo mating described above may be a reflection of the frequency of gene transfer. Recombinants inheriting proximally transferred markers occurred in consistently higher titers than those inheriting distally transferred markers. These results are somewhat similar to those obtained from in vitro matings. If conjugation proceeds in vivo as it does in vitro, the frequency of recombinants inheriting proximally transferred markers would be higher than those for distally transferred markers because of prezygotic exclusion. Another possibility for the above results is that for some reason the recombinants inheriting proximally transferred markers had a selective advantage in vivo. Additional experiments are needed to determine the role of selection following in vivo genetic exchange.

The results of the F^+ × F^- mating indicate that F^+ populations can mediate chromosomal transfer with subsequent recombinant formation in an in vivo environment. One would predict from the results of in vitro matings that the probability of obtaining recombinants for any marker in an F^+ × F^- mating would be much lower than for an Hfr × F^- mating. This prediction, of course, does not take into account the possibility that certain recombinant classes would be selected in vivo and reach high levels. In the F^+ × F^- in vivo mating described above, only low levels of recombinants were detected over the 12-day period; this observation might suggest that selection does not play an important role in these short-term experiments and that what we are observing is a reflection of the frequency of competent donors within an F^+ population.

In natural environments, the selection and survival of various recombinant classes probably assumes an important role. It would be of interest to set up experiments similar to those described above and to monitor the animals for recombinants over long periods of time. This would allow time for the selection of recombinants best adapted to the in vivo environment and might better represent the conditions which exist in natural environments.

The F′ factor carried by strain ORF-210 was readily transferred to the F^- recipient strain in vivo. It appears that F′ transfer (which we considered to be representative of F transfer) under the proper conditions in vivo is a very efficient process. Six hours after feeding the ORF-210F′ culture, almost the entire F^- population in the mouse intestine had received the *purE* locus associated with the F′. Recombinants for chromosomally located markers indicated that this F′ strain was also able to mediate chromosome transfer. The presence of *Lactobacillus* appeared to have no influence on gene transfer and recombinant formation in this F′ × F^- in vivo mating.

Our results generally support the findings of Ducluzeau and Galinka (11) with regard to the Hfr × F^- in vivo mating. These authors isolated recombinants for proximal markers from germ-free mice contaminated with Hfr and F^- *E. coli* K-12 strains. Recombinants inheriting more terminally transferred fermentation markers were rarely recovered. Schneider et al. (32) isolated hybrids from the intestinal tract of anti-

biotic-treated conventional mice after the addition of an *E. coli* K-12 donor and a *Salmonella typhimurium* recipient. In their experiments, the hybrid classes often gained ascendancy over both parental strains, possibly indicating that the hybrids had some selective advantage in vivo. More work is needed to determine the biochemical and physiological conditions which influence microbial populations in vivo and the selection of strains with different genotypes.

The in vivo transfer of the R factor ROR-1 to the χ820 recipient is in agreement with the results reported by Reed et al. (28) and Salzman and Klemm (30). R factor-containing clones of χ820 were detected within 6 hr after the addition of the donor strain M7-18, after which time the proportion of Tc^r χ820 clones slowly increased in the recipient population. These experiments are being expanded to considerations of the influence of diet, other bacterial species, and drugs on the in vivo transfer of R factors. Such studies will be helpful in understanding the ecological significance of R factor transfer in nature.

Our primary goal in the future will be to reproduce in germ-free mice a microbial flora which is similar in complexity to that found in nature but in which all of the components are well characterized. We will continue in our attempts to isolate naturally occurring fertility factors or plasmids from *E. coli* strains isolated from mice and to use these to study genetic exchange in germ-free mice contaminated with selected genera of mouse origin. Such a system, in which the organisms used are isolated from a common ecological niche and also in which the host organism employed (mouse) is the same one in which the bacterial genera had evolved, should more closely simulate the ecosystem as it exists in nature.

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