

## R-Plasmid Transfer to and from *Escherichia coli* Strains Isolated from Human Fecal Samples

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Strains of *Escherichia coli* recently isolated from human feces were examined for the frequency with which they accept an R factor (R1) from a derepressed  $fi^+$  strain of *E. coli* K-12 and transfer it to fecal and laboratory strains. Colicins produced by some of the isolates rapidly killed the other half of the mating pair; therefore, conjugation was conducted by a membrane filtration procedure whereby this effect was minimized. The majority of fecal *E. coli* isolates accepted the R factor at lower frequencies than K-12  $F^-$ , varying from  $10^{-2}$  per donor cell to undetectable levels. The frequencies with which certain fecal recipients received the R-plasmid were increased when its  $R^+$  transconjugant was either cured of the R1-plasmid and remated with the  $fi^+$  strain or backcrossed into the parental strain. The former suggests the loss of an incompatibility plasmid, and the latter suggests the modification of the R1-plasmid deoxyribonucleic acid (DNA). In general, the fecal  $R^+$  *E. coli* transconjugants were less effective donors for K-12  $F^-$  and heterologous fecal strains than was the  $fi^+$  K-12 strain, whereas the single strain of *Citrobacter freundii* examined was generally more competent. Passage of the R1-plasmid to strains of salmonellae reached mating frequencies of  $10^{-1}$  per donor cell when the recipient was a *Salmonella typhi* previously cured of its resident R-plasmid. However, two recently isolated strains of *Salmonella* accepted the R1-plasmid from *E. coli* K-12  $R^+$  or the  $R^+$  *E. coli* transconjugants at frequencies of  $5 \times 10^{-7}$  or less.

The presence of antibiotic resistance transfer factors (R-plasmids) in the intestinal pathogens, *Salmonella* and *Shigella* (2, 11, 12), presents a problem in therapeutics. There is both epidemiological (12, 16) and experimental (4, 17, 25, 26) evidence suggesting that one source of these plasmids for the pathogens is  $R^+$  coliforms. Because of this and because  $R^+$  coliforms, especially  $R^+$  *Escherichia coli* strains, can be recovered in significant numbers from domestic sewage and its receiving waters (13, 15, 21), there has been concern that the use of sewage-polluted waters as sources of recreation is a significant route for the dissemination of R-plasmids from excretors back into the general population. This, in turn, would increase the probability of its contact with salmonellae or shigellae.

Transfer of the plasmids from  $R^+$  coliforms to salmonellae or shigellae in sewage or environmental waters is a special case of a general situation controlled by existing guidelines and standards formulated to prevent the discharge of unacceptably large numbers of these pathogens in sewage effluents. Another possibility, transfer of the R-plasmid in the human gastrointestinal tract, is not prevented by these guide-

lines. For this possibility to be realized, however, ingested  $R^+$  coliforms must colonize the gastrointestinal tract or pass their plasmids to the resident fecal flora (18). In both cases, the  $R^+$  bacteria must remain as reservoirs for transfer of their R-plasmids to shigellae or salmonellae subsequently ingested (via food, drinking water, contact, etc.) in sufficient numbers to cause disease.

Both situations mentioned above require information on the abilities of  $R^+$  *E. coli* environmental isolates to donate their plasmids to the resident *E. coli* strains and of the resultant  $R^+$  transconjugants to pass the R-plasmid to salmonellae or shigellae. Most of the available data relating to this question are deceptive in their implications in that the percentage of  $R^+$  isolates capable of donating R-plasmids rather than the transfer frequencies are presented (6, 8, 12, 21, 30). Furthermore, the recipients generally were laboratory strains of *E. coli* (K-12) or the enteric pathogens selected because they were good recipients rather than coliforms of fecal origin (8, 16, 27).

In response to the need for more realistic information, a study was conducted to determine

the R-plasmid transfer frequencies with fecal strains of *E. coli* as both the recipients and donors. A second objective was to investigate the intrinsic characteristics which allow an *E. coli* strain of fecal origin to either accept or reject an R-plasmid. In addition, some limited information was obtained on the abilities of environmental salmonella isolates to accept the R1-plasmid from fecal R<sup>+</sup> transconjugants.

#### MATERIALS AND METHODS

**Bacterial strains.** The donor (RS-2) initially used to test the competency of the fecal strains of *E. coli* to accept an R-plasmid was an *E. coli* K-12 *lac* strain containing an fi<sup>+</sup> derepressed R factor. This plasmid, designated as R1, is included in the compatibility class FII (23). It specifies F-like pili and carries chloramphenicol, kanamycin, streptomycin, ampicillin, and sulfonamide resistance genes.

The reference recipients were *E. coli* K-12 F<sup>-</sup> strains J5 and CSH-26. The latter, a lactose-negative mutant, was used in experiments to examine the capability of the lactose-positive fecal transconjugants containing R1 to donate this plasmid. Strains RS-2, J5, and CSH-26 were obtained from Grace Thorne, Tufts Medical School, Boston, Mass.

Twenty-three fecal isolates, 22 strains of *E. coli* and one strain of *Citrobacter freundii* (F-76), were examined as the R-plasmid recipients. They had been isolated by the method of Dufour et al. (10) from fecal samples obtained from students at the University of Rhode Island. Their identities were confirmed by the API *Enterobacteriaceae* system. All strains fermented lactose. Of the 22 *E. coli* strains, 12 produced colicins active against the *E. coli* K-12 donor, RS-2, as determined by the zone of inhibition when "spotted" on a lawn of the donor.

A *Salmonella typhi* isolated from an epidemic of typhoid fever in Mexico (14) was cured of its resident R-plasmid. It and strains of *S. paratyphi* and *S. enteritidis* isolated from marine surface waters in Great South Bay, Long Island, were examined for their abilities to accept the R1-plasmid from R<sup>+</sup> transconjugants.

*E. coli* K-12 HfrH was used as the host in obtaining preparations of the male-specific phage f2.

**Media.** Liquid cultures were prepared in Penassay broth (Difco Laboratories, Detroit, Mich.) unless otherwise specified. Selective media employed in the R factor transfer experiments consisted of Mueller-Hinton medium (Difco), powdered antibiotics (Sigma Chemical Co., St. Louis, Mo.), 1% lactose, and 80 µg of bromothymol blue (MCB, Norwood, Ohio) per ml as a pH indicator. Antibiotics utilized were chloramphenicol (30 µg/ml), nalidixic acid (50 µg/ml), and sodium azide (250 µg/ml). The medium used in the preparation of bacteriophage stocks and in the sensitivity tests, tryptone-glucose-yeast extract (TGY), consisted of 1% tryptone (Difco), 0.85% NaCl (Fischer, Fair Lawn, N. J.), 0.1% yeast extract (Difco), 0.1% glucose (Difco), and 0.022% CaCl<sub>2</sub> (Sigma). Hard agar plates contained 1.5% agar, and the soft agar overlay contained 0.8%.

**Plate test for colicin production.** The indicator strain used to detect colicin production by the fecal isolates was the *E. coli* K-12 R<sup>+</sup> donor, RS-2. Cells from Penassay broth culture incubated for 24 h at 37°C were spread on the surface of a brain heart infusion (BHI) agar plate (Difco) with a cotton swab. The strain to be examined for colicin production was grown for 24 h at 37°C on a BHI plate from which a heavy inoculum was stabbed into the plate containing the indicator strain, RS-2. After incubation at 37°C, the zone of inhibition around the colony of the test strain was recorded as - to +++, depending on its size.

**Antibiotic susceptibility tests.** Drug sensitivity was determined with the following antibiotic disks (Difco): chloramphenicol (10 µg), ampicillin (10 µg), kanamycin (10 µg), streptomycin (10 µg), sulfadiazine (300 µg), and nalidixic acid (30 µg) (5).

**Genetic markers.** Recipient bacteria in the conjugation experiments were chromosomally marked with resistance to nalidixic acid (Na<sup>r</sup>, 100 µg/ml) or sodium azide (300 µg/ml). Nalidixic acid was an ideal selective agent for inhibiting the donor cells and, in combination with the *lac* characteristic, was used to quantify the transconjugants wherever possible. The inability to employ the *lac* marker in some crosses was overcome by a control on the mutation frequency for Na<sup>r</sup> in the donor. The mutation frequency to Na<sup>r</sup> was found to be <10<sup>-7</sup> in all cases. Sodium azide was used to obtain the R<sup>+</sup> fecal donors for the "transconjugant crosses" from RS-2 × fecal isolate matings. Since these R<sup>+</sup> donors were resistant to sodium azide, nalidixic acid resistance could be the selective marker on the recipient.

Only chloramphenicol was used to inhibit the recipients in the selective medium for the mating experiments. Transconjugants from low-frequency crosses (≤10<sup>-6</sup>) were picked and tested for other drug resistance markers coded by the plasmid. Resistance to at least two of the drugs was considered as indicative of conjugation rather than spontaneous mutation of the recipient.

A summary of the conjugation experiments performed, the strains crossed, and the genetic markers utilized is given in Table 1.

**R factor transfer experiments.** The conjugation experiments were carried out on the surface of membrane filters. A modification of the membrane filter mating technique of Matney and Achenbach (22) was developed to minimize the lethal effects of colicin production by either the donor or the recipient on a sensitive mating partner. A 0.9-ml amount of recipient cells and 0.1 ml of donor cells in the logarithmic growth phase were each diluted in 10 ml of Penassay broth. The suspension of the colicin-producing member of the mating pair was passed through a polycarbonate membrane filter (Nuclepore, 0.4 µm). The filter containing the impinged cells was then rinsed with 20 ml of liquid medium E wash (28) to reduce the quantity of absorbed colicin protein. Then, an appropriate portion of the colicin-sensitive partner was similarly impinged onto the filter, followed by a final 10-ml medium E wash. The filter was removed and placed on a medium E plate (0.75% agar, no dextrose supplement)

TABLE 1. *In vitro* conjugation experiments

Conjugation expt	Donor strain	Recipient strains	Genetic markers in transconjugant recovery <sup>a</sup>
Recipient test cross	RS-2	1. Fecal isolates 2. Cured transconjugant <sup>b</sup>	Cm <sup>r</sup> , Na <sup>r</sup> Lactose reactions
Transconjugant control cross	Fecal R <sup>+</sup> transconjugant	CSH-26	Cm <sup>r</sup> , Na <sup>r</sup> Lactose reactions
Transconjugant backcross	Fecal R <sup>+</sup> transconjugant	1. Parental fecal isolates 2. Cured homologous transconjugants	Cm <sup>r</sup> , Na <sup>r</sup> Mutation check <sup>c</sup>
Transconjugant-interstrain cross	Fecal R <sup>+</sup> transconjugant	1. Nonhomologous fecal isolates 2. Cured nonhomologous R <sup>+</sup> transconjugants	Cm <sup>r</sup> , Na <sup>r</sup> Mutation check
Transconjugant-pathogenic cross	Fecal R <sup>+</sup> transconjugant	<i>Salmonella</i> sp.	Cm <sup>r</sup> , Na <sup>r</sup> Lactose reactions

<sup>a</sup> Cm, Chloramphenicol resistance (plasmid-linked marker); Na<sup>r</sup>, nalidixic acid resistance (chromosomal marker).

<sup>b</sup> Transconjugants from matings (fecal strains × RS-2) which were subsequently cured of the R1 plasmid.

<sup>c</sup> At the time of each mating the donor strains were placed alone on the selective media to assess the mutation rate to Na<sup>r</sup>. The mutation frequency was <10<sup>-7</sup> in all cases.

which was incubated at 37°C. After a 1-h mating period, one of the two following techniques was employed. In the screening procedure, the membrane filter was transferred directly from medium E to a selective medium appropriate for transconjugant recovery and incubated at 37°C. Transconjugant colonies on the filter were counted after 24 h. In the dilution procedure, the membrane filter was placed in 10 ml of phosphate-buffered saline (19) and shaken with a Vortex mixer for 1 min to resuspend the cells (10<sup>-1</sup> dilution). Dilutions of the suspension were placed onto selective media and incubated at 37°C for 36 h. The R-plasmid transfer frequency was calculated as the number of transconjugants per donor cell.

In the broth matings, 9 ml of recipient and 1 ml of donor cells were mixed and incubated at 37°C for 1 h to allow for the R-plasmid transfer.

**Curing R<sup>+</sup> strains.** Tubes of Penassay broth containing various concentrations of acridine orange or acriflavine were inoculated with the R<sup>+</sup> strains (29). Spread plates on MacConkey medium (Difco) were prepared from dilutions of those tubes exhibiting approximately a 30% reduction in growth. Replica plating onto Mueller-Hinton medium containing 30 µg of chloramphenicol per ml was then utilized to search for cured strains. A strain was considered cured of the R factor if no R1 resistance determinants were detectable.

When required, an alternative method of R factor curing was utilized. R<sup>+</sup> strains inoculated into Penassay broth were incubated for 2 h at 37°C and then 48 h at 45°C; the resulting cultures were then refrigerated at 4°C for 24 h. The cycle of preincubation, high-temperature incubation, and refrigeration was repeated several times before the cultures were scored for cured organisms as in the acridine orange treatment.

**Bacteriophage sensitivity.** The male-specific

RNA phage, f2, was used as a rapid method to assess F-like pilus formation by R<sup>+</sup> bacteria strains (7). An inoculum of the bacterial cells was suspended in soft agar (TGY) which was overlaid on a hard agar base (TGY). A loopful of high-titer phage preparation (≥10<sup>9</sup> plaque-forming units per ml) was placed on the plate, which was then incubated for 24 h at 37°C. A cleared zone in the bacterial lawn indicated a sensitive strain. Stocks of the male-specific bacteriophage f2 were prepared after the method of Adams (1).

## RESULTS

**Broth versus filter matings.** The number of donor cells (RS-2) which survived a 1-h mating period in broth and on membrane filters with several colicinogenic fecal *E. coli* recipients is shown in Table 2. The control recipient was a noncolicinogenic *E. coli* K-12 F<sup>-</sup> lac<sup>+</sup> (J5). The donor recovery was higher from fecal crosses performed on membrane filters than in broth. Moreover, with the more competent recipient (F-79), higher R-plasmid transfer frequencies were obtained by the membrane filter procedure. Consequently, all subsequent crosses were performed on filters.

**Competence of fecal strains as recipients.** The frequencies (transconjugants/donor) with which the fecal isolates received the R1-plasmid from RS-2 are shown in Fig. 1 and 2. The most striking feature was the extreme strain-to-strain variability in the frequencies observed. About 70% were relatively poor recipients (<10<sup>-4</sup>), and the remaining 30% were relatively efficient recipients. Of the latter, one strain (F-309) was as competent a recipient as the reference strain, J5;

TABLE 2. Broth versus filter matings

Recipient strains	Donor recovered		R-Plasmid transfer frequency <sup>a</sup>	
	Broth mating	Filter mating	Broth mating	Filter mating
J5 (control)	$2.5 \times 10^7$	$2.1 \times 10^7$	$5.0 \times 10^{-2}$	$3.0 \times 10^{-1}$
F-17	$7.1 \times 10^2$	$5.2 \times 10^5$	NTR <sup>b</sup>	NTR
F-79	$5.9 \times 10^3$	$9.4 \times 10^6$	NTR	$1.0 \times 10^{-6}$
F-79 cured	$1.8 \times 10^4$	$1.7 \times 10^7$	$4.0 \times 10^{-6}$	$8.0 \times 10^{-2}$

<sup>a</sup> Number of transconjugants per donor cell added.  
<sup>b</sup> No transconjugants recovered.

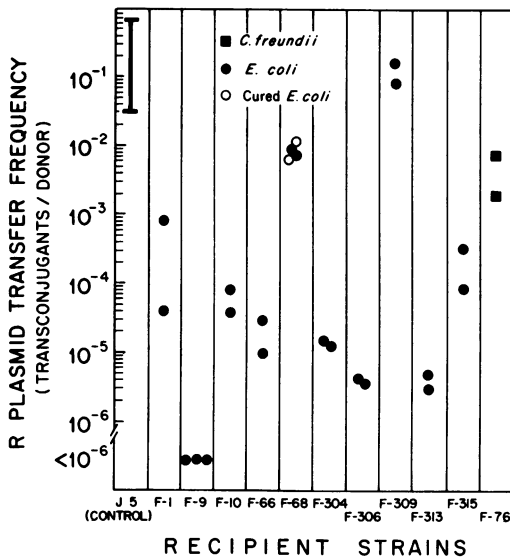


FIG. 1. Transfer frequencies for the R1-plasmid from *E. coli* K-12 R<sup>+</sup>, fi<sup>+</sup>, drd (RS-2) to non-colicinogenic fecal strains. Each value represents the results of an individual conjugation experiment. Crosses were performed at least in duplicate.

and two strains, F-68 and F-76 (the strain of *C. freundii*), were only slightly defective in this regard. In general, the transfer frequencies were higher for the noncolicinogenic than the colicinogenic strains.

Transconjugants were isolated from the mating between five fecal recipients and RS-2. They were cured of the R-plasmid and remated with RS-2 in an attempt to determine whether the initial mating (or the subsequent curing process) selected for more competent recipients. The remating of cured transconjugants with the original donor, RS-2, resulted in an increase in transfer frequency in some cases. Both of the cured F-79 transconjugants became excellent recipients ( $\geq 10^{-2}$ ); and a 2 to 3 log increase in transfer frequency was observed with both F-18 transconjugants (Fig. 2). However, with strains F-68,

F-7, and F-17, no increase in recipient competency was detectable after mating, curing, and remating (Fig. 1 and 2). Both of the cured F-79 transconjugants still produced colicins lethal to RS-2, as did one of the cured F-18 strains.

**Competence of fecal strains as donors in transconjugant backcrosses.** R<sup>+</sup> transconjugants from several fecal strains were backcrossed to their parental strains (i.e., F-17R<sup>+</sup> × F-17) and mated with their homologous cured transconjugants (i.e., F-17R<sup>+</sup> × F-17 cured). The results of these matings are illustrated in Fig. 3 along with the transfer frequencies of the R1-plasmid from the control donor (RS-2) to the same fecal recipient. The majority of R<sup>+</sup> fecal strains demonstrated donor abilities approximately equal to that of RS-2. The notable exception was strain F-17R<sup>+</sup> whose R1 transfer frequency was five orders of magnitude greater than that of the control (RS-2). F-18R<sup>+</sup> and F-79R<sup>+</sup> were defective donors relative to the laboratory strain RS-2. This could be observed with assurance only in the mating experiments with the cured transconjugants as the recipients since these two strains initially were also defective recipients.

**Competence of fecal strains as donors in transconjugant test crosses.** The R<sup>+</sup> transconjugants from the seven fecal strains were

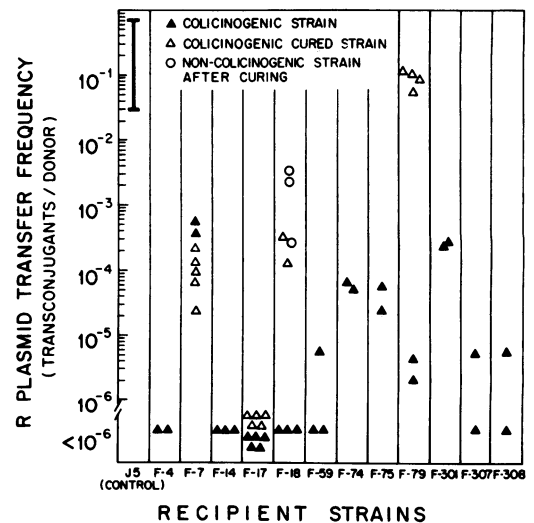


FIG. 2. Transfer frequencies for the R1-plasmid from *E. coli* K-12 fi<sup>+</sup>, drd (RS-2) to initially colicinogenic fecal strains. Each value represents the results of an individual conjugation experiment. Crosses were performed at least in duplicate with the same mating strains, except cross RS-2 × F-17 cured, in which five different cured F-17 recipients were tested once. Two different strains of the cured recipients, F-7, F-18, and F-79, were crossed at least twice.

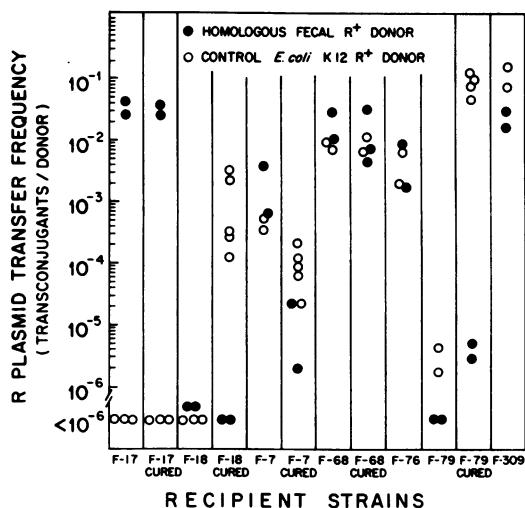


FIG. 3. Transfer frequencies for the R1-plasmid from R<sup>+</sup> transconjugants to their parental strains (backcrosses) and to cured R<sup>+</sup> transconjugants of the parental strains. The frequencies with which the R1-plasmid was transferred from the original donor, K-12 f<sup>i</sup>\*, drd, (RS-2), as given in Fig. 1 and 2 are also included for comparison. Each value represents the results of an individual conjugation experiment. Crosses were performed at least in duplicate.

crossed with an *E. coli* K-12 F<sup>-</sup> to determine the relative donor ability of each strain when mated to a competent laboratory recipient. These transfer frequencies and, for comparison, those from transconjugants "backcrosses" are shown in Table 3. The defective donors in the backcrosses, F-18R<sup>+</sup> and F-79R<sup>+</sup>, again exhibited very low rates of R transfer to the competent recipient K-12 F<sup>-</sup>. The remaining fecal donors were somewhat less efficient than RS-2 in transferring the R1-plasmid to the laboratory recipient K-12 F<sup>-</sup>. The exception was the transconjugant from the *C. freundii* strain (F-76R<sup>+</sup>) which appeared to be a more competent donor for the *E. coli* K-12 F<sup>-</sup> recipient than the original R1

donor, RS-2. In general, the fecal R<sup>+</sup> transconjugants transferred the R1-plasmid to their parental strains at frequencies greater than they did to K-12 F<sup>-</sup>.

Competence of fecal strains as donors in interstrain crosses. Interstrain crosses of the R<sup>+</sup> donors with some of the more competent fecal recipients were performed to determine the potential for secondary transmission of the R1-plasmid to other fecal strains (Table 4). The fecal strains F-7R<sup>+</sup>, F-18R<sup>+</sup>, and F-79R<sup>+</sup> were not studied since they had been shown to be poor or partially defective donors in the backcrosses or when mated with *E. coli* K-12 F<sup>-</sup> (CSH-26). Only the fecal donors attaining mating frequencies of  $\geq 10^{-2}$  in these crosses were considered capable of high-frequency interstrain R1 passage. F-76R<sup>+</sup>, the *C. freundii* strain, proved to be an extremely competent donor; it transferred the R1-plasmid to itself, three other fecal isolates, and K-12 F<sup>-</sup> (Table 3) more efficiently than did RS-2. In general, the remaining

TABLE 3. R-plasmid transfer in transconjugant backcrosses and control crosses

Donor strains	Recipient strains		f2 sensitivity of donor strain <sup>b</sup>
	<i>E. coli</i> K-12 F <sup>-</sup> (CSH-26)	Parental fecal recipients <sup>a</sup>	
RS-2	$4.7 \times 10^{-2}$		C
F-76 R <sup>+</sup>	$1.6 \times 10^{-1}$	$5.6 \times 10^{-3}$	C
F-17 R <sup>+</sup>	$3.8 \times 10^{-3}$	$3.6 \times 10^{-2}$	C
F-309 R <sup>+</sup>	$5.3 \times 10^{-4}$	$2.7 \times 10^{-2}$	H
F-68 R <sup>+</sup>	$2.3 \times 10^{-4}$	$2.1 \times 10^{-2}$	H
F-7 R <sup>+</sup>	$4.8 \times 10^{-4}$	$1.6 \times 10^{-3}$	H
F-18 R <sup>+</sup>	$2.8 \times 10^{-7}$	NTR <sup>c</sup>	—
F-79 R <sup>+</sup>	NTR	$4.3 \times 10^{-6}$	—

<sup>a</sup> Recipients utilized in the case of F-18 and F-79 are the homologous cured transconjugants.

<sup>b</sup> Sensitivity determined by the phage spotting technique. C, clear plaque; H, hazy plaque; —, no plaque formation.

<sup>c</sup> No transconjugants recovered.

TABLE 4. R-plasmid transfer in interstrain fecal crosses

Recipient strains	<i>E. coli</i> K-12 R <sup>+</sup> donor (control)	Fecal donors			
		F-17 R <sup>+</sup>	F-68 R <sup>+</sup>	F-76 R <sup>+</sup>	F-309 R <sup>+</sup>
F-79 cured	$9.4 \times 10^{-2}$	$4.5 \times 10^{-2}$	$6.6 \times 10^{-3}$	$2.9 \times 10^{-1}$	$8.4 \times 10^{-2}$
F-309	$9.2 \times 10^{-2}$	$1.8 \times 10^{-3}$	$2.1 \times 10^{-2}$	$1.1 \times 10^{-1}$	$2.7 \times 10^{-2}$
F-68	$8.7 \times 10^{-3}$	$1.6 \times 10^{-3}$	$2.1 \times 10^{-2}$	$5.1 \times 10^{-3}$	$1.0 \times 10^{-3}$
F-76	$2.0 \times 10^{-3}$	$1.4 \times 10^{-5}$	$3.8 \times 10^{-4}$	$5.6 \times 10^{-3}$	$1.4 \times 10^{-4}$
F-18 cured colicin (-)	$2.0 \times 10^{-3}$	$3.8 \times 10^{-6}$	$2.8 \times 10^{-6}$	$3.3 \times 10^{-5}$	$3.3 \times 10^{-5}$
F-18 cured colicin (+)	$2.2 \times 10^{-4}$	$6.7 \times 10^{-4}$	$4.2 \times 10^{-5}$	$2.9 \times 10^{-5}$	$6.8 \times 10^{-5}$
F-7	$4.6 \times 10^{-4}$	$2.4 \times 10^{-4}$	$1.2 \times 10^{-4}$	$1.1 \times 10^{-3}$	$1.6 \times 10^{-5}$
F-17	$\leq 10^{-7a}$	$3.6 \times 10^{-2}$	NTR <sup>b</sup>	NTR	$3.0 \times 10^{-7}$

<sup>a</sup> One transconjugant recovered in a series of crosses.

<sup>b</sup> No transconjugants recovered.

fecal donors, F-17R<sup>+</sup>, F-68R<sup>+</sup>, and F-309R<sup>+</sup>, transferred the R1-plasmid to heterologous fecal strains at somewhat lower frequencies than did the RS-2 control. The differences were especially marked with F-18 Col<sup>-</sup> as the recipient.

**Bacteriophage sensitivity.** The fecal transconjugants were screened for F-pilus production by testing their sensitivity to the bacteriophage f2. Clear plaques were obtained with F-76R<sup>+</sup>, F-17R<sup>+</sup>, and the positive controls (RS-2, K-12 Hfr, and J5R<sup>+</sup>); hazy plaques were observed with F-68R<sup>+</sup>, F-309R<sup>+</sup>, F-7R<sup>+</sup>, and the negative control J5. Sensitivity to this male-specific phage appeared to correspond to the frequency with which a strain donated its R1-plasmid to K-12 F<sup>-</sup> (CSH-26) (Table 3).

**Pathogenic crosses.** An *S. typhi* strain isolated in an epidemic of typhoid fever in Mexico was cured of its resident R-plasmid. This strain and two salmonella environmental isolates were crossed with the seven fecal R<sup>+</sup> transconjugants (Table 5). High-frequency transfer to the *S. typhi* strain was observed with six of the eight R<sup>+</sup> donors. In general, it was a better recipient than K-12 F<sup>-</sup> or the homologous fecal strains (Table 3). However, the two environmental isolates from marine waters were very poor recipients when crossed with laboratory strain (RS-2) and all the fecal donors examined (Table 5).

## DISCUSSION

Most of the fecal isolates were found to be poor recipients for the fi<sup>+</sup>, derepressed R1-plasmid from the laboratory donor RS-2. Since they were obtained from membrane filter assays of serially diluted human fecal samples, it is assumed that each of the isolates was representative of a major component of the resident coliform population of a given individual at the time of sampling and, hence, was a competent colonizer of the human gut. Thus, it would appear

that, in general, the ability to colonize the human gastrointestinal tract and accept the R1-plasmid at high frequency is not a commonly shared trait. However, there were three strains which were relatively good recipients, notably F-309, which accepted R1 at a frequency comparable to K-12 F<sup>-</sup>. These may be exceptions to the generalization. It is also possible that a relatively infrequent cell was carried along in the dilution sequence or that, for some unknown reason, an individual may carry a large number of relatively poor colonizers or transient organisms at a given time. This is consistent with the identity of one of the strains, F-76. It was identified as *C. freundii*, and this species rarely colonizes the human gastrointestinal tract (9).

Three observations suggest that production by the recipients of colicins active on the *E. coli* K-12 R<sup>+</sup> donor was marginally, if at all, responsible for the generally low R1 transfer frequencies to the fecal strains. First, the use of the membrane filter mating procedure minimized any inhibitory effect on the colicin-sensitive donor. Second, R<sup>+</sup> transconjugants of a colicin-positive, poor recipient (F-79), when cured of the R1-plasmid, remained colicin positive but accepted the R1-plasmid from RS-2 on remating at a very high frequency (10<sup>-1</sup>). Thirdly, the colicinogenic and noncolicinogenic variants of the cured F-18 transconjugants accepted R1 at similar frequencies when remated with RS-2.

R-plasmid transfer can be prevented by interference with plasmid entry or establishment, generally by systems referred to as a superinfection inhibition (24). In one such system, the incompatibility of the R-plasmid with a resident plasmid in the cell results in the loss of one or the other during division of the transconjugant cell. Fecal strains of *E. coli* are known to harbor many plasmids; and it is not unreasonable to assume that some of them are incompatible with R1 and that they are lost in rare individual cells before or upon acceptance of the R1-plasmid from an R<sup>+</sup> donor. This would account for the low rates observed in the initial matings and the higher transfer frequencies obtained when cured transconjugants were remated (i.e., F-18, F-79). An equally plausible explanation is a mutational event. However, the cured F-17 and F-7 transconjugants were no better recipients for R1 than their parents, and cured F-18 still did not receive R1 at frequencies comparable to that of the control (K-12 F<sup>-</sup>) or F-309. This suggests the presence of an additional system which interferes with the ability of the fecal strains to accept R. The obvious explanation is that all the indigenous, interfering plasmids were not eliminated in the curing process.

Another explanation is the presence of an

TABLE 5. R<sup>+</sup> plasmid transfer from fecal strains to *Salmonella*

Donor strains	R <sup>+</sup> transfer frequency in recipient strain:		
	<i>S. typhi</i>	<i>S. paratyphi B</i>	<i>S. enteritidis</i>
F-76 R <sup>+</sup>	4.9 × 10 <sup>-1</sup>	ND <sup>a</sup>	ND
F-17 R <sup>+</sup>	5.5 × 10 <sup>-2</sup>	1.2 × 10 <sup>-7</sup>	≤10 <sup>-7b</sup>
F-309 R <sup>+</sup>	3.5 × 10 <sup>-2</sup>	NTR <sup>c</sup>	2.2 × 10 <sup>-7</sup>
F-79 R <sup>+</sup>	7.1 × 10 <sup>-6</sup>	NTR	NTR
F-7 R <sup>+</sup>	2.4 × 10 <sup>-3</sup>	NTR	4.3 × 10 <sup>-7</sup>
R-18 R <sup>+</sup>	2.9 × 10 <sup>-6</sup>	NTR	NTR

<sup>a</sup> ND, No data.

<sup>b</sup> Transconjugants recovered in one cross, frequency = 10<sup>-7</sup>.

<sup>c</sup> NTR, No transconjugants recovered.

endonuclease in the recipient which destroys the R1 deoxyribonucleic acid (DNA) (20). The results of the backcross and interstrain cross experiments indicate that this restriction system was present in F-17. With the R<sup>+</sup> transconjugants from three other strains (F-309, F-68, F-7), the frequency of the transfer of R1 into its homologous recipient was greater than it was into K-12 F<sup>-</sup> or, when tested, into heterologous fecal recipients (the exception was cross F-309R<sup>+</sup> × F-79 cured). This would suggest that restriction-modification systems effective against R1-plasmid DNA are found in these strains, although less active than those observed in F-17.

The fecal transconjugants F-18R<sup>+</sup> and F-79R<sup>+</sup> exhibited very poor donor ability in crosses with their parental strains and with *E. coli* K-12. All the R1 antibiotic resistance determinants were present in these defective donors; thus a repression of the conjugative function is suspected. Neither strain was sensitive to the male-specific bacteriophage f2, indicating either phage absorption to the F-pili without cell lysis or the absence of F-pili in the majority of cells. Absorption experiments or electron microscopy studies or both are necessary to distinguish between these two possibilities. The absence of pili can be explained by the presence of indigenous plasmids in the donor strains, F-79R<sup>+</sup> and F-18R<sup>+</sup>, which produce a repressor active on the regulator gene of the R1 sex factor. A model for such systems is the fi<sup>+</sup> (fertility inhibition) character of many R factors. An fi<sup>+</sup> R factor, when introduced into an F<sup>+</sup> strain, will often drastically reduce the ability of the strain to conjugate and abolishes lysis by F-specific phage (23).

Among *Enterobacteriaceae*, sex pilus production by donor cells is a necessary prerequisite for R-plasmid transfer during conjugation. However, sex pilus formation in *E. coli*, the coliform which normally colonizes the human colon, would appear to be a disadvantage since it makes the strain susceptible to the male-specific bacteriophages, and these constitute a significant proportion of the phage in sewage (G. McBride, unpublished data). Furthermore, R-factor carriage or at least the production of sex pilus seems to adversely affect colonization ability (3). Thus, it is not unreasonable that many fecally derived strains have mechanisms to repress sex pilus production. This would not be the case with an organism such as *C. freundii*. The one strain examined was the only isolate whose R<sup>+</sup> transconjugant was very susceptible to f2, an excellent R-plasmid donor, and a relatively good recipient for the R1-plasmid from RS-2. More data are needed with regard to this observation.

High-frequency passage of the R1-plasmid from R<sup>+</sup> fecal isolates to a strain of *S. typhi*

affirmed the possibility of potential therapeutic problems arising during infection by this pathogen. However, the near absence of R1 acceptance among the two environmental isolates of *Salmonella* indicates that barriers to plasmid entry and establishment exist in this genus as in the coliform strains studied. Obviously, more strains of *Salmonella* and *Shigella* directly isolated from clinical and environmental sources must be tested for R-plasmid receptivity.

Neither the quantity nor the types of required data are available from this or previous studies to completely model a system in which the inputs are the ingestion of R<sup>+</sup> *E. coli* cells (much less R<sup>+</sup> coliforms) during shellfish consumption or swimming and the output is the probability that the R-plasmid will be transferred to salmonellae subsequently ingested via another route. However, the data obtained clearly show that the potential "health risks" are markedly overstated when made from data on the percentage of environmental coliform isolates that will transfer their plasmids to laboratory strains of *E. coli*, *Salmonella*, or *Shigella* without regard to the transfer frequencies involved.

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