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Gene Transmission Among Strains of *Erwinia amylovora*

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Stable donor strains of *Erwinia amylovora* were obtained from strain EA178R$_1$ (harboring an *Escherichia coli* F'*lac*) by selection for clones resistant to curing by acridine orange. These donor strains (EA178R$_1$-99 and EA178R$_1$-111) transfer chromosomal markers (*arg, cys, gua, ilv, met, pro, ser, trp*); the frequency of the appearance of recombinants prototrophic for Cys, Gua, Met, Ser, and Trp is highest ($>10^{-5}$), followed by recombinants prototrophic for Arg, Ilv, and Pro (10^{-7} to 10^{-5}). The results of interrupted matings, as well as the frequency of transmission of various markers, suggest that *cys* is transferred as an early marker by both donor strains. The Hfr state of these donor strains is rather likely on the basis of the following observations. The donor strains exhibit a relatively efficient and possibly oriented chromosome transfer; the Lac$^+$ character is not cured by acridine orange in these donor strains; and these donor strains do not transfer F.

The transfers of the episomic element F'*lac* from *Escherichia coli* and of R factors from *E. coli* and a clinical isolate of *Shigella flexneri* 1a to *Erwinia* spp. have been reported by us (4, 5). Subsequently, we sought and found some Lac$^+$ heterogenotes of *Erwinia amylovora* and *E. herbicola* (derived from crosses between *E. coli* F'*lac* and the *Erwinia* spp.), which resisted curing by acridine orange. This finding suggested the possibility that the element might exist in an integrated state in these heterogenotes and that they might be Hfr derivatives such as are formed in *E. coli* by the integration of F merogenote and chromosome (7, 11). We describe here the isolation of donor strains selected by acridine orange treatment of an *E. amylovora* strain harboring an *E. coli* F'*lac*; these donor strains are being used by us in studies on genetic transfer among *Erwinia* spp. and other enterobacteria, with particular reference to mapping gene order and to pathogenicity for plants and animals (9, 19). Although the behavior of these donor strains suggests the likelihood that they are in the Hfr state, we prefer for the present to consider this point as moot until further characterization can be completed.

MATERIALS AND METHODS

Bacterial strains. The strains of bacteria used in the present study and their characteristics are listed in Table 1. Refer also to our previous papers (4, 5) for additional information.

Media. The compositions of media have been described in our earlier report (4). The minimal medium was supplemented by amino acids (50 μg/ml) and vitamins (nicotinic acid, 50 μg/ml; thiamine, 1 μg/ml), depending upon the growth requirement of the organisms.

Isolation and characterization of auxotrophic mutants of E. amylovora strain EA178. Auxotrophic mutants were induced by *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (NTG) treatment (1). The mutants were isolated by replica plating after penicillin enrichment, and their nutritional requirements were then determined by using nutrient-impregnated disks (J. L. Ingraham, personal communication). The multiple markers (Table 1) were introduced by repeating the above procedure with NTG as the mutagen.

Isolation of donor strains of E. amylovora strain EA178. Cells of *E. amylovora* strain EA178R$_1$ (Table 1) were treated with acridine orange in Penassay broth (Difco) by a procedure already described by us (4). The Lac$^+$ clones which survived this treatment were inoculated into Penassay broth (1 ml/tube) by touching the colony lightly with a needle. After overnight growth at 30 C, the donor ability of the cultures was tested by the procedure of Mäkelä (13). A sample of the culture (about 0.02 ml) was spotted on a minimal agar plate containing streptomycin (200 μg/ml) and prespread with the recipient culture (log-phase cells of EA178-M64S$_1$ [*ser*] or EA178-M107S$_1$ [*his*]). The donor streptomycin-sensitive cells were counterselected by the streptomycin added to the medium, and the recipient cells were counter-

TABLE 1. *Bacterial strains*

Strain	ICPB[a] designation	Characteristics[b]	Source
Erwinia amylovora	EA178	Wild type; Lac$^-$, requires nicotinic acid for growth, virulent (9)	ICPB (ATCC 19381)
	$EA178R_1$	F'*lac*$^+$/Lac$^-$, Str^s	Derived from a cross between *E. coli* F'*lac* × *E. amylovora* EA178 (4)
	$EA178R_1$-99	Donor strain, Lac$^+$, Str^s	Derived from $EA178R_1$, this paper
	$EA178R_1$-111	Donor strain, Lac$^+$, Str^s	Derived from $EA178R_1$, this paper
	EA178-$M64S_1$	*ser*-1, Lac$^-$, Str^r	NTG mutagenesis (1) of EA178 and selection of Str^r
	EA178-$M64S_1$-9-5	*ser*-1, *thr*-1, *ilv*-1, Lac$^-$, Str^r	NTG mutagenesis of EA178-$M64S_1$
	EA178-$M64S_1$-15	*ser*-1, *pro*-1, Lac$^-$, Str^r	NTG mutagenesis of EA178-$M64S_1$
	EA178-$M64S_1$-18-2	*ser*-1, *his*-1, *cys*-1, Lac$^-$, Str^r	NTG mutagenesis of EA178-$M64S_1$
	EA178-$M64S_1$-19	*ser*-1, *met*-1, Lac$^-$, Str^r	NTG mutagenesis of EA178-$M64S_1$
	EA178-$M64S_1$-35	*ser*-1, *met*-2, Lac$^-$, Str^r	NTG mutagenesis of EA178-$M64S_1$
	EA178-$M64S_1$-41	*ser*-1, *trp*-1, Lac$^-$, Str^r	NTG mutagenesis of EA178-$M64S_1$
	EA178-$M64S_1$-68	*ser*-1, *arg*-1, Lac$^-$, Str^r	NTG mutagenesis of EA178-$M64S_1$
	EA178-$M64S_1$-102	*ser*-1, *gua*-1, Lac$^-$, Str^r	NTG mutagenesis of EA178-$M64S_1$
	EA178-$M107S_1$	*his*-1, Lac$^-$, Str^r	NTG mutagenesis of EA178
Escherichia coli K-12	2451	F$^-$, *thi*, *his*, *ilv*, *arg*, *met*, *xyl*, *gal*, *mal*, T_6^r, Lac$^-$, Str^r	ICPB (AB1450)

[a] International Collection of Phytopathogenic Bacteria (Department of Bacteriology, University of California, Davis, Calif. 95616; M. P. Starr, Curator).

[b] In the case of Lac, the superscripts + or − indicate, respectively, the ability or inability to utilize lactose as the sole source of carbon; in the case of Str, the superscripts r and s, respectively, indicate resistance or sensitivity to streptomycin (200 μg/ml).

selected because of their auxotrophic state. The plates were incubated at 30 C and examined, after 48 to 72 h of incubation, for the appearance of recombinants in the region where the prospective donor culture was spotted. The clones which gave rise to recombinants (50 or more colonies) were retested for donor ability by mating on membranes (see procedure B below).

Mating procedure A: in broth. The mating procedure in broth, which we described earlier (4), was used.

Mating procedure B: on membranes. The mating procedure on membranes, employed by Beck and Ingraham (2) for *Salmonella typhimurium*, was used in the following version. Log-phase cultures (1 ml each) of the donor (about 5×10^7 cells/ml) and recipient (about 5×10^8 cells/ml) were transferred to a tube, and the cells were collected on a membrane filter (Millipore Corp., 0.22-μm pore size, 25-mm diameter) under negative pressure, care being taken not to allow the membrane surface to dry. The membrane and the cells were transferred onto the surface of a Penassay base agar (Difco) plate previously overlaid with 2.5 ml of soft Penassay agar. After incubation at 30 C for 3 h, the cells on the membranes were suspended in 1.0 ml of phosphate buffer (pH 7.2, 0.01 M), agitated on a Vortex Jr., mixer for 30 s, and serially diluted (decimally) in phosphate buffer. The controls, which consisted of donor and recipient cells alone, were treated similarly. Samples (0.1 ml) were spread on the selective medium. The plates were incubated at 30 C and examined for the appearance of recombinants after 3 to 4 days of incubation.

Mating procedure C: in broth after adsorption on membranes. The third procedure, described here, was tried in an effort to improve the pairing efficiency between the donor and recipient cells by adsorption onto a membrane surface. Log-phase cultures (1 ml each) of the donor and recipient were mixed, and the cells were collected on the membranes by the procedure described above. The membranes were incubated on the surface of soft nutrient agar at 30 C, and after 10 min of incubation the membrane was transferred to nutrient broth (10 ml) contained in an Erlenmeyer flask (125-ml capacity). The cells were dislodged into the broth by gentle shaking at 30 C, the membrane was removed after 5 min of incubation, and the incubation was continued. Samples (0.5 ml) were transferred, after incubation for a total period of 3 h, to phosphate buffer (4.5 ml, pH 7.2, 0.01 M) and shaken vigorously for 30 s on a Vortex Jr., mixer. The serial, decimal dilutions (0.1 ml), which were made starting with this sample (1:10 dilution), were spread on selective agar media. The plates were incubated at 30 C and examined for the appearance of the recombinant colonies after 3 to 4 days of incubation.

Interrupted matings. Interrupted matings were done according to procedure C described above. The

mating process was considered to be initiated (zero time) when the cells were dislodged into the broth and the membrane was removed from the mating medium. Samples (0.2 ml) were removed at the initiation of the matings (zero time), and at intervals of 15 min thereafter, and transferred to phosphate buffer containing 200 μg of streptomycin per ml (1.8 ml, pH 7.2, 0.01 M; 1:10 dilution). The cells, which were shaken vigorously on a Vortex Jr., mixer for 30 s, were further diluted and plated (0.1 ml) on the selective medium. The plates were incubated at 30 C, and the recombinant colonies were counted after 3 to 4 days of incubation.

Sensitivity to F-specific phage M13. The procedure for determining sensitivity to F-specific phage M13, described earlier (4), was used.

Acridine orange "curing." The effect of acridine orange on the elimination of *lac* was determined in Penassay broth by the procedure described earlier (4).

Stability of the donor property in isolates EA178R$_1$-99 and EA178R$_1$-111. Stability of the donor property was tested by a procedure slightly modified from that of Broda (3). The donor strains EA178R$_1$-99 and EA178R$_1$-111 were grown overnight at 30 C in Penassay broth to a cell density of approximately 5×10^8 cells/ml. The growth was serially diluted in phosphate buffer (pH 7.2; 0.01 M), and the diluted sample (0.1 ml) was spread on Penassay base agar plates. The well-separated single colonies which appeared after 36 h of incubation at 30 C were touched lightly with a needle and transferred to Penassay broth (1.5 ml). A sample (0.02 ml) of the growth in Penassay broth after 18 h of incubation at 30 C was spotted on minimal agar containing streptomycin (200 μg/ml), and prespread with the log-phase culture (0.1 ml; about 5×10^7 cells) of the recipient (EA178-M64S$_1$; *ser*) (six to nine donor cultures were spotted on each plate). The plates were incubated at 30 C and examined after 48 h of incubation for the appearance of recombinant clones in the region where the donor cultures were spotted. The clones which developed in Penassay broth were subjected to a second cycle of single-colony isolation and growth in Penassay broth, and were then tested for donor ability according to the foregoing procedure.

RESULTS

Isolation of donor strains of E. amylovora. The results of the curing of *lac* from *E. amylovora* strain EA178R$_1$, in the presence and absence of acridine orange, are presented in Table 2. The majority (about 90%) of the clones were Lac$^-$ in the presence of the dye (1 μg/ml). The surviving Lac$^+$ clones (about 200) were examined for their donor ability with strains EA178-M64S$_1$ and EA178-M107S$_1$ (refer to Table 1 for characteristics) as the recipient cultures. Five out of 200 clones gave rise to recombinants (50 or more colonies per spot) with EA178-M64S$_1$ as the recipient; with two of these isolates (EA178R$_1$-99 and EA178R$_1$-111), the recombinants were far greater in number

TABLE 2. *Effect of acridine orange on the elimination of lac from the Erwinia amylovora Lac$^+$ heterogenote EA178R$_1$*[a]

Acridine orange concn (μg/ml)	Growth[b]	No. of colonies examined		Lac$^-$ (% of the total no. of colonies)
		Total	Lac$^-$	
0.0	+	2,376	18	0.75
0.2	+	1,960	87	4.4
1.0	+	1,409	1,254	89.0
2.0	Nil	NT[c]	NT[c]	NT[c]

[a] For the details of experimental conditions, refer to Materials and Methods and also to Chatterjee and Starr (4).

[b] Growth of the cultures, after overnight incubation, was assessed by visual examination.

[c] Not tested.

(confluent growth in the region where the donor cultures were spotted) than with the other clones. No recombinant clones were observed when EA178-M107S$_1$ was used as the recipient. These five clones were tested subsequently for their donor ability by mating on membranes with EA178-M64S$_1$ as the recipient. Whereas three of the isolates produced recombinants at a low frequency (about 10^{-7} to 10^{-6} recombinants formed per donor cell), recombinants were formed at relatively higher frequency with isolates EA178R$_1$-99 and EA178R$_1$-111 as donors (about 10^{-4} recombinants formed per donor cell). The properties of these isolates (referred to as strains or isolates 99 and 111) were studied subsequently in some detail.

Stability of the donor property in isolates 99 and 111. Our previous experience (A. K. Chatterjee and M. P. Starr, unpublished data) with donor cultures derived by the procedure described above had indicated their unstable state in that the donor property was gradually lost upon successive transfers on nutrient media. Therefore, we were initially rather sceptical and tested quite rigorously the stability with respect to their donor ability of the new isolates (99 and 111). The frequency of the appearance of serine prototrophs (in crosses between isolates 99 and 111 as donors and EA178-M64S$_1$ as the recipient) decreased after maintenance of the donor strains on nutrient media for prolonged periods of time. To test the stability of the donor state under lyophilized conditions, the donor cultures were regenerated after 5 months of storage in lyophils, and the donor ability (*ser* transfer) of 50 single-colony derivatives was determined; all the tested clones retained the ability to transfer *ser*. This indicates that the donor state in *E. amylovora* strains 99 and 111 is more stable under lyophi-

lized condition as compared to maintenance on nutrient media. Analogous observations have been reported (18) with *S. typhimurium* Hfr strain SA722 (Hfr K10).

The proportion of the possible revertants which are unable to transfer chromosomal markers in the donor population was also determined. Fifty-three single-colony isolates each from strains 99 and 111 were tested for the ability to transfer *ser* after two and four cycles of growth on nutrient media. All the tested isolates of strain 99 retained the donor ability; 51 out of 53 isolates of strain 111 retained the donor ability after the second and the fourth cycles of growth on nutrient media.

Effect of acridine orange on Lac$^+$ property of isolates 99 and 111 and the parent strain EA178R$_1$. The *lac* genes are not eliminated by acridine orange from isolates 99 and 111 (Table 3). The frequency of Lac$^-$ clones is low in the absence of acridine orange (about 0.5 to 0.6%), and these values do not increase significantly in the presence of the dye (the frequency of Lac$^-$ clones ranges from 0.6 to 0.7%). On the other hand, acridine orange had a marked effect on the production of Lac$^-$ clones in the parent strain EA178R$_1$ (harboring an *E. coli* F'*lac*)—Lac$^-$ clones constituted 95% of the population in the presence of 1 μg/ml of the dye, whereas only 0.5% of the population was Lac$^-$ in the absence of the dye (Table 3).

Effect of mating conditions on the frequency of recombinant formation. In the case of matings among strains of *Pasteurella pseudotuberculosis* (10), the transmission of genetic material occurs when the matings are performed on solid surfaces (membranes) and does not occur in liquid medium (broth). The frequency of gene transmission in *E. coli* (F_2 donors) and in *Salmonella* spp. also is increased by mating on membranes as compared with mating in broth (14, 18). Because of these reports, we examined the effect of mating conditions on the frequency of gene transmission in *Erwinia amylovora*. The results (Table 4) clearly indicate that the transmission of *ser* from *E. amylovora* isolates 99 and 111 to *E. amylovora* strain EA178-M64S$_1$ occurs in matings in broth (procedure A) as well as on solid surfaces (membranes); however, the frequency is increased significantly when the matings are performed on membranes (procedure B). Once the cells are adsorbed onto membranes, resulting possibly in efficient mating pair formation, the subsequent incubation conditions (in broth [procedure C] or on membranes [procedure B]) appear to have no significant effect on the frequency of the formation of recombinants (Table 4). Therefore, in subsequent experiments, matings were routinely performed on membranes by procedure B.

Transmission of chromosomal markers by E. amylovora isolates 99 and 111. The ability of isolates 99 and 111 to transfer markers to *E. amylovora* recipient strains (multiple-marker strains, Table 1) was tested by matings on membranes (Table 5). It can be seen that the isolates (99 and 111) transfer *arg*, *cys*, *gua*, *ilv*, *met*, *pro*, *ser*, and *trp* to the recipient cultures and that the frequencies of the transfer of these markers are comparable with both donor cultures.

Representative clones from all the recombinant classes resulting from crosses between strain 111 and appropriate recipient cultures (Table 5) were tested for the Lac$^+$ and F properties, the latter determined by sensitivity to the F-specific phage M13. All the tested clones were found to be Lac$^-$ and F$^-$. Preliminary attempts to transfer chromosomal markers

TABLE 3. *Effect of acridine orange on the elimination of lac from Erwinia amylovora parent strain EA178R$_1$ and donor strains EA178R$_1$-99 and EA178R$_1$-111*

Cultures	Acridine orange (μg/ml)	Total no. of colonies tested	No. of Lac$^-$ colonies	Percent Lac$^-$ of the total
EA178R$_1$	0.0	1,147	7	0.6
	0.5	928	540	58.1
	1.0	844	804	95.2
EA178R$_1$-99	0.0	1,555	10	0.6
	0.5	1,428	9	0.6
	1.0	1,092	7	0.6
EA178R$_1$-111	0.0	1,024	5	0.5
	0.5	1,311	9	0.7
	1.0	1,198	9	0.7

TABLE 4. *Effect of mating conditions on the transfer of ser from Erwinia amylovora donor strains (EA178R$_1$-99 and EA178R$_1$-111) to Erwinia amylovora recipient strain (EA178-M64S$_1$)*

Mating conditions[a]	Cross	Frequency of *ser*$^+$ per input donor cell
Broth (procedure A)	99 × M64S$_1$	2.0×10^{-6}
	111 × M64S$_1$	5.0×10^{-6}
Adsorption and incubation on membranes (procedure B)	99 × M64S$_1$	3.1×10^{-5}
	111 × M64S$_1$	9.0×10^{-5}
Adsorption on membrane followed by incubation in broth (procedure C)	99 × M64S$_1$	3.6×10^{-5}
	111 × M64S$_1$	7.8×10^{-5}

[a] For details of mating conditions, refer to Materials and Methods.

TABLE 5. *Transfer of chromosomal markers by conjugation in strains of Erwinia amylovora*[a]

Recipient strains[b]	Selection	Frequency of transfer per donor cell with strains[b]		
		EA178R$_1$-99	EA178R$_1$-111	EA178R$_1$
M64S$_1$-9-5	Ilv$^+$	2.0×10^{-6}	4.0×10^{-6}	$<10^{-7}$
M64S$_1$-15	Pro$^+$	1.2×10^{-7}	4.4×10^{-7}	$<10^{-7}$
M64S$_1$-18-2	Cys$^+$	2.0×10^{-4}	4.6×10^{-4}	$<10^{-7}$
M64S$_1$-18-2	Ser$^+$	3.8×10^{-5}	8.0×10^{-5}	$<10^{-7}$
M64S$_1$-19	Met$^+$	1.7×10^{-4}	2.0×10^{-4}	5.8×10^{-7}
M64S$_1$-35	Met$^+$	2.2×10^{-5}	7.4×10^{-5}	4.6×10^{-7}
M64S$_1$-41	Trp$^+$	1.2×10^{-4}	2.6×10^{-4}	$<10^{-7}$
M64S$_1$-68	Arg$^+$	4.2×10^{-6}	8.0×10^{-6}	$<10^{-7}$
M64S$_1$-102	Gua$^+$	1.0×10^{-5}	8.5×10^{-5}	$<10^{-7}$

[a] Matings were performed on membranes for 3 h by procedure B, as described in Materials and Methods.

[b] The various M64S$_1$ strains are auxotrophs derived from *Erwinia amylovora* strain EA178. EA178R$_1$ is an *Erwinia amylovora* strain derived from EA178 and harboring an *E. coli* F'*lac* (4). Donor strains EA178R$_1$-99 and EA178R$_1$-111 were derived from EA178R$_1$ by acridine orange treatment. See Table 1 and text for details.

from the *E. amylovora* donor strains (99 and 111) to an *E. coli* F$^-$ strain (2451) were unsuccessful. The parent strain, EA178R$_1$, of *Erwinia amylovora*—which harbors an *E. coli* F'*lac* (and is consequently Lac$^+$ and sensitive to phage M13) and which had not been exposed to acridine orange selection—could transfer only *met* at a very low frequency (Table 5).

Times of entry of cys and ser markers in strains of E. amylovora. The results of interrupted matings between *E. amylovora* strain 111 and *E. amylovora* strain EA178-M64S$_1$-18-2 are shown in Fig. 1. The *cys* marker is transferred after 6 min and the *ser* marker is transferred after 20 min of contact. Strain 99 also behaved in a similar manner (detailed data are not presented here). Although we are not ready to present detailed linkage data, the results of these preliminary trials indicate that mapping by time of entry is quite likely feasible in *E. amylovora*.

DISCUSSION

Stable donor strains (99 and 111) of *E. amylovora* were isolated from strain EA178R$_1$ (which harbors F'*lac* from *E. coli*; reference 4) by treatment of the cells with acridine orange (a procedure known to cure the cells of F factors and F' elements; reference 8), the subsequent selection of Lac$^+$ clones, and the testing of the survivors for their donor ability. The following observations substantiate our preferred notion that these donor cultures arose as a result of a reciprocal crossing-over event between the F merogenote and the chromosome resulting in a stable Hfr state. (i) The donor cultures show a relatively efficient and possibly oriented chromosome transfer (Table 5; Fig. 1), which is characteristic of the Hfr mating type. (ii) The *lac* genes are not eliminated by treatment with acridine orange from strains 99 and 111 (both F factor and *lac* genes are readily eliminated from EA178R$_1$, the F'*lac* parent culture from which strains 99 and 111 were derived). (iii) The donor cultures do not transfer F, as determined by the insensitivity of the recombinants to the F-specific phage, M13.

However, we cannot at present disprove an alternative explanation for the donor ability of these strains, namely, that the exposure of *E. amylovora* strain EA178R$_1$ (harboring F'*lac*) to acridine orange might have brought about changes leading to (i) the stability (both spontaneous and when further exposed to acridine orange) of the F merogenote in a proportion of the cells and (ii) the ability to mobilize the chromosome in these cells. Although they may have different bases, other cases of stability are known; for example, *E. coli* strain AB1206 (F$_{14}$)

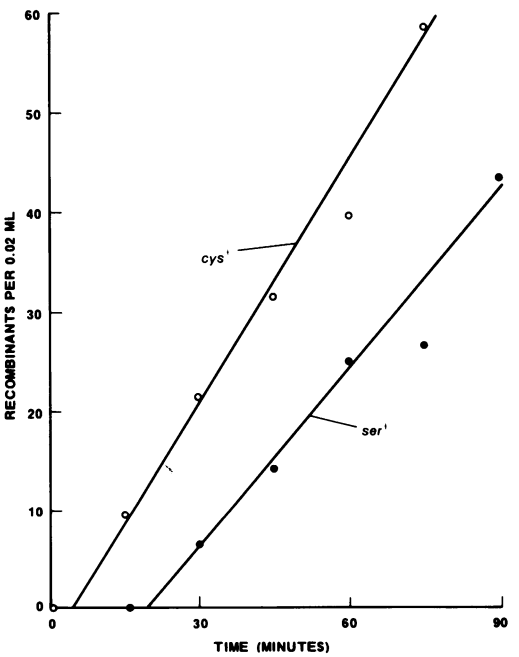

FIG. 1. *Times of entry of cys and ser in Erwinia amylovora. The times of entry of cys and ser were determined by interrupted matings between donor strain EA178R$_1$-111 and recipient strain EA178-M64S$_1$-18-2. For the details of experimental conditions, refer to Materials and Methods section. Symbols: O, cys$^+$ recombinants; ●, ser$^+$ recombinants.*

does not produce F^- or ilv^- either spontaneously or after treatment with acridine orange (16). Another example is F*lacS*, insensitive to plasmid-curing agents including acridine orange, which was isolated from a strain of *S. typhimurium* harboring F_{ts114} *lac* (12). Pittard and Adelberg (15) have also demonstrated that, in an *E. coli* mating system, chromosome transfer prevented the transfer of F merogenote markers which had not reached the zygote before chromosome transfer began. It is possible that chromosome transfer in *E. amylovora* strains 99 and 111 begins simultaneously with or before F merogenote transfer and that this process prevents the entry of F merogenote markers. A mechanism such as this might explain the lack of inheritance of the F or Lac^+ properties in the *E. amylovora* recombinants examined. Taking all these uncertainties into account, we prefer for the present to remain agnostic about whether the Hfr state exists in strains 99 and 111 and choose to refer to them somewhat noncommittally as "donor strains," which they indeed are.

The procedure employed in the present study has resulted in the isolation of donor strains with similar directions of injection (O → *cys*). If strains 99 and 111 are actually Hfr, it should be possible, by including recipient strains with different markers in the screening of the possible donor cultures, that Hfr strains having different origins and directions of injection might be obtained. This aspect is currently being examined.

The transfer of F'*lac* from *E. coli* to *Erwinia* spp. in general and to *E. amylovora* in particular (4), the transfer of antibiotic resistance on R factors from *E. coli* and *Shigella flexneri* 1a to *Erwinia* spp. (5), and the chromosome transfer by the donor strains of *E. amylovora* (as documented in this report) clearly indicate the relatedness among these organisms. Moreover, the frequencies of transmission of several of the markers (*cys*, *met*, *ser*, *arg*, *ilv*, and *pro*) suggest in a very tentative way the general similarity in gene order among *E. amylovora* EA178, *E. coli* K-12 (20), and *S. typhimurium* (17). However, this point is still uncertain because different recipient strains were used in the present study (except for *cys* and *ser* markers; Table 5 and Fig. 1). We intend to use polyauxotrophic recipient strains for precise genetic analyses. If the similarity in gene order is indeed confirmed by use of polyauxotrophic strains, this would further support our notion that these *E. amylovora* strains, although phytopathogenic, are indeed enterobacteria (19). It is expected that this *E. amylovora* gene transmission system would make possible detailed mapping of gene order as well as analyses of phytopathogenicity (9) in these organisms—possibilities which we are currently exploring.

We have found, and are reporting elsewhere (6), that Lac^+ strains of *Erwinia* spp. from human clinical material can transfer *lac* by conjugation involving an unusual sex factor (E) to a variety of *Erwinia* spp. (both plant strains and isolates from human clinical samples) and to other enterobacteria (*E. coli*, *Paracolobactrum arizonae*, *S. typhimurium*, and *Shigella dysenteriae*). We have already demonstrated (4, 5) the transfer of F'*lac* and R factors to plant strains of *E. herbicola*, the species one most often implicated in human infections (19), and the donor ability of the exconjugants for *lac* and R factors. Our preliminary observations (A. K. Chatterjee and M. P. Starr, in preparation) suggest that a gene transmission system similar to the one reported here exists in some Lac^+ exconjugants of *E. herbicola* (harboring an *E. coli* F'-lac). All these findings point strongly to the likely common occurrence of competence for genetic interaction among these *Erwinia* spp. and other enterobacteria, and the probable contribution of such interactions to the pathogenicity of these organisms for plants and animals.

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LITERATURE CITED

1. Adelberg, E. A., M. Mandel, and G. C. C. Chen. 1965. Optimal conditions for mutagenesis by N-methyl-N'-nitro-N-nitrosoguanidine in *Escherichia coli* K12. Biochem. Biophys. Res. Commun. 18:788–795.
2. Beck, C. F., and J. L. Ingraham. 1971. Location on the chromosome of *Salmonella typhimurium* of genes governing pyrimidine metabolism. Mol. Gen. Genet. 111:303–316.
3. Broda, P. 1967. The formation of Hfr strains in *Escherichia coli* K12. Genet. Res. 9:35–47.
4. Chatterjee, A. K., and M. P. Starr. 1972. Genetic transfer of episomic elements among *Erwinia* species and other enterobacteria: F'*lac*$^+$. J. Bacteriol. 111:169–176.
5. Chatterjee, A. K., and M. P. Starr. 1972. Transfer among *Erwinia* spp. and other enterobacteria of antibiotic resistance carried on R factors. J. Bacteriol. 112:576–584.
6. Chatterjee, A. K., and M. P. Starr. 1973. Transmission of *lac* by the sex factor E in *Erwinia* strains from human

clinical sources. Infect. Immunity 8:563-572.
7. Cuzin, F., and F. Jacob. 1963. Integration réversible de l'épisome sexuel F' chez *Escherichia coli* K12. C.R. Acad. Sci. **257**:795-797.
8. Hirota, Y. 1960. The effect of acridine dyes on mating type factors in *Escherichia coli*. Proc. Nat. Acad. Sci. U.S.A. **46**:57-64.
9. Lakso, J. U., and M. P. Starr. 1970. Comparative injuriousness to plants of *Erwinia* spp. and other enterobacteria from plants and animals. J. Appl. Bacteriol. **33**:692-707.
10. Lawton, W. D., B. C. Morris, and T. W. Burrows. 1968. Gene transfer in strains of *Pasteurella pseudotuberculosis*. J. Gen. Microbiol. **52**:25-34.
11. Low, K. B. 1972. *Escherichia coli* K-12 F-prime factors, old and new. Bacteriol. Rev. **36**:587-607.
12. Macrina, F. L., and E. Balbinder. 1972. Genetic characterization of a stable F'*lac* plasmid. J. Bacteriol. **112**:503-512.
13. Mäkelä, P. H. 1963. Hfr males in *Salmonella abony*. Genetics **48**:423-429.
14. Matney, T. S., and N. E. Achenbach. 1962. A comment on the fertility of F_2 donor types of *Escherichia coli* K12. Biochem. Biophys. Res. Commun. **9**:285-287.
15. Pittard, J., and E. A. Adelberg. 1963. Gene transfer by F' strains of *Escherichia coli* K-12. II. Interaction between F-merogenote and chromosome during transfer. J. Bacteriol. **85**:1402-1408.
16. Pittard, J., J. S. Loutit, and E. A. Adelberg. 1963. Gene transfer by F' strains of *Escherichia coli* K-12. I. Delay in initiation of chromosome transfer. J. Bacteriol. **85**:1394-1401.
17. Sanderson, K. E. 1972. Linkage map of *Salmonella typhimurium*, edition IV. Bacteriol. Rev. **36**:558-586.
18. Sanderson, K. E., H. Ross, L. Ziegler, and P. H. Mäkelä. 1972. F^+, Hfr, and F' strains of *Salmonella typhimurium* and *Salmonella abony*. Bacteriol. Rev. **36**:608-637.
19. Starr, M. P., and A. K. Chatterjee. 1972. The genus *Erwinia:* enterobacteria pathogenic to plants and animals. Annu. Rev. Microbiol. **26**:389-426.
20. Taylor, A. L., and C. D. Trotter. 1972. Linkage map of *Escherichia coli* strain K-12. Bacteriol. Rev. **36**:504-524.