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Donor Strains of the Soft-Rot Bacterium *Erwinia chrysanthemi* and Conjugational Transfer of the Pectolytic Capacity

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Donor strains of *Erwinia chrysanthemi* ICPB EC16, a member of the soft-rot (pectolytic) section of the enterobacterial genus *Erwinia*, were obtained by chromosomal integration of an F′*lac*+ plasmid originating from *Escherichia coli*. These stable donor strains, selected from an unstable F′*lac*+ heterogenote by repeated platings of single Lac+ colonies on lactose minimal agar, do not segregate (as does the parent F′*lac*+ heterogenote) into Lac− or F− clones, in either the presence or absence of acridine orange. One representative donor strain (from the 12 that have been selected) has been examined in more detail; it can transfer *ade*+, *gal*+, *gtu*+ (utilization of galacturonate), *his*+, *lac*+, *leu*+, *lys*+, *mcu*+ (multiple carbohydrate utilization), *pat*+ (production of polygalacturonic acid *trans*-eliminase), *thr*+, and *trp*+ in a polarized manner to appropriate recipient strains of *E. chrysanthemi*; the frequencies of *ade*+, *leu*+, and *thr*+ transfer were higher than those of the other markers tested to date. This donor strain transfers *lac*+ genes during a 6-h mating on membranes; most of the Lac+ recombinants are donors of chromosomal markers. The kinetics of entry as well as the frequencies of transfer of chromosomal markers indicate that *thr*+ and *leu*+ enter the recipient as proximal markers and that *lac*+ enters as a distal marker. Analysis of the recombinants demonstrates close linkage between *thr* and *leu*, *ade* and *thr*, *his* and *pat*, and *his* and *trp* loci. The results suggest that the integration of F′*lac*+ into the chromosome of *E. chrysanthemi* has occurred at a region adjacent to the *leu-thr* loci, and that the chromosome is transferred in the following sequence: origin----*leu*--*thr*--*ade*--*lys*--*mcu*--*pat*--*his*--*trp*--*gal*--*gtu*--*lac*--F. Plant-tissue maceration occurs in Pat+ recombinants and not in Pat− recombinants, even though both form another pectolytic enzyme, hydrolytic polygalacturonase. This genetic evidence supports the idea that the *E. chrysanthemi* polygalacturonic acid *trans*-eliminase plays an essential role in bringing about plant-tissue maceration.

The soft-rot or pectolytic section of the genus *Erwinia* (25) contains those phytopathogenic enterobacteria that have a marked capability to macerate plant tissues and thus to bring about soft-rot diseases and storage rots of plants or plant parts. The plant-tissue maceration is mediated, at least in part, by a set of pectolytic enzymes that collectively digest the pectic substances of the plant cell walls (2–4).

As part of our exploration of the physiology and genetics of the genus *Erwinia* (5–9, 21, 25), we have undertaken to develop a conjugational gene transfer system in the soft-rot section of that genus. We report here (i) the successful isolation of donor strains from one member of this group, *Erwinia chrysanthemi*; (ii) some of the properties of one of these donor strains, including its ability to transfer a gene (*pat*) that controls the production and expression of a pectolytic enzyme, polygalacturonic acid *trans*-eliminase (PATE); and (iii) some genetic evidence pointing toward an essential role of PATE in causing plant-tissue maceration.

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MATERIALS AND METHODS

Bacterial strains. The strains of *Erwinia chrysanthemi* used in this study are listed in Table 1, together with information about their origin and other relevant properties. The M-13 male-specific bacteriophage preparation was obtained from David Pratt, University of California, Davis.

Culture media and reagents. The media and reagents used in our laboratory are detailed in our previous publications (5–9, 21, 26).

Assay methods for pectolytic ability, pectolytic enzymes, and plant-tissue maceration. The procedures described by Starr et al. (26) were used.

Isolation of donor and recipient strains. The principles and procedures used to isolate donor and

TABLE 1. *Erwinia chrysanthemi strains*

Strain	Characters[a]	Source[b]
EC16	Wild-type, prototrophic, Str^s *lac*, virulent, pectolytic[c]	ICPB; available as ATCC 11662
EC16 F'*lac*$^+$	As for EC16, also carrying F'*lac*$^+$ plasmid derived from *E. coli*	Chatterjee and Starr (ref. 6)
AC4001	*thr-1* Str^r	EMS mutagenesis of EC16 and spontaneous resistance to streptomycin
AC4005	*thr-1 ade-2* Str^r	EMS mutagenesis of AC4001
AC4008	*thr-1 leu-1* Str^r	EMS mutagenesis of AC4001
AC4009	*thr-1 leu-2* Str^r	EMS mutagenesis of AC4001
AC4011	*thr-1 trp-1* Str^r	EMS mutagenesis of AC4001
AC4012	*thr-1 lys-1* Str^r	EMS mutagenesis of AC4001
AC4013	*thr-1 his-1* Str^r	EMS mutagenesis of AC4001
AC4019	*thr-1 gtu-1* Str^r	EMS mutagenesis of AC4001
AC4020	*thr-1 gal-1* Str^r	EMS mutagenesis of AC4001
AC4022	*thr-1 leu-1 mcu-1* Str^r	EMS mutagenesis of AC4008
AC4023	*thr-1 his-1 trp-1* Str^r	EMS mutagenesis of AC4013
AC4024	*thr-1 his-1 pat-1* Str^r	EMS mutagenesis of AC4013
AC4052	Hfr-8, prototrophic, Str^s	This paper; derived from EC16 F'*lac*$^+$

[a] Designation of uncommon markers: *gtu*$^+$, D-galacturonic acid utilization; *pat*$^+$, PATE production and excretion; *mcu*$^+$, multiple carbohydrate utilization (glycerol, arabinose, xylose, galacturonic acid, polygalacturonic acid). All strains listed are *hpg*$^+$; that is, they produce hydrolytic PG.

[b] ICPB, International Collection of Phytopathogenic Bacteria, Davis, Calif.; EMS, ethyl methane sulfonate.

[c] EC16 is a naturally isolated Lac$^-$ strain.

recipient strains of *Erwinia chrysanthemi* were similar to those we have used with *Erwinia amylovora* (8). Ethyl methane sulfonate (Eastman Kodak Co., Rochester, N.Y.) mutagenesis was carried out according to the procedure of Lin et al. (15). Penicillin enrichment was done by the technique described by Clowes and Hayes (10). Strains of *E. chrysanthemi* that no longer are able to degrade polygalacturonic acid were detected on polygalacturonate-yeast extract agar (PEC-YA; 26). Replicate plates of PEC-YA medium at pH 8.0 were flooded with 2 N HCl after 24 h of growth to visualize the nonpectolytic colonies, which then were recovered from the corresponding loci on the untreated replicate plates. In this procedure (26), pectolytic colonies form clear halos in a turbid background and nonpectolytic colonies do not form such halos (Fig. 1).

Mating conditions. Crosses were performed both in broth and on membrane filters by the procedures described by us elsewhere (6-9). Interrupted matings were done on membrane filters, using the following modification of procedure B detailed by Chatterjee and Starr (8). Cells of donor and recipient strains (in a ratio of 1:10) were collected on membranes. The membranes were then placed on soft Luria agar and incubated at 30°C. A drop of Luria broth was placed on the membranes at hourly intervals to prevent excessive drying of the cells. At desired intervals, membranes were removed and cells were resuspended in phosphate buffer (55 mM, pH 7.2). Mating was interrupted by vigorously agitating the cell suspension for 1 min on a MaxiMix (Thermolyne Sybron Corp., Dubuque, Iowa), and appropriate dilutions (0.1 ml) were spread on the surface of the selective agar medium. The plates were incubated at 30°C, and recombinants were enumerated after 3 to 4 days of incubation. Controls, which consisted of donor and recipient cells separately, were always included.

RESULTS

Isolation of donor strains. We had shown earlier that the F'*lac*$^+$ plasmid was readily transferred to *Erwinia chrysanthemi* strain ICPB EC16 from *Escherichia coli* (6). However, the F' factor was very unstable in the resulting *E. chrysanthemi* heterogenotes. Our current, successful efforts to isolate *E. chrysanthemi* clones in which the F' factor is stable resulted from repeated single-colony selections of Lac$^+$ clones when the heterogenote strain EC16 F'*lac*$^+$ was grown on minimal agar medium containing lactose as the sole source of carbon and energy. After several such transfers and selections, clones were obtained that were stable with respect to the Lac$^+$ property. Twelve such Lac$^+$ clones were tested for segregation to the Lac$^-$ and F$^-$ states in the presence and absence of acridine orange. None of these clones produced Lac$^-$ or F$^-$ segregants (the latter determined by insensitivity to the male-specific phage, M-13), whereas the parent heterogenote (EC16 F'*lac*$^+$, containing the F'*lac*$^+$ plasmid) continued—in the presence of acridine orange—to produce Lac$^-$ and F$^-$ clones at a very high frequency (90 to 95% of the clones tested were both Lac$^-$ and F$^-$). These 12 clones (but not the parent heterogenote, strain EC16 F'*lac*$^+$) had the capacity to transfer chromosomal markers (see below). One such clone, AC4052 (Hfr-8), was examined in more detail.

Transfer of chromosomal genes. (i) Auxotrophic markers. The results summarized in Table 2 demonstrate the transfer of the following

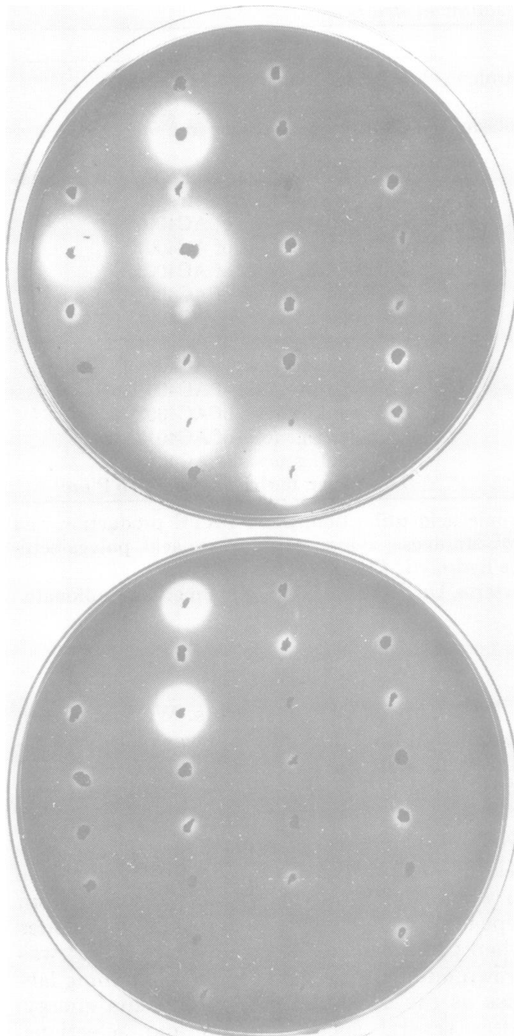

FIG. 1. *Method for the scoring of the inheritance of pat^+ as an unselected marker among different classes of Erwinia chrysanthemi recombinants. The medium is PEC-YA at pH 8.0 (26), containing streptomycin (200 μg/ml) to prevent the multiplication of Str^s donor cells that might have been carried over from the initial selective medium. The recombinants (His^+ on top plate, Thr^+ on bottom plate) from a cross between Hfr-8 (Pat^+, prototrophic, Str^s) and AC4024 (Pat^- Thr^- His^- Str^r) were patched on PEC-YA medium (at pH 8.0) containing streptomycin. The plates were flooded with 2 N HCl after overnight incubation at 30°C. Note that five out of 26 His^+ clones (top plate) and two out of 26 Thr^+ clones (bottom plate) have degraded polygalacturonic acid, as is shown by the clear halos around the Pat^+ colonies in an otherwise turbid background.*

genes by Hfr-8: *ade^+, gal^+, gtu^+, his^+, leu^+, lys^+*, and *thr^+*. The transfer of these genes from the parent heterogenote strain (EC16 F′*lac^+*) or from the wild-type strain (EC16) did not occur (detailed data not presented). The *ade^+*, *thr^+*, and *leu^+* markers were transferred at higher frequencies than the other markers. Clones representative of the various recombinant classes from crosses between Hfr-8 and appropriate recipient strains (Table 2) were purified by repeated streakings on the selective medium and tested for the inheritance of *lac^+* and F genes and the ability to degrade polygalacturonic acid. All the recombinants tested (except those selected for Gtu^+ and Lac^+; see [ii] below) were Lac^- and F^-, and all could degrade polygalacturonic acid.

The kinetics of the marker transfer are shown in Fig. 2. The *thr^+* and *leu^+* markers are transferred at about 42 min, *lys^+* at 84 min, and *his^+* at 118 min subsequent to adsorption of the donor and recipient cells onto the membrane. It should be noted that these time estimates are relative, because the recombinants were derived from independent mating mixtures collected on separate membranes.

The results (Table 3) summarizing the effects of mating conditions on the frequency of marker transfer indicate the need for a continued membrane mating for the distal markers to be transferred from the donor strain (*his^+* is transferred only when the mating is carried out on a membrane and not when the mating is performed in broth). These results (Table 3) also suggest that

TABLE 2. *Transfer of chromosomal markers from Erwinia chrysanthemi donor strain (Hfr-8) to E. chrysanthemi recipient strains*[a]

Recipient	Selection[b]	Frequency of transfer (recombinants/input donor cell)
AC4005	Thr^+	2.2×10^{-4}
	Ade^+	1.0×10^{-4}
AC4008	Thr^+	2.0×10^{-4}
	Leu^+	3.5×10^{-4}
AC4011	Thr^+	2.2×10^{-4}
	Trp^+	3.2×10^{-5}
AC4012	Thr^+	2.5×10^{-4}
	Lys^+	8.0×10^{-5}
AC4013	Thr^+	2.0×10^{-4}
	His^+	5.0×10^{-5}
AC4019	Gtu^+	2.0×10^{-8}
AC4020	Gal^+	1.0×10^{-8}
AC4022	Thr^+	1.0×10^{-4}
	Leu^+	9.0×10^{-5}
	Mcu^+	7.5×10^{-5}
AC4023	Thr^+	1.5×10^{-4}
	His^+	2.0×10^{-5}
	Trp^+	1.5×10^{-5}
	Lac^+	3.0×10^{-8}

[a] Matings were done on membrane filters for 6 h at 30°C.

[b] See footnote *a*, Table 1, for uncommon marker designations.

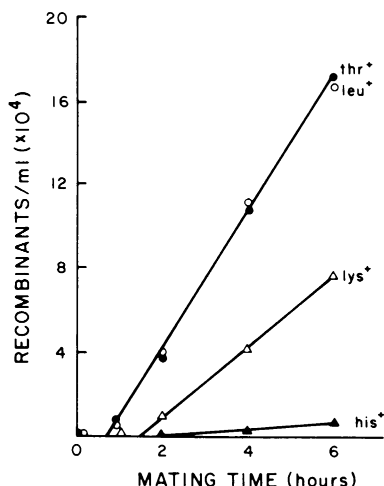

FIG. 2. *Kinetics of entry of chromosomal markers from Erwinia chrysanthemi Hfr-8 to E. chrysanthemi recipient strains:* ●, *AC4013 (thr);* ○, *AC4008 (leu);* △, *AC4012 (lys);* ▲, *AC4013 (his). For details of experimental conditions, refer to the text.*

TABLE 3. *Effect of mating conditions on the frequency of transfer of chromosomal markers from Erwinia chrysanthemi Hfr-8 to E. chrysanthemi recipient strain AC4013*

Mating conditions[a]	Frequency of transfer (recombinants/input donor cell) of:	
	thr$^+$	*his*$^+$
In broth[b]		
Static	5.0×10^{-7}	$<10^{-8}$
Shaken	7.5×10^{-6}	$<10^{-8}$
Adsorption on membranes, followed by incubation in broth[c]	1.5×10^{-5}	$<10^{-8}$
Adsorption and incubation on membranes[d]	1.4×10^{-5}	6.0×10^{-7}

[a] For details of experimental conditions, refer to Chatterjee and Starr (8). Mating was carried out for 5 h at 30°C.
[b] Procedure A of Chatterjee and Starr (8).
[c] Procedure C of Chatterjee and Starr (8).
[d] Procedure B of Chatterjee and Starr (8).

the effective mating pair (11) is formed after adsorption of the donor and recipient cells onto the membrane, but that the mating pair formed is fragile and is broken when the cells are dislodged into the broth even under gentle conditions. Despite this limitation, it is clear that the donor strain transfers the chromosomal markers in a polarized fashion: *thr*$^+$ and *leu*$^+$ enter the recipient cells as early markers and *his*$^+$ enters as a late marker. This conclusion is further substantiated by the data on the frequency of transfer of these markers in a 6-h mating (Table 2). The data in Table 2 also reveal that *lac*$^+$ genes are transferred from Hfr-8 to AC4023 at a very low frequency. The purified Lac$^+$ clones were subsequently tested for donor ability of the *leu*$^+$ marker by cross-streaking against the recipient strain (AC4009) and also by spotting on a lawn of the recipient strain. Nineteen out of 20 recombinant clones tested (95% of the total recombinant clones) transferred *leu*$^+$ at a high frequency; the one remaining Lac$^+$ recombinant transferred *leu*$^+$ at a low frequency. These results suggest that the *lac*$^+$ genes are transferred as a terminal marker, and that the majority of the Lac$^+$ recombinants also inherit F genes and behave as Hfr strains, probably as a result of the integration of the *lac-* and F-containing region of the exogenote.

The data on the inheritance of unselected markers in recombinants from 6-h matings (Table 4) reveal a close linkage between *thr* and *leu*, *ade* and *thr*, and *trp* and *his*; some linkage between *thr* and *lys*, *thr* and *his*, *thr* and *trp*, and *gtu* and *lac*; no linkage between *thr* and *gtu*, *lac* and *his*, and *lac* and *trp*.

(ii) Transfer of *mcu*$^+$. The recipient strain AC4022 was derived from AC4009 after ethyl methane sulfonate treatment. This mutant strain (AC4022) was originally detected as an Xyl$^-$ clone. However, subsequent studies revealed that (i) AC4022 failed to utilize an assortment of carbon sources (arabinose, galactose, galacturonic acid, glycerol, polygalacturonic acid, and xylose) that supported the growth of the parent strain (AC4009) and the wild-type strain (EC16), (ii) it did not degrade polygalacturonic acid, and (iii) it was totally avirulent (it did not cause tissue maceration on celery petioles, potato tuber slices, and carrot root slices). Growth of AC4022 on any of the aforementioned carbon sources also did not occur where exogenous cyclic adenosine 3',5'-monophosphate (Sigma) was added to the growth medium (16), but growth did occur on glucose in the presence or absence of cyclic adenosine 3',5'-monophosphate. Spontaneous revertants of AC4022 that grew on galacturonic acid or polygalacturonic acid could also utilize the other carbon sources (arabinose, galactose, glycerol, and xylose). These properties of this mutant strain (AC4022) are apparently similar to those of *crp* (cyclic adenosine 3',5'-monophosphate receptor protein; 1) mutants of *Escherichia coli* (20). However, since the nature of the gene product affected in *Erwinia chrysanthemi* is yet to be identified, we have tentatively and noncommittally designated the mutant gene locus *mcu* (multiple carbohydrate utilization). The *mcu* locus (as judged by xylose utilization) is linked to the *thr* and *leu* loci (29% coinheritance with Thr$^+$ and 22% coinheritance with Leu$^+$; Table 4).

(iii) Transfer of *pat*$^+$. The gene responsible for the production of PATE is here designated

TABLE 4. *Frequency of unselected markers among recombinants from matings between Erwinia chrysanthemi Hfr-8 donor strain (prototrophic, Str^s) and various E. chrysanthemi recipient strains*[a]

Recipient strain (relevant genotype)	Selected phenotype (no. of clones analyzed)	Unselected phenotype	Frequency of unselected phenotype (% of total clones tested)
AC4005 (*thr-1 ade-2* Str^r)	Ade⁺ (149)	Thr⁺	20
	Thr⁺ (90)	Ade⁺	26
AC4008 (*thr-1 leu-1* Str^r)	Leu⁺ (46)	Thr⁺	61
	Thr⁺ (47)	Leu⁺	68
AC4011 (*thr-1 trp-1* Str^r)	Trp⁺ (50)	Thr⁺	20
	Thr⁺ (18)	Trp⁺	0
AC4012 (*thr-1 lys-1* Str^r)	Lys⁺ (50)	Thr⁺	16
	Thr⁺ (11)	Lys⁺	10
AC4013 (*thr-1 his-1* Str^r)	His⁺ (50)	Thr⁺	14
	Thr⁺ (44)	His⁺	2
AC4019 (*thr-1 gtu-1* Str^r)	Gtu⁺ (6)	Thr⁺	0
		Lac⁺	17
AC4022 (*thr-1 leu-1 mcu-1* Str^r)	Mcu⁺[b] (100)	Thr⁺	29
		Leu⁺	22
AC4023 (*thr-1 his-1 trp-2* Str^r)	Thr⁺ (104)	His⁺	5
		Trp⁺	0
		Lac⁺	0
	His⁺ (88)	Thr⁺	9
		Trp⁺	31
		Lac⁺	0
	Trp⁺ (203)	His⁺	63
		Thr⁺	15
		Lac⁺	0
AC4024 (*thr-1 his-1 pat-1* Str^r)	Thr⁺ (78)	Pat⁺[c]	6
		His⁺	3
	His⁺ (78)	Pat⁺[c]	19
		Thr⁺	11

[a] Matings were performed on membranes for 6 h at 30°C, according to the procedure described in the text. See footnote *a*, Table 1, for uncommon marker designations.

[b] Mcu⁺ selection was based upon the ability of the Mcu⁺ recombinant and the inability of the Mcu⁻ parent recipient strain to grow in the presence of xylose as the sole source of carbon.

[c] The Pat⁺ phenotype was scored on PEC-YA medium at pH 8.0 (26) containing streptomycin (200 μg/ml); see Results.

pat⁺. Pat⁻ mutants defective in PATE synthesis and excretion were obtained by scoring (Fig. 1) mutant clones on PEC-YA medium at pH 8.0 (26), after ethyl methane sulfonate treatment of the culture (16). Our choice of pH 8.0 for screening Pat⁻ mutants was dictated by the vastly different pH optima of the two major pectolytic enzymes produced by the soft-rot enterobacteria: *Erwinia* PATE acts best at about pH 8.3 (17), and *Erwinia* hydrolytic polygalacturonase (PG) acts best at about pH 5.4 (19). Using this procedure (26), we have isolated an assortment of mutants that exhibit different patterns of synthesis and excretion of PATE. For the present studies, we chose one particular mutant strain, AC4024, that has about 15% of the total PATE activity relative to the AC4013 parent strain (Table 5). Most of this reduced PATE activity is located in the cells of the AC4024 mutant strain (less than 6% of the total PATE activity is detectable in the culture supernatant) as opposed to the parent strain, in which about 99% of the much higher total PATE activity is present in the culture supernatant (Table 5). The *pat* mutant strain (AC4024) and the *pat*⁺ parent strain (AC4013) have similar levels of the hydrolytic PG activity (detailed data not presented here), and hence both strains can utilize polygalacturonic acid as the sole source of carbon, AC4013 by means of both PATE and PG and AC4024 by means mainly of PG. As might be expected from the differing pH optima of the two pectolytic enzymes alluded to above, this mutant strain (AC4024) shows very little polygalacturonic acid degradation on PEC-YA medium at pH 8.0. This behavior provided a means to score PATE production and excretion as an unselected marker on PEC-YA medium at pH 8.0 (Fig. 1). The linkage data summarized in Table 4 reveal that *pat*⁺ is inherited by 19%

TABLE 5. *PATE activity and plant-tissue macerating ability in various strains of Erwinia chrysanthemi including representative recombinant clones from a cross between E. chrysanthemi Hfr-8 and E. chrysanthemi recipient strain AC4024*[a]

Phenotype[b]	Strain (clone)	PATE: U/ml of culture: Total	PATE: U/ml of culture: Supernatant	PATE: U/ml of culture: % of total in supernatant	PATE: Sp act: Cells	PATE: Sp act: Supernatant	Plant-tissue maceration[c]
Wild-type, prototrophic, Pat⁺ Str^s, virulent	EC16	9.1	7.5	83.0	1.0	23.0	+
Prototrophic, Lac⁺, donor male, Pat⁺ Str^s, virulent	Hfr-8	10.5	9.5	95.5	0.9	35.0	+
Thr⁻ His⁻ Pat⁺ Str^r, virulent	AC4013	12.5	12.4	99.0	0.2	39.0	+
Thr⁻ His⁻ Pat⁻ Str^r, avirulent	AC4024	1.8	0.1	5.4	1.7	0.5	−
Thr⁻ His⁺ Pat⁺ Str^r	Recombinant 2[d]	16.7	16.5	99.0	0.2	75.0	+
Thr⁺ His⁺ Pat⁺ Str^r	Recombinant 11[d]	17.0	16.8	99.0	0.2	70.0	+
Thr⁺ His⁺ Pat⁻ Str^r	Recombinant 18[d]	1.3	0.1	7.7	1.0	0.5	−
Thr⁻ His⁺ Pat⁻ Str^r	Recombinant 19[d]	1.3	0.1	7.7	0.9	0.6	−
Thr⁺ His⁻ Pat⁻ Str^r	Recombinant 26[d]	1.5	0.1	6.7	1.3	0.6	−

[a] For details of experimental conditions, see Materials and Methods. The cultures were grown overnight (16 h) on polygalacturonic acid-salts-Casamino Acids medium (26) at 30°C. The cells were removed by centrifugation (17,000 × *g*) for 10 min at 4°C, washed with tris(hydroxymethyl)aminomethane (Tris)-hydrochloride buffer (0.1 M, pH 7.0), resuspended in the same buffer, sonically treated, and assayed for activity. The supernatants were dialyzed against 200 volumes of Tris-hydrochloride (5 mM, pH 7.0) buffer and assayed for activity. Refer to Starr et al. (26) for assay conditions.

[b] All strains listed are *hpg*⁺; that is, they all produce hydrolytic PG.

[c] Maceration (rotting) of plant tissues was tested on celery petioles, potato tuber slices, and carrot root slices according to the procedures detailed by Starr et al. (26). Results with all three plant tissues were essentially the same, but the results listed here are those obtained with potato tuber slices.

[d] The recombinant clones were grown on polygalacturonate-salts-Casamino Acids medium (26) for enzyme assays, after purification by repeated streaking on the selective medium. Assays were done on several clones belonging to each of the recombinant classes. The data for a single recombinant clone that represents a particular recombinant class are presented here.

of the His⁺ clones and 6% of the Thr⁺ clones; Thr⁺ is inherited by 10% of His⁺ clones; and His⁺ is inherited by 3% of Thr⁺ clones. This pattern suggests that *pat* is located between *thr* and *his* and that *pat* is somewhat closer to *his* than to *thr*. It should be noted that the results of enzyme assays (Table 5) on the various classes of recombinants (that were delineated by scoring on PEC-YA medium at pH 8.0) clearly demonstrate the validity of the PEC-YA medium (at pH 8.0) in the plate assay technique for scoring Pat⁺ and Pat⁻ phenotypes. The recombinant clones that are scored as Pat⁺ on PEC-YA (Fig. 1) have levels (Table 5) of the PATE enzyme similar to that of the Pat⁺ parent strain (AC4013) from which the recipient strain was derived, whereas those scored as Pat⁻ on PEC-YA medium at pH 8.0 (Fig. 1) have about the same reduced level (Table 5) of the PATE enzyme as the recipient strain (AC4024).

Plant-tissue maceration by Pat⁺ recombinants. The results summarized in Table 5 show that Pat⁺ recombinants from crosses between the donor strain (Hfr-8, *pat*⁺, *hpg*⁺, virulent) and the recipient strain (AC4024, *pat hpg*⁺, avirulent) cause maceration (rotting) of celery petioles, potato tuber slices, and carrot root slices. The Pat⁻ recombinants that have inherited either one or both of the auxotrophic markers (*thr*⁺ *his*⁺) but not *pat*⁺ do not cause maceration of the plant tissues, even though they do have the capacity to produce the hydrolytic PG (marker symbol *hpg*⁺).

DISCUSSION

The results of the present study demonstrate the isolation of donor strains of *Erwinia chrysanthemi* using an *Escherichia coli* F′*lac*⁺ plasmid. Although we do not at present have direct molecular or genetic evidence for the integration of the F′ factor into the chromosome of *E. chrysanthemi*, the following indirect evidence strongly suggests that the donor (Hfr) strains arose from the F′ parent strain as a result of recombinational events between the chromosome and the F′ factor: (i) both Lac⁺ and F⁺ properties are stable in the parent F′ heterogenote strain; (ii) acridine orange does not cause the elimination of these properties from donor strains but does cause loss of these properties from the parent strain; and (iii) chromosomal markers are transferred by the donor strains in a polarized manner, whereas none of the chromosomal markers tested can be transferred by the parent strain. Furthermore, (iv) although the F′ and *lac*⁺ genes are readily transferred from the *E. chrysanthemi* F′*lac*⁺ heterogenote strain to recipient *E. chrysanthemi* strains (6),

neither is readily transferred from the donor strain (AC4052 = Hfr-8); the latter strain has both F genes (as judged by M-13 sensitivity and donor ability) and lac^+ genes (Table 3). The present study suggests that lac^+ is transferred from Hfr-8 as a terminal marker. Whereas Lac$^-$ recombinants are infertile, most of the Lac$^+$ recombinants inherit F genes and are capable of transferring chromosomal markers at a high frequency. These properties are typical of *E. coli* recombinants that have inherited the terminal markers from Hfr donor strains (4). The acquisition of the Hfr state upon integration of F′ factors into the chromosome is known in other enterobacterial systems (23, 24), such as *Citrobacter freundii* (12) and *Erwinia amylovora* (8), and thus supports our assumption.

Given the strong conservation of gene order in the enterobacteria (23), we expect that the gene order on the chromosome of *Erwinia chrysanthemi* is similar to that of *Escherichia coli* (1) and *Salmonella typhimurium* (22–24). Based on the evidence we have obtained thus far and the foregoing reasonable assumption, it seems likely that the integration of F′lac^+ in *E. chrysanthemi* Hfr-8 has occurred close to the *leu* region and that the deoxyribonucleic acid is transferred with leu^+ and thr^+ as proximal markers followed by ade^+, lys^+, mcu^+, pat^+, his^+, trp^+, gal^+, gtu^+, lac^+, and F genes.

It is evident that this genetic system can be used for locating the genes on the chromosome of *E. chrysanthemi*. In this preliminary study, we have demonstrated close linkage between the *thr* and *leu* loci, the *ade* and *thr* loci, and the *trp* and *his* loci; the results of the times-of-entry experiments also support this conclusion. It should be noted that these pairs of loci are closely linked also on the *E. coli* chromosome (1) and the *S. typhimurium* chromosome (22, 23).

Our results reveal that the gene (*pat*) that controls the production and excretion of the enzyme PATE is linked to the *his* locus on the chromosome of *E. chrysanthemi*. In addition, the results suggest that the production of PATE and the production of hydrolytic PG, two quite different enzymes that are involved in the degradation of polygalacturonic acid (17, 19, 25, 26), are controlled (at least in part) by separate gene loci in *E. chrysanthemi*.

The data on the PATE enzyme assays (Table 5) suggest that a single-site mutation in the recipient strains has altered both the synthesis and the excretion of the PATE protein molecule. The AC4024 recipient strain possesses about 15% of the total PATE activity found in the AC4013 parent pat^+ strain, and less than 6% of this reduced enzyme activity is present in the culture supernatant. The recombinant class scored as His$^+$ Pat$^+$ produces PATE levels similar to the parent strain (AC4013) from which the recipient strain (AC4024) was derived (both total PATE units and PATE units in supernatants are comparable), whereas all those recombinants scored as His$^+$ Pat$^-$ have much lower enzyme levels, similar to that found in the recipient strain.

It should be noted that thus far we have not obtained a recombinant or a mutant in which the processes of PATE synthesis and excretion are separable. This fact, taken together with our finding that the processes are coupled tightly in *E. chrysanthemi* strain EC16 but loosely in *E. carotovora* strain EC153 (26; A. K. Chatterjee, G. E. Buchanan, M. K. Behrens, and M. P. Starr, manuscript in preparation), leads us to believe that in *E. chrysanthemi* the nature of the protein (PATE) molecule determines both the transport across the membrane of the molecule and, at least in part, the *trans*-eliminase activity. If there were two linked genes, one for PATE synthesis and another for PATE excretion, we would have expected the segregation of these genes in some of the recombinants. We have not detected, in this preliminary analysis, any recombinant *E. chrysanthemi* clones that produce a wild-type level of PATE and yet do not excrete the PATE activity. Another possibility, which we have not yet ruled out, is that AC4024 might have a polar mutation that results in a pleiotropic effect in terms of decreased synthesis and excretion of PATE.

In spite of the uncertainty regarding the specific site of mutation (i.e., whether in a regulator locus or in the *pat* structural gene) in AC4024, the results summarized in Table 5 suggest the essential role of PATE activity in plant-tissue maceration caused by *E. chrysanthemi* (2, 3, 13, 18). The contributions of other *Erwinia* degradative enzymes to the plant-tissue maceration process are not yet understood. Clearly, more work is needed to gain an adequate understanding of the patterns of synthesis and excretion of PATE and of other extracellular enzymes that have been implicated in the plant virulence of the soft-rot enterobacteria. This conjugational system can be used to construct desired strains for pathobiological studies, and it undoubtedly will prove to be a valuable tool in analyzing the physiology of virulence in these pectolytic soft-rot bacteria. Our current efforts are directed to this end, as well as towards the construction of a gene linkage map in *E. chrysanthemi*.

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