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Infection of *Salmonella typhimurium* with Coliphage Mu *d*1 (Ap^r *lac*): Construction of *pyr*::*lac* Gene Fusions

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A procedure was developed for introducing the coliphage Mu *d*1 (Ap^r *lac*) into *Salmonella typhimurium* in order to construct gene fusions that place the structural genes of the *lac* operon under the control of the promoter-regulatory region of other genes. To introduce Mu *d*1 from *Escherichia coli* K-12 into *S. typhimurium*, which is normally not a host for Mu, we first constructed an *E. coli* double lysogen carrying the defective Mu *d*1 phage and a Mu-P1 hybrid helper phage (MuhP1) that confers the P1 host range. A lysate prepared from this strain was used to infect a P1-sensitive (i.e., *galE*), restriction-deficient, modification-proficient strain of *S. typhimurium*, and a double lysogen carrying Mu *d*1 and MuhP1 was isolated. Induction of the latter strain produced lysates capable of infecting and generating gene fusions in P1-sensitive strains of *S. typhimurium*. In this paper we describe the construction of *pyr*::*lac* fusions by this technique.

The recently developed techniques for fusing in vivo the structural genes of the lactose (*lac*) operon to the promoter region of any gene or operon of interest have provided a powerful tool for studying the regulation of gene expression (5, 7-9). The products of such fused genes are easily measured, and mutations affecting their expression can be readily selected. The simplest technique for fusing genes is the one-step procedure developed by Casadaban and Cohen (9). This procedure uses a specialized transducing Mu phage, Mu *d*1 (Ap^r *lac* cts62), which carries near one end of the Mu genome the structural genes of the *lac* operon without the *lac* promoter. Upon infection of Mu-sensitive bacteria, the Mu *d*1 genome can integrate into the host chromosome at apparently random sites. If it becomes integrated into a transcribed region in the proper orientation, transcription directed by the adjacent promoter-regulatory region proceeds into the Mu *d*1 genome to express the *lac* structural genes.

The one-step procedure has been useful in constructing gene fusions in *Escherichia coli* K-12, but its use so far has been restricted to this organism because of the limited host range of Mu; for example, Mu does not infect *Salmonella typhimurium* (23), *E. coli* B (23), or *Klebsiella pneumoniae* (4, 21). In this paper we describe a technique for introducing Mu *d*1 into *S. typhimurium*. This technique takes advantage of the fact that the essential Mu genes that determine the phage host range (24) are apparently deleted from Mu *d*1 (M. Casadaban, personal communication). The host range of Mu *d*1 is determined by the helper phage which provides the deleted functions. By using as the helper phage a Mu-P1 hybrid in which the Mu host range genes have been replaced by the analogous genes of phage P1, we have been able to prepare Mu *d*1 lysates capable of infecting P1-sensitive (i.e., *galE* [19]) strains of *S. typhimurium*. In this paper we describe the construction and characterization of *pyr*::*lac* fusions that were obtained by this technique.

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MATERIALS AND METHODS

Chemicals and other materials. Dilithium carbamylphosphate (purity, 90 to 95%), penicillin G (sodium salt), ampicillin trihydrate, *o*-nitrophenyl-β-D-galactopyranoside, and X-gal (5-bromo-4-chloro-3-indolyl-β-D-galactoside) were purchased from Sigma Chemical Co. L-[*U*-^{14}C]aspartic acid (>200 mCi/mmol) and [^{14}C]sodium carbonate (2 to 10 mCi/mmol) were from New England Nuclear Corp. The [^{14}C]aspartic acid was purified before use (20). Analytical-grade cation-exchange resin AG 50W-X12 (200 to 400 mesh, hydrogen form) was obtained from Bio-Rad Laboratories and was washed before use (20). All other chemicals were reagent grade and commerically available.

Bacteria and bacteriophages. The bacterial strains used in this study are listed in Table 1. Mu cts62 hP1≠1, called MuhP1, was isolated as an S$^+$ recombinant arising after infection by Mu cts62 Sam1004 (13) of an Su$^-$ *E. coli* K-12 strain lysogenic for phage P1c1.100CMh (14, 24). MuhP1 has Mu immunity and forms plaques on a number of P1-sensitive, Mu-resistant *E. coli* K-12 strains. Mu cts62 h7629 is a spontaneous mutant of Mu cts62 that can infect *galE*

TABLE 1. *Bacterial strains*

Strain	Genotype	Source and comments
E. coli K-12		
MAL103	F⁻ Mu *d*1 (Ap^r *lac cts*62) Mu *cts*62 Δ(*proAB lacIPOZYA*) *XIII rpsL*	M. Casadaban (9)
JA194	F⁻ *hsdR* Δ*trpE5 thr-1 leuB6 thi-1 lacY1 supE44*	J. Jaynes
JC3272/R751	F⁻ *his lys-301 trp rpsL malA, tsx-317 lacX74 gal-300*/R751	R. A. J. Warren (15)
MH2923	F⁺ Mu *cts*62 hP1≠1 *araD*	M. Howe
KC87	JC3272 [Mu *d*1 (Ap^r *lac cts*62)]/R751	See Results
KC89	MH2923/R751::Mu *d*1 (Ap^r *lac cts*62)	See Results
S. typhimurium LT2		
LT2-Z	Wild type	B. N. Ames
LB82	*zab-403*::Tn*10*	J. Ingraham
JL3103	*argI1843*::Tn*10*	J. Ingraham
JL3404	*galE1122*	J. Ingraham FO^r and P1^s
JL3473	SL4213[Mu *d*1 (Ap^r *lac cts*62) Mu *cts*62 hP1≠1]	See Results
JL3484	*galE1122 pyrA1517*::Mu *d*1 (Ap^r *lac cts*62)	See Results
JL3486	*galE1122 pyrB1518*::Mu *d*1 (Ap^r *lac cts*62)	See Results
JL3487	*pyrB1518*::Mu *d*1 (Ap^r *lac cts*62)	P22(LT2-Z) × JL3486
JL3500	*galE1122 pyrB1519*::Mu *d*1 (Ap^r *lac cts*62)	See Results
JL3501	*galE1122 pyrB1520*::Mu *d*1 (Ap^r *lac cts*62)	See Results
pyrAΔ81	Δ*pyrA81*	B. N. Ames
pyrBΔ655	Δ*pyrB655*	J. Roth
purFΔ145	Δ*purF145*	B. N. Ames
SL4213	*galE496 metA22 metE55 rpsL120 xyl-404* (Fels2)⁻ *H1-b nml⁻ H2-enx*, (*ilv*?) *hsdL6 hsdSA29*	B. A. D. Stocker (10)
TL154	JL3473 transduced to *srl-2*::Tn*10 recA1*	P22(TT521) × JL3473
TL155	SL4213 transduced to *galE*⁺	P22(LT2-Z) × SL4213
TL156	SL4213 (Mu *cts*62 h7629)	See Results
TT521	*srl-2*::Tn*10 recA1*	J. Roth

strains of *S. typhimurium*. Phage P22 HT105/1 *int*201 (3, 22) was provided by John Roth.

Media and culture methods. Nutrient broth (NB), VBCG (0.4% glucose) minimal medium, and N⁻C⁻ minimal medium were prepared as previously described (25). LB medium, R medium, R top agar containing 6.5 g of agar per liter, and H top agar were prepared according to Miller (17). SB medium contained 32 g of tryptone (Difco Laboratories), 20 g of yeast extract (Difco), 5 g of NaCl, 2.5 g of $MgSO_4 \cdot 7H_2O$, and 0.2 g of NaOH per liter. Solid medium contained 15 g of agar (Difco) per liter. Ampicillin was added to liquid and solid media at 25 μg/ml. X-gal indicator plates were N⁻C⁻ medium supplemented with 10 mM NH_4Cl, 2% glucose, 0.5 mM arginine, and 40 μg of X-gal per ml.

All liquid cultures and plates were incubated at 30°C unless indicated otherwise. Liquid cultures were grown with shaking.

Preparation of Mu *cts* lysates by heat induction. Mu *cts* lysogens were grown at 28 to 30°C in SB to a density of 10^8 cells/ml, shifted to 43°C for 30 min, and then incubated at 37°C until lysis (approximately 1 h). Chloroform (1/100 volume) was added, and the lysate was shaken vigorously. Cell debris was removed by centrifugation. Because Mu lysates lose infectivity rapidly (13), they were used immediately. Occasionally, complete clearing was not observed with heat-induced Mu *cts* lysogens of *S. typhimurium*, but titers of approximately 10^9 plaque-forming units/ml were obtained.

Titration of Mu lysates and Mu *d*1-mediated transductions. Mu lysates were titrated by mixing 0.1-ml samples of dilutions in LB with 0.1 ml of a fresh culture of a recipient strain that had been grown to saturation in LB plus 10 mM $CaCl_2$ at 37°C. After incubation for 20 min at 37°C, 2.5 ml of molten (45°C) R top agar was added and the mixture was poured on R plates. Plates were incubated overnight at 37°C, and plaques were counted. Mu *d*1-mediated transductions were performed by mixing 0.2-ml samples of appropriate dilutions of lysate with an equal volume of a fresh culture of a recipient strain that had been grown to saturation in LB plus 10 mM $CaCl_2$ at 37°C. The mixtures were incubated at 30°C for 20 min, and 0.2 ml was spread on an LB plus ampicillin plate.

Preparation of P22 lysates and P22-mediated transductions. P22 HT105/1 *int*201 lysates were prepared as described (12). Mu *cts* lysogenic donor strains were grown at 28°C; all other donor strains were grown at 37°C. Transductions were performed by spreading 10^8 recipient bacteria and dilutions of phage lysates directly on selective medium plates. When the recipient or donor stain was *galE*, it was grown in NB supplemented with 0.2% glucose and 0.1% galactose, which restores P22 sensitivity (11).

Penicillin counterselection of Mu *d*1-induced pyrimidine auxotrophs. A stationary-phase LB cul-

ture of *S. typhimurium* JL3404 (*galE*) (0.1 ml) was plated with 2.5 ml of H top agar containing 10 mM $MgSO_4$ and 5 mM $CaCl_2$ on an LB plus ampicillin plate. Small drops (50 per plate) of a fresh Mu lysate prepared from JL3473 were spotted on the top agar overlay, and the plate was incubated overnight at 28°C. Lysogenic cells were scraped from the centers of 50 spots and used to inoculate a 5-ml VBCG plus ampicillin, 0.2 mM uracil, and 1 mM arginine culture (note: *pyrA* strains require arginine and uracil), which was incubated at 28°C with shaking until fully grown. Cells in a 0.1-ml sample of this culture were collected by filtration on a sterile 0.45-μm membrane filter (Millipore Corp.) and were washed with 30 ml of VBCG. The filter was placed in 10 ml of VBCG plus 1 mM arginine (note: arginine was added here and during penicillin treatment to select against arginine auxotrophs) and incubated at 28°C for 2 h. One milliliter of this culture was added to 1 ml of VBCG containing 1 mM arginine and 10,000 U of penicillin. (Mu *d*1-lysogenic strains are sensitive to the high concentration of penicillin used here.) After incubating at 28°C for 3 h, the entire penicillin-treated culture was collected by filtration and washed as above. The filter was placed in 10 ml of VBCG plus 1 mM arginine and 0.2 mM uracil and incubated at 28°C until fully grown. The culture was diluted 10^6-fold, and 0.1 ml was spread on VBCG plus ampicillin, 0.5 mM arginine, and 0.2 mM uracil plates. The plates were incubated at 28°C for 2 days, and colonies were screened for a requirement for uracil or uracil and arginine.

Preparation of cell extracts for enzyme assays. Extracts for carbamylphosphate synthetase (CPSase) assays were prepared as described (1) with slight modification. Harvested cells were washed and extracted in buffer (4°C) without phenylmethylsulfonyl fluoride. Extracts were dialyzed (4°C) for at least 8 h against two changes of buffer. For aspartate transcarbamylase (ATCase) assays, samples (40 ml) of culture were centrifuged at 20,000 × *g* for 10 min at 4°C. The cells were washed with cold extraction buffer (0.1 M Tris-acetate–0.1 mM EDTA, pH 8.0), centrifuged as above, and stored at −20°C for 1 day with no loss of enzymatic activity. The pelleted cells were suspended in 5 ml of cold extraction buffer and disrupted by sonic oscillation at 0°C. Extracts were centrifuged at 17,000 × *g* for 30 min at 4°C, and the supernatant was used for enzyme and protein assays.

Enzyme assays. β-Galactosidase activity was assayed as described by Miller (17). CPSase activity was determined at 30°C as described (2). ATCase activity was measured by a modification of the assay of Porter et al. (20). The reaction mixture contained 5 mM carbamylphosphate, 20 mM L-aspartic acid, 100,000 cpm of uniformly labeled L-[^{14}C]aspartic acid, 0.2 M imidazole acetate (pH 7.0), and cell extract (up to 150 μl) in a final volume of 0.5 ml. Reaction mixtures were incubated at 30°C for 20 min, and the reaction was stopped by the addition of 0.15 ml of 1 N acetic acid. The entire mixture was then applied to a 5-cm Pasteur pipette column of AG 50W (H^+ form) cation-exchange resin, and the effluent along with three column washings each with 0.7 ml of deionized water were collected, mixed with 10 ml of Bray scintillation fluid (6), and counted. A control in which carbamylphosphate was omitted from the reaction mixture was run for each determination.

Protein determination. Protein was measured by the method of Lowry et al. (16), using crystalline bovine serum albumin as the standard. Samples in Tris buffer were precipitated in 5% trichloroacetic acid before being assayed.

RESULTS

Construction of an *E. coli* double lysogen for making Mu *d*1 lysates capable of infecting *S. typhimurium*. The first step in introducing Mu *d*1 into *S. typhimurium* was the construction of an *E. coli* strain carrying both Mu *d*1 and a Mu helper phage with the host range of P1. To do this, the *E. coli* K-12 strain JC3272/R751, which carries the self-transmissible, broad-host-range plasmid R751 (15), was transduced to ampicillin resistance by Mu *d*1 from MAL103. The resultant strain, KC87, carrying Mu *d*1 at an unknown site, was grown to a density of 10^8 cells/ml in LB at 30°C and then shifted to 37°C for 5 h to effect limited induction of the prophage, thus generating transpositions to new sites (including R751). A 0.5-ml sample of this culture was mixed with 1 ml of a culture of *E. coli* K-12 strain MH2923 growing exponentially in LB at a density of 10^9 cells/ml. This strain is lysogenic for Mu *cts*62 hP1≠1 (MuhP1), a Mu phage with P1 host range. Dilutions of the mating mixture were spread on VBCG plus ampicillin plates to select prototrophic, ampicillin-resistant transconjugants which presumably arose by transfer of an R751::Mu *d*1 to MH2923. Such transconjugants occurred at a frequency of 4 per 10^5 recipient cells; one of these was called KC89.

Introduction of Mu *d*1 into *S. typhimurium*. The P1-sensitive (i.e., *galE*), restriction-deficient, modification-proficient *S. typhimurium* LT2 strain SL4213 was transduced to ampicillin resistance using a heat-induced lysate from KC89. Fifty transductants were grown and heat induced to test for the presence of the helper phage. One transductant produced a lysate capable of infecting SL4213. This putative double lysogen was designated JL3473; its *recA* derivative, TL154, was used to prepare lysates to test whether other *S. typhimurium* strains were sensitive to phage infection (Table 2, column 1). Heat-induced lysates of TL154 formed plaques on the *galE S. typhimurium* strains SL4213 and JL3404 but not on *galE*$^+$ strains TL155 and LT2-Z. Similar results were obtained by selecting for ampicillin resistance: the *galE* strains were transduced at a 10^5-fold-greater efficiency than the *galE*$^+$ strains.

The requirement for the recipient to carry *galE* was also apparent when the Mu *d*1/

TABLE 2. *Infection of S. typhimurium with phage Mu*

Recipient strain	Mu *d*1/Mu *cts*62 hP1#1 from *S. typhimurium* TL154		Mu *d*1/Mu *cts*62 hP1#1 from *E. coli* KC89		Mu *d*1/Mu *cts*62 from *E. coli* MAL103	
	Plaques/ml	Ap^r transductants/ml	Plaques/ml	Ap^r transductants/ml	Plaques/ml	Ap^r transductants/ml
JA194 (*E. coli* K-12) *hsdR*	1.4×10^{10}	1.3×10^{8}	2.1×10^{10}	1.2×10^{8}	6.8×10^{10}	2.2×10^{8}
SL4213 (*S. typhimurium*) *galE496 hsdL6 hsdSA29*	5.8×10^{9}	3.8×10^{7}	3.8×10^{9}	2.9×10^{7}	4.2×10^{4}	4.5×10^{5}
JL3404 (*S. typhimurium*) *galE1122 hsdL*$^+$ *hsdSA*$^+$	1.8×10^{9}	3.0×10^{7}	5.8×10^{4}	7.5×10^{3}	<10	30
TL155 (*S. typhimurium*) *galE*$^+$ *hsdL6 hsdSA29*	<10	90	<10	40	<10	9.5×10^{3}
LT2-Z (*S. typhimurium*) *galE*$^+$ *hsdL*$^+$ *hsdSA*$^+$	<10	70	<10	<10	<10	<10

MuhP1 lysate was derived from *E. coli* K-12 strain KC89 (Table 2, column 2). The phage formed plaques on *S. typhimurium* strains only if they were P1 sensitive (*galE*), and the transducing efficiency of P1-sensitive strains was at least 5×10^3-fold greater than that of the corresponding P1-resistant strains. However, the *E. coli*-grown phage is apparently sensitive to the *S. typhimurium* restriction systems: the plaquing as well as the transducing efficiency of the lysate was at least 4×10^3-fold greater on strain SL4213, which is deficient in DNA restriction, than on strain JL3404, which possesses intact DNA restriction. (We assume that the relevant difference between the nonisogenic strains SL4213 and JL3404 is the state of the restriction systems.)

That the helper phage must have a P1 host range in order to form plaques with high efficiency even on *galE* strains of *S. typhimurium* was demonstrated using a mixed lysate of Mu *d*1 and Mu *cts*62 (wild-type host range) derived by heat inducing *E. coli* K-12 strain MAL103 (Table 2, column 3). This lysate formed plaques at very low efficiency on SL4213. Also, the Mu *d*1/Mu *cts*62 lysate transduced this strain at a decreased efficiency.

Mutational events rendering *S. typhimurium* sensitive to Mu. As demonstrated, a *galE* mutation is sufficient to render *S. typhimurium* sensitive to phage Mu with P1 host range. However, Mu *d*1/MuhP1 lysates can transduce *galE*$^+$ strains at reduced efficiency. To examine the genetic basis for this sensitivity, 21 ampicillin-resistant transductants (12 derived from TL155 and 9 derived from LT2-Z; Table 2, column 1) were further characterized. All were lysed at 43°C, indicating the presence of Mu *d*1. Seven were Gal$^-$, inhibited by galactose, sensitive to phage P1CM, and resistant to phages FO and P22—properties that are typical of *galE* or *galU* strains (19). Nine were Gal$^+$ and sensitive to phages P1CM, FO, and P22; two were Gal$^+$, sensitive to P1CM, and resistant to FO and P22. These strains may carry a mutation affecting lipopolysaccharide synthesis (26). The remaining three transductants were Gal$^+$, resistant to P1CM, and sensitive to FO and P22. They may be products of rare infections of *galE*$^+$ *S. typhimurium* by Mu with P1 host range.

The low-level plaquing efficiency seen with Mu *cts*62 (wild-type host range) from *E. coli* strain MAL103 on *S. typhimurium* strain SL4213 (Table 2, column 3) is probably a consequence of mutant Mu phages in the population. Examination of five of the plaques revealed that they contained phages that could plaque with high efficiency on *galE S. typhimurium* and *E. coli* K-12 strains, indicating that they carried a mutation extending their host range. Strain TL156 (see below) was obtained by lysogenization of SL4213 by one such mutant phage. In several attempts, we were unable to find a spontaneous mutant of Mu *cts*62 or MuhP1 that could plaque on *galE*$^+$ *S. typhimurium*.

Also, phage from MAL103 could transduce *S. typhimurium* strains at a low efficiency. To investigate how this was possible, 20 ampicillin-resistant transductants of TL155 (Table 2, column 3) were examined. All 20 were Gal$^+$ and resistant to phage FO 11 were sensitive to P1CM and P22 (at least partially); 5 were sensitive to P1CM and resistant to P22; 3 were resistant to P1CM and fully or partially sensitive to P22; and 1 was resistant to both P1CM and P22. Thus, several types of mutations in *S. typhimurium* appear to confer sensitivity to phage Mu even if it has the wild-type host range.

Growth of Mu in *S. typhimurium*. The growth of phage Mu with the host range of P1 in *galE* strains of *S. typhimurium* was similar to that found in *E. coli* K-12. MuhP1 formed turbid

plaques at 30°C, clear plaques at 43°C, and plaques of intermediate turbidity at 37°C. In one experiment in which JL3404 was infected with a Mu *d*1/MuhP1 lysate from TL154, the ampicillin-resistant transductants showed variable ability to ferment lactose, 0.6% were shown to be auxotrophic, and all were killed at 43°C. Thus, as it does in *E. coli* K-12, Mu *d*1 can integrate into the chromosome of *S. typhimurium* at many sites.

Construction and characterization of *pyr*::*lac* gene fusions in *S. typhimurium* using Mu *d*1. Mu *d*1-induced pyrimidine auxotrophs were isolated as described in Materials and Methods and were divided into four classes (tentatively identified as *pyrA*, *pyrB*, *pyrC* or *-D*, and *pyrE* or *-F*) by virtue of their nutrient requirements and growth characteristics (data not shown). Only the putative *pyrA* and *pyrB* auxotrophs were characterized further. To determine whether Mu *d*1 integrated in the orientation yielding *pyr*::*lac* fusions, cells were streaked on X-gal indicator plates supplemented with either 1 mM uracil or 1 mM orotate. Orotate is a poor pyrimidine source and causes derepressed *pyr* gene expression, whereas uracil is a good pyrimidine source and causes repression. Strains which were dark blue on orotate plates and light blue on uracil plates were judged to be *pyr*::*lac* fusions. With only independently derived mutant strains, 7 of the 14 *pyrA* and 3 of the 6 *pyrB* auxotrophs were scored as *pyr*::*lac* fusions, consistent with two possible Mu *d*1 orientations.

One *pyrA*::*lac* fusion strain (JL3484) and all three *pyrB*::*lac* fusion strains (JL3486, JL3500, and JL3501) were tested for the presence of helper phage. Cultures of each strain were heat induced, but none produced a lysate capable of forming plaques on JL3404, indicating that they lacked the helper phage. To test for the presence of additional copies of Mu *d*1, each strain was transduced to Pyr$^+$ with P22 grown on LT2-Z (*pyrA*$^+$ *pyrB*$^+$). All transductants tested (16 from each fusion strain) were ampicillin sensitive and white on the X-gal indicator plates, indicating that they had no additional copies of Mu *d*1.

Two strains, JL3484 (*pyrA*::*lac*) and JL3486 (*pyrB*::*lac*), were used to characterize further the *pyr*::*lac* fusions. The tentative *pyr* gene assignments for the fusions in these strains were confirmed by the mapping experiments summarized in Table 3 and by showing that P22 grown on strains *pyrAΔ81* and *pyrBΔ655* could not transduce JL3484 and JL3486, respectively, to Pyr$^+$. The synthesis of β-galactosidase, CPSase, and ATCase (encoded by *lacZ*, *pyrA*, and *pyrB*, respectively) was compared in the fusion strains grown under conditions that result in either repression or derepression of *pyr* gene expression (Table 4). The repression/derepression ratios for β-galactosidase in JL3484 (*pyrA*::*lac*) and CPSase in JL3486 (*pyrA*$^+$) were essentially identical, as were the ratios for β-galacto-

TABLE 3. *P22 transductional mapping of pyr::lac fusions*

Donor	Recipient	Selected phenotype	Distribution of unselected phenotype in recipients
LB82 (*zab-403*::Tn*10*)	*pyrAΔ81*	Tet^r	83/100 Pyr$^+$
		Pyr$^+$	77/100 Tet^r
	JL3484 (*pyrA*::*lac*)	Tet^r	89/100 Pyr$^+$
		Pyr$^+$	55/100 Tet^r
JL3103 (*argI1843*::Tn*10*)	*pryBΔ655*	Tet^r	24/100 Pyr$^+$
		Pyr$^+$	28/100 Tet^r
	JL3486 (*pyrB*::*lac*)	Tet^r	58/100 Pyr$^+$
		Pyr$^+$	31/100 Tet^r

TABLE 4. *Synthesis of CPSase, ATCase, and β-galactosidase in pyr::lac fusion strains*[a]

Strain	Pyrimidine source	CPSase (nmol/min per mg of protein)	ATCase (nmol/min per mg of protein)	β-Galactosidase (U)
JL3484 (*pyrA*::*lac*)	Uracil	ND[b]	14.0 (1)[c]	128 (1)
	Orotate	ND	1,695 (121)	4,575 (36)
JL3486 (*pyrB*::*lac*)	Uracil	0.408 (1)	ND	21.8 (1)
	Orotate	16.82 (41)	ND	2,542 (117)

[a] Cultures were grown in MOPS (morpholinepropanesulfonic acid) minimal medium (18) supplemented with 0.5 mM arginine, 0.4% glucose, and either 0.2 mM uracil or 0.5 mM orotate as indicated. Samples were harvested at an optical density (650 nm) of 0.5.

[b] ND, Not detectable.

[c] Numbers in parentheses represent relative specific activities for that enzyme in the indicated strain.

sidase in JL3486 (*pyrB*::*lac*) and ATCase in JL3484 (*pyrB*$^+$).

Phage P22-mediated transduction of *pyr*::*lac* fusions. A P22 lysate grown on JL3487 (*pyrB*::*lac*), a *galE*$^+$ transductant of JL3486, was used to transduce TL156 (Mu *cts*62 h7629 lysogen) to ampicillin resistance. Twelve of 13 transductants characterized were Pyr$^-$ and were shown to contain the intact *pyrB*::*lac* fusion. The sole Pyr$^+$ transductant did not carry the *pyrB*::*lac* fusion. This transductant may have arisen after homologous recombination between Mu *d*1 and the endogenous Mu *cts*62 h7629 or by the translocation of Mu *d*1 from a transducing particle to a nonessential region on the chromosome. When the JL3487 lysate was used to transduce nonlysogenic strains to ampicillin resistance, none of the transductants characterized contained the *pyrB*::*lac* fusion. Of 56 transductants of strain *purFΔ145* and 16 transductants of SL4213, all were *pyrB*$^+$. Three transductants, two from *purFΔ145* and one from SL4213, had acquired an additional Mu *d*1-induced auxotrophic requirement. It appears likely that all 72 transductants of *purFΔ145* and SL4213 were generated by translocation of Mu *d*1 to random sites on the chromosome after zygotic induction of Mu *d*1-carrying transducing particles. The number of transductants acquiring new auxotrophic requirements (3 of 72) is consistent with this interpretation (23).

DISCUSSION

In this paper we have presented a method for introducing the coliphage Mu *d*1 (Ap^r *lac cts*62) into *S. typhimurium* in order to construct gene fusions. This method extends the host range of Mu *d*1 by using a Mu helper phage (MuhP1) that confers the P1 host range and by using P1-sensitive strains of *S. typhimurium* as recipients. To overcome DNA restriction, a Mu *d*1/MuhP1 mixed lysate from *E. coli* was used to infect a P1-sensitive, restriction-deficient, modification-proficient strain of *S. typhimurium*. A double lysogen of this strain carrying Mu *d*1 and MuhP1 was then used to make lysates capable of infecting and generating fusions between *S. typhimurium* genes and the *lac* operon in other P1-sensitive strains of *S. typhimurium*.

This procedure for constructing gene fusions is simple and appears to be generally useful. The only limitation in using this method is that the recipient bacteria be sensitive to phage P1. All *galE* strains of *S. typhimurium* are P1 sensitive, and these strains are common in many laboratories. They can also be isolated conveniently by selecting strains that are resistant to phage FO (26). In addition, P1 sensitivity can be transduced into strains of *S. typhimurium* by exploiting the close proximity of *galE* to the *bio-203*::Tn*10* insertion; in P22-mediated transduction, the linkage of *bio-203*::Tn*10* to *galE1122* is 15% (unpublished data).

In addition to the method presented here, two other procedures for introducing Mu *d*1 into *S. typhimurium* have been developed recently. Lee and co-workers (J. H. Lee, L. Heffernan, and G. Wilcox, manuscript in preparation) constructed an F::Mu *d*1 in *E. coli* which was then transferred into *S. typhimurium*. They obtained gene fusions by inducing Mu *d*1 to translocate from the F to other sites on the chromosome. A second technique (S. Rosenfeld and J. Brenchley, personal communication) used P1 to transduce Mu *d*1 from *E. coli* into a *galE*, restriction-deficient strain of *S. typhimurium*. Then a P1 lysate grown on this strain was used to transduce Mu *d*1 to other *galE S. typhimurium* strains, and gene fusions were obtained as a consequence of zygotic induction.

Using the procedure described in this paper, we constructed *pyr*::*lac* fusions for several of the *pyr* genes in *S. typhimurium*. Two of these fusion strains, JL3484 (*pyrA*::*lac*) and JL3486 (*pyrB*::*lac*), were characterized with respect to the synthesis of β-galactosidase (encoded by *lacZ*) under conditions of pyrimidine limitation and excess. The regulation of β-galactosidase synthesis in JL3484 and JL3486 was identical to that of CPSase (encoded by *pyrA*) and ATCase (encoded by *pyrB*), respectively. These *pyr*::*lac* fusion strains should be useful for the further study of the regulation of the expression of *pyr* genes and for the selection of regulatory mutations.

In such studies, it would be useful to move gene fusions constructed by our procedure into other strains of *S. typhimurium*. We have shown that this is possible by P22-mediated transduction, if the recipient strain is lysogenic for Mu. If the recipient is nonlysogenic, it appears that the Mu *d*1 phage on infecting transducing particles translocates to other sites on the chromosome, probably as a consequence of zygotic induction. Although this makes it difficult to transfer fusions into nonlysogenic strains, it provides yet another way of constructing gene fusions.

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