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Isolating Tryptophan Regulatory Mutants in *Escherichia coli* by Using a *trp-lac* Fusion Strain

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A *trp-lac* fusion strain of *Escherichia coli* in which the *lac* structural genes are part of the tryptophan operon has been used to isolate *trp* regulatory mutants. This was accomplished by isolating *lac*$^+$ colonies on either lactose-minimal agar or lactose-MacConkey indicator agar. Seventy-seven of 78 *lac*$^+$ isolates contained mutations which mapped near the *ara* locus and most of these isolates were found to be 5-methyltryptophan-resistant after introduction of an F-*trp* episome. The *lac*$^+$ phenotypes of these 77 isolates were therefore probably the result of *trpR*$^-$ mutations. The one remaining isolate carried a mutation which was not part of the *trp* regulatory system.

Beckwith and Signer (1) have described the isolation of strains in which the lactose (*lac*) region of the *Escherichia coli* chromosome has been transposed to a site close to the tryptophan (*trp*) operon. Subsequent genetic manipulations (20) allowed the isolation of *trp-lac* fusion strains among those carrying *tonB*$^-$ deletions (2, 17). From a *lac* transposition strain in which the *lac* operon is in the same orientation as *trp*, a special class of *trp-lac* fusion deletions has been isolated. These are deletions which cut from the *trp* structural genes to the *lac*-controlling elements (19). Strains carrying such deletions make *lac* messenger ribonucleic acid (mRNA) which is covalently fused to *trp* mRNA (6). A specific example of such a deletion is W1 shown in Fig. 1. This deletion cuts from *trpE* to beyond *lac p* leaving part of *lac o* and *lac z*, *y*, and *a* intact and thereby bringing the *lac* structural genes under the control of *trpP1* and *trpO* (19).

Since the expression of the *lac* genes in strains carrying the W1 deletion is a direct reflection of the functioning of the *trp* operon, it might be expected that one could use this *lac* phenotype as a means of isolating strains carrying *trp* regulatory mutations. The work reported in this paper confirms this expectation and suggests that this novel system may offer certain advantages over previously described techniques for isolating *trp* regulatory mutants.

MATERIALS AND METHODS

Strains. *E. coli* X8060 is an F$^-$Sm^r strain which carries a deletion of the *lac* region (X74), an *ara*$^-$ mutation (J3), and a $\phi 80 d_1 lac$ prophage which has been partially deleted by the W1 *trp*$^-$-*tonB*$^-$-*lac p*$^-$ deletion. Figure 1 pictorially represents the chromosome of X8060. The *lac*$^-$ deletion X74 was isolated by Cuzin and Jacob (4) and the *ara*$^-$ mutation J3 is an ultraviolet-induced mutation isolated by J. Miller. The strain X8060-*trpR*$^-$ is a derivative of X8060 which carries the *trpR*$^-$ mutation described by Imamoto, Ito, and Yanofsky (12). This strain was constructed by mating X8060 with an Hfr Cavalli carrying the *trpR*$^-$ mutation and selecting *lac*$^+$ recombinants. XW205-*trpE*$^-$9851 is a strain which carries a *trp-lac o* fusion deletion cutting either very late in or just after the end of *trpA* to just before *lac z* such that the *lac* structural genes are fused to the *trp* operon but the strain is still *trp*$^+$. The isolation and characterization of this deletion will be described in a subsequent communication by Mitchell, Reznikoff, Beckwith, and Michels. The *trpE*$^-$9851 mutation is an operator proximal *E* nonsense mutation (24) known not to be covered by the W1 deletion (19). WD5017 is a *gal*$^-$, Sm^s strain which carries the F-*trp* episome described by Fredericq (7). CA77 is an Hfr Hayes derivative which is Sm^s, *ara*$^+$ and carries the X74 *lac* deletion.

The phage P1*vir* is a virulent mutant of P1*kc* which was isolated by B. Wolf and obtained from B. Konrad.

Media. The buffer λ-Ca is composed of 0.01 M $MgSO_4$, 0.005 M $CaCl_2$, and 0.1 M tris(hydroxymethyl)aminomethane (Tris)hydrochloride, *p*H 7.9. Other media have been described previously (19).

Chemicals. Pyridoxal-5'-phosphate, *o*-nitrophenyl-β-D-galactopyranoside and DL-5-methyltryptophan were purchased from Sigma Chemical Co.

Assays: tryptophan synthetase A. Exponentially growing cultures (in minimal PB medium containing 40 μg of L-tryptophan per ml) were harvested by centrifugation at 5 C. They were resuspended in 0.1 M Tris-hydrochloride, *p*H 7.8, in one-tenth the original volume. The cells were sonically disrupted by using a sonifier (Heat Systems—Ultrasonics, Inc.). The activity of tryptophan synthetase A in the extracts was determined by using the indole + serine → tryptophan assay as described by Smith and Yanofsky (21), modified to have 0.8 μmole of indole and 60 μmoles of DL-serine in a reaction volume of 1.1 ml. A unit of tryptophan synthetase A has been set equal to that quantity of enzyme which will convert 0.1 μmole of indole to tryptophan in 20 min at 37 C. The protein concentration was determined by the procedure of Lowry et al. (15) with bovine serum albumin (Pentex) as a standard.

β-Galactosidase. β-Galactosidase was assayed as described previously (19) and specific activities were normalized to X8060-*trpR*$^-$ = 100 except for those assays reported in Table 3. In this case, a unit of β-galactosidase has been equated to that quantity of enzyme which will hydrolyze enough *o*-nitrophenyl-β-D-galactopyranoside in 1 min to give an absorbancy of 1 at 420 nm.

Transduction experiments. The transduction experiments were performed by a technique similar to one described by B. Konrad (Ph.D. Thesis, Harvard University, 1969). A saturated culture of the recipient was centrifuged and resuspended in an equal volume of λ-Ca buffer. This suspension was incubated for 15 min at 37 C with aeration. Samples of 0.1 ml of this suspension were mixed with 0.1-ml volumes of P1*vira* phage diluted, if necessary, with λ-Ca buffer. These mixtures were incubated at 37 C for 20 min, and then plated out in 2 ml of F top agar supplemented with 0.12% sodium citrate onto selective plates which also contained 0.12% sodium citrate.

Ultraviolet mutagenesis and selection of lac$^+$ mutants. A suspension of X8060 grown to a concentration of approximately 2 × 10^8/ml in LB broth was centrifuged, and the bacterial pellet was resuspended in an equal volume of 0.1 M $MgSO_4$. Five milliliters of the suspension was irradiated for 60 sec by using a General Electric 15-w germicidal lamp, G15T8, at a distance of 27 inches (ca. 68.6 cm), resulting in 99.5% killing. A volume of 0.5 ml of this irradiated suspension was directly plated out either on lactose-minimal agar plates containing 40 μg of DL-tryptophan per ml and 0.12% sodium citrate or onto lactose-MacConkey agar. The lactose-minimal plate selections (CUV500 series) were incubated for 2 days (CUV501 to 522) or 3 days (CUV523 to 533) at 42 C before the *lac*$^+$ colonies were picked and purified. The lactose-MacConkey plates (CUV1000 series) were incubated for 3 days at 42 C after which time the "reddish" papillae were picked and purified on lactose-MacConkey agar.

Physiological classification of mutants. All isolates were tested for their phenotype on lactose-minimal agar and lactose-MacConkey agar at 37 C. The amount of β-galactosidase produced at 37 C by each *lac*$^+$ isolate as well as by X8060 and X8060-*trpR*$^-$ was also determined.

Each mutant was examined to see whether the particular *lac*$^+$ CUV mutation also resulted in the simultaneous attainment of a cytoplasmic property which would effect a derepression of an unlinked *trp* operon. This was done by (i) introducing the F-*trp* episome from WD5017 by mating WD5017 with each CUV strain and selecting partial diploids on galactose-Sm minimal agar plates and (ii) test-streaking each merozygote so constructed on glucose-minimal agar plates containing 100 μg of DL-5-methyltryptophan (5-MT) per ml followed by incubation at 42 C. In the case of mutant strains CUV532 and 533, the merozygotes were further characterized by determining the specific activity of tryptophan synthetase A and β-galactosidase produced in these merozygotes relative to that produced by F-*trp*/X8060.

Genetic screening of mutants. The mutations giving rise to the *lac*$^+$ phenotype were screened for possible linkage to the *ara* locus by selecting *ara*$^+$ recombinants following a 20-min mating with CA77. The exact techniques have been described previously (19). The *lac* phenotype of the *ara*$^+$ recombinants

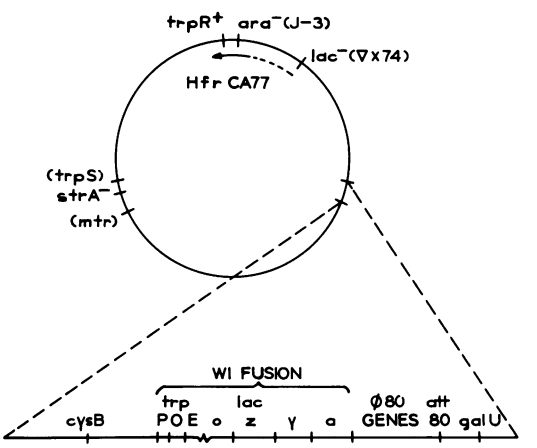

FIG. 1. *Genetic map of X8060. The locations of genetic markers carried by X8060 are placed on the E. coli circular genetic map in the approximate positions described by Taylor (22). The region containing the W1 trp-lac fusion deletion [described in more detail by Reznikoff et al. (19)] is expanded below the pictured circular chromosome. Arrow inside the circle indicates the origin and direction of transfer of Hfr CA77 used to map the lac$^+$ CUV mutations. The locations of two other markers are included for reference purposes: the trpS (tryptophanyl-tRNA synthetase) locus, which may play some regulatory role and whose mutants have been used to isolate trp-constitutive mutations (10); and mtr, which plays no known trp regulatory function but whose mutants have a 5-MT-resistant phenotype (11).*

were then checked on lactose-MacConkey or lactose-minimal agar.

The one CUV mutation unlinked to *ara* was then tested for linkage to *trpE* by using P1*vira*, which had been propagated through two rounds of plate lysates on the mutant strain, to transduce XW205-*trpE*$^-$ 9851 to a *trp*$^+$ genotype. The *trp*$^+$ transductants were tested for their sensitivity to 5-MT, and their *lac* phenotype was examined.

RESULTS

Isolation of lac$^+$ mutants. When *E. coli* X8060 was mutagenized with ultraviolet light, *lac*$^+$ derivatives were discovered on both the lactose-minimal and the lactose-MacConkey plates at a frequency of approximately 3×10^{-5}. With the exception of three papillae picked from the lactose-MacConkey plates, all were successfully purified by streaking them out on the selective or on the indicator medium.

Preliminary physiological classification of the lac$^+$ mutants. The mutants obtained by the above procedure were first characterized as to their phenotype on lactose-minimal and lactose-MacConkey plates. As expected, all of the CUV500 series grew on lactose-minimal medium (Table 1). Five CUV500 isolates gave pink to light-red colonies on lactose-MacConkey plates (Table 1), whereas the other CUV500 isolates gave *lac*$^+$ (dark red) indicator results. These pink colonies were distinctly redder than the control X8060. Most CUV1000 isolates were also *lac*$^+$ on both lactose-minimal and lactose-MacConkey plates when incubated at 37 C (Table 2). The exceptions to this generalization are CUV1003, 1012, and 1044 which yielded pink or light-red colonies on lactose-MacConkey plates.

β-Galactosidase assays. The CUV isolates were assayed for β-galactosidase production after growth at 37 C (Tables 1 and 2). As expected, all of the strains produced more β-galactosidase than the parental X8060. Different strains produced different levels of the enzyme, reflecting different levels of *lac* expression in the isolates. Most of the strains which gave pink colonies on lactose-MacConkey agar. produced relatively low levels of β-galactosidase. There were some surprising results. Several of the CUV strains (CUV501, 506, 516, 520, 529, 530, 1010, 1013, 1016, 1020, 1022, 1029, and 1037) produced significantly more β-galactosidase (i.e., $>1.15 \times$) than the control strain X8060-*trpR*$^-$ which contains the *trpR*$^-$ mutation described by Imamoto et al. (12). It was thought that this *trpR*$^-$ mutation resulted in the maximum possible genetic derepression of the *trp* operon (C. Yanofsky, *personal communication*). It would be tempting to suggest that some of these higher level mutants manifest the true maximum level of genetic derepression of the *trp* operon, but the possibility of a difference in the genetic background of these strains and the X8060-*trpR*$^-$ strain, due to the method of constructing the latter or the ultraviolet mutagenesis of the former, has not been ruled out. The mutant CUV532 has a *lac*$^-$ phenotype on lactose-MacConkey plates and yet shows β-galactosidase levels comparable to many other strains with *lac*$^+$ lactose-MacConkey phenotypes. We have no explanation for this finding.

Cytoplasmic derepression of the trp operon. The possibility that the *lac*$^+$ phenotype of the CUV mutations might be the result of a failure to produce an active *trp* repressor molecule was examined by introducing an F-*trp* episome into each *lac*$^+$ strain and then testing for constitutive expression of the episomal *trp* operon as manifested by resistance to 5-MT. Most F-*trp*/CUV strains tested were resistant to the tryptophan analogue (Tables 1 and 2), suggesting that the *lac*$^+$ phenotypes of these strains were due to *trpR*$^-$ mutations (or at least due to a change in a cytoplasmically active product related to *trp* repression). The F-*trp* derivatives of four strains (CUV507, 532, 1003, and 1044) were sensitive to 5-MT. This result could be explained by the following possibilities. (i) The *lac*$^+$ phenotype(s) might have nothing to do with the tryptophan regulatory system. (ii) The *lac*$^+$ phenotype(s) is the result of defects in the *trp* regulatory system, but the defects are not of sufficient magnitude to yield resistance to the 5-MT. (iii) The *lac*$^+$ phenotype(s) is related to defects in the *trp* regulatory system, but they are not defects in a cytoplasmically active part of that system. Although we have not ruled out the first possibility for strains CUV-507, 1003, and 1044, possibility ii best explains their properties since all three make relatively low levels of β-galactosidase (Tables 1 and 2) and since (as will be described later) their mutations all map near the *ara* locus.

The observation that F-*trp*/CUV532 was sensitive to 5-MT could not be explained by possibility ii since this strain produced more β-galactosidase than other CUV strains whose F-*trp* derivatives were resistant to 5-MT. To confirm that the episomal *trp* operon was not derepressed in F-*trp*/CUV532, the content of tryptophan synthetase A in this merozygote was determined. As can be seen in Table 3, tryptophan synthetase A was not produced by F-*trp*/CUV532 in significantly higher amounts

TABLE 1. *Characterization of CUV500 isolates*[a]

CUV strain	*lac*- MacConkey phenotype	*lac*- Minimal phenotype	β-Galactosidase activity	Cytoplasmic derepression of F-*trp*	*ara* Linkage (*lac*⁻/*ara*⁺)
501	Red	+	150	+	34/40
502	Red	+	105	+	34/40
503	Red	+	105	+	37/40
504	Red	+	105	+	37/40
505	Red	+	90	+	36/40
506	Red	+	120	+	36/40
507	Pink	+	20	−	36/40
508	Red	+	75	+	37/40
509	Red	+	90	+	31/40
510	Red	+	65	+	32/40
511	Red	+	50	+	32/40
512	Red	+	105	+	36/40
513	Red	+	85	+	35/40
514	Red	+	95	+	35/40
515	Red	+	105	+	34/40
516	Red	+	120	+	39/40
517	Red	+	100	+	38/40
518	Red	+	105	+	38/40
519	Pink	+	35	+	35/40
520	Red	+	145	+	36/40
521	Red	+	105	+	36/40
522	Red	+	110	+	39/40
523	Red	+	100	+	38/40
524	Red	+	105	+	37/40
525	Pink	+	40	+	35/40
526	Red	+	100	+	35/40
527	Red	+	95	+	32/40
528	Red	+	80	+	35/40
529	Red	+	130	+	35/40
530	Red	+	130	+	37/40
531	Red	+	105	+	34/40
532	Pink	+	70	−	0/40*
533	Light red	+	60	+	36/40
X8060	White	−	5	−	

[a] β-Galactosidase levels were normalized to X8060-trpR⁻ = 100 and were rounded off to the nearest unit of 5. These assays were done at least in duplicate, and, in cases where strains showed levels of enzyme greater than 115 or less than 70, assays were repeated several times in triplicate. Cytoplasmic derepression of F-*trp*/CUV merozygotes was determined by examining their sensitivity to DL-5-methyltryptophan. The *lac* phenotype of *ara*⁺ recombinants was checked on lactose- MacConkey agar or on lactose-minimal agar (*).

TABLE 2. *Characterization of CUV 1000 isolates*[a]

CUV strain	*lac*- MacConkey phenotype	*lac*- Minimal phenotype	β-Galactosidase activity	Cytoplasmic derepression of F-*trp*	*ara* Linkage (*lac*⁻/*ara*⁺)
1001	Red	+	80	+	38/40
1002	Red	+	85	+	39/40
1003	Pink	+	15	−	19/20*
1004	Red	+	55	+	36/40
1005	Red	+	100	+	36/40
1006	Red	+	90	+	37/40
1007	Red	+	90	+	36/40
1008	Red	+	90	+	32/40
1009	Red	+	90	+	35/40
1010	Red	+	150	+	33/38
1011	Red	+	100	+	37/40
1012	Pink	+	20	+	17/20*
1013	Red	+	125	+	34/40
1014	Red	+	100	+	37/40
1015	Red	+	110	+	33/40
1016	Red	+	125	+	16/20
1017	Red	+	110	+	30/40
1018	Red	+	100	+	34/40
1019	Red	+	80	+	33/40
1020	Red	+	120	+	38/40
1021	Red	+	115	+	37/40
1022	Red	+	120	+	38/40
1023	Red	+	100	+	35/40
1024	Red	+	90	+	34/40
1025	Red	+	80	+	35/50
1026	Red	+	80	+	36/40
1027	Red	+	100	+	38/40
1028	Red	+	85	+	37/40
1029	Red	+	120	+	36/40
1030	Red	+	75	+	32/40
1031	Red	+	100	+	37/40
1032	Red	+	100	+	36/40
1033	Red	+	105	+	37/40
1034	Red	+	95	+	38/40
1035	Red	+	90	+	38/40
1036	Red	+	100	+	36/40
1037	Red	+	130	+	37/40
1038	Red	+	75	+	36/40
1039	Red	+	80	+	35/40
1040	Red	+	95	+	33/40
1041	Red	+	40	+	36/40
1042	Red	+	50	+	37/40
1043	Red	+	65	+	18/20
1044	Light red	+	40	−	16/20
1045	Red	+	85	+	32/40

[a] See footnote to Table 1.

than by F-*trp*/X8060, whereas F-*trp*/CUV533 does produce increased amounts of tryptophan synthetase A. These results indicate that the mutation creating the *lac*⁺ phenotype in CUV532 is incapable of causing derepression of an episomally located *trp* operon and suggest that its phenotype can best be explained by either possibility i or iii.

Genetic characterization. A very simple genetic screening test for possible *trpR*⁻ mutations exists in the CUV strains. One can mate the given CUV isolate which carries an *ara*⁻

TABLE 3. *Tryptophan synthetase A and β-galactosidase activities in F-trp/CUV merozygotes*[a]

Merozygote	Tryptophan synthetase A (units/mg of protein)	β-Galactosidase (units/mg of protein)
F-*trp*/X8060-1	2.16	.105
F-*trp*/X8060-2	2.25	.084
F-*trp*/CUV532-1	3.16	1.172
F-*trp*/CUV532-2	2.42	1.092
F-*trp*/CUV533-1	5.10	.398
F-*trp*/CUV533-2	5.61	.428

[a] The assays reported were accomplished on two independently derived F-*trp* merozygotes for each strain. The lower derepression ratio for tryptophan synthetase A than for β-galactosidase in F-*trp*/CUV533 probably reflects the fact that the tryptophan synthetase A, but not the β-galactosidase, is under the control of both the operator proximal *trp* promoter and the internal *trp* promoter.

marker with an Hfr Hayes derivative such as CA77. For any CUV strain in which the *lac*$^+$ phenotype is a manifestation of a *trpR*$^-$ mutation, one would expect a large fraction of CA77×CUV *ara*$^+$ recombinants to be *lac*$^-$, reflecting the known tight linkage between the *ara* region and the *trpR* locus and the fact that the *trpR* locus is between *ara* and the origin of transfer (14) (see Fig. 1). As indicated in Tables 1 and 2, this expectation was realized for all but one CUV strain, suggesting that in 77 of the 78 cases studied in this paper, the *lac*$^+$ phenotype was in fact due to a *trpR*$^-$ mutation. This suggestion is confirmed by the fact that all but three of these *ara*-linked CUV mutations can also effect a cytoplasmic derepression of an episomally located *trp* operon. The low levels of *lac* expression in these three exceptional strains (CUV507, 1003, and 1044) suggest that the failure to detect cytoplasmic derepression in these cases might be due to a threshold detection problem, i.e., these mutants might be weak *trpR*$^-$ constitutives.

The one exception noticed in this mapping study is CUV532. The *lac* phenotype in this strain failed to show linkage with the *ara* marker. We also know that even though there is significant expression of the *lac* genes in this strain, as measured by β-galactosidase activity, there is no detectable cytoplasmic derepression of an episomal *trp* operon. One possible explanation of these data is that the *lac*$^+$ phenotype of CUV532 is due to a *cis*-dominant, *trans*-recessive mutation such as a *trpO*^{c} mutation.

If CUV532 does carry a *trpO*^{c} mutation, one would predict that this mutation would be closely linked to the residual *trp* operon (9). This possibility has been studied by isolating *trp*$^+$ transductants of XW205-*trpE*$^-$9851 generated by P1*vira* grown on CUV532 (P1*vira*·CUV532). As a negative control, the same transduction was accomplished by using P1*vira* grown on X8060 (P1*vira*·X8060). All 20 of the P1*vira*·CUV532 *trp*$^+$ transductants tested were sensitive to 5-MT as were those generated by P1*vira*·X8060. All 20 of the P1*vira*·CUV532 *trp*$^+$ transductants also produced the same level of β-galactosidase as did the P1*vira*·X8060 transductants. These results suggest that strain CUV532 probably carries a mutation which is not part of the *trp* regulatory system. Further considerations on the nature of this mutation will be given below.

DISCUSSION

The results described in this paper and similar findings by R. Somerville (*personal communication*) indicate that the *lac* phenotype in *trp-lac* fusion strains can be a powerful tool for the isolation and characterization of *trp* constitutive mutations. Of the 78 *lac*$^+$ isolates derived from ultraviolet-treated X8060, 77 were shown to be *trpR*$^-$ mutations. These mutations were of many different physiological types with different levels of constitutivity, some of which may be higher than those previously reported, some of which were too low for detection on plates containing 5-MT, and some of which were at intermediate levels. A rigorous determination of the constitutive levels of these strains would, of course, demand introducing all of them into identical genetic backgrounds. In another selection, not described in this communication, we were able to isolate a temperature-sensitive CUV strain (CUV107) which made 5% of the X8060-*trpR*$^-$ level of β-galactosidase at 30 C and 60% at 42 C.

The one *lac*$^+$ strain which appears not to carry a *trpR*$^-$ mutation (CUV532) may contain a mutation which has generated a new promoter signal within the residual *trpE* gene similar to that found by Morse and Yanofsky (18). Such a mutation would be expected to be an *E*$^-$ mutation and, therefore, would not have been detected in the *trp* transduction experiment. Alternatively, CUV532 might carry a mutation creating a new promoter site in the residual *lac o* region. The observation that different *trp-lac* fusion strains manifest different levels of read through (19 and *unpublished data* by Mitchell, Reznikoff, Beckwith, and Michels) suggests another possibility. An extension of the fusion deletion may increase the

efficiency of read through. Finally, the mutation may be an unlinked suppressor, specific for the *lac* p^- character of the W1 deletion (e.g., a mutation in ribonucleic acid polymerase or a ribonucleic acid polymerase factor). It is obvious that this mutation may be of significant interest and so we are continuing work to see whether it is linked to the fusion (thereby distinguishing the first three possibilities from the fourth one), and if so whether it can be removed by recombination with *trp* genetic material (defining possibility one) or with *lac* genetic material (defining possibility two).

Our failure to find a *trp* O^c mutant was somewhat surprising but might be explained by one or both of the following reasons. Operator constitutive mutations of the *trp* operon may be less frequent than *trpR*$^-$ mutations. This result, though not consistent with the observations of Hiraga (9), has been generally observed by other workers (13). *trp* Operator constitutive mutants are in general only partially constitutive (9); thus, though low-level partial constitutive mutants can be detected in our system, a slight inadvertant prejudice towards picking very *lac*$^+$ colonies would bias our selection towards *trpR*$^-$ mutants.

There are certain obvious advantages to using the *trp-lac* fusion system for isolating and studying *trp* constitutive mutations. (i) It seems to be a very efficient system. (ii) It allows the use of complex indicator agars which permit the isolation of *trp* constitutives which may be simultaneously auxotrophic. (iii) It may be a more sensitive selective technique than those used before as witnessed by the isolation of mutants CUV507, 1003, and 1044. This sensitivity might be further increased by using the very sensitive indicator dye 5-bromo-4-chloro-3-indolyl-β-D-galactoside. (iv) Since there exist means of selecting for the *lac*$^-$ phenotype (5, 8, 16, 23), it should be possible to select for *trpR*$^+$ or *trpO*$^+$ genotypes, facilitating reversion and recombination studies of *trp*-constitutive mutations. (v) By the use of modifications to be described in subsequent communications, it should be a useful system for studying other genetic signals associated with the *trp* operon. (vi) Since one can use indicator plates of various types, it is a colorful system.

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