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SHORT COMMUNICATION

Expression of the Lactose Transposon Tn951 in *Escherichia coli*, *Proteus* and *Pseudomonas*

By SIMON BAUMBERG,[1]* GUY CORNELIS,[2]
MARIA PANAGIOTAKOPOULOS[2] AND MARGO ROBERTS[1]

[1] *Department of Genetics, University of Leeds, Leeds LS2 9JT*
[2] *Unité de Microbiologie, Université de Louvain, UCL* 30.58, *B*-1200 *Brussels, Belgium*

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The control of β-galactosidase specified by the lactose transposon Tn951 (inserted into RP1 to give pGC9114) has been studied in *Escherichia coli* K12, *Proteus mirabilis*, *Pseudomonas aeruginosa* and *Pseudomonas putida*; in the first two species comparison could be made with F*lac*. In *E. coli* K12, the Tn951 and chromosomally encoded enzymes showed marked qualitative differences in regulation, the former giving a substantially lower maximum induced level and induction ratio. Several parameters were slightly affected by strain background. In *P. mirabilis*, β-galactosidase control determined by both F*lac* (in accord with earlier work) and pGC9114 was markedly different from *E. coli* in that maximal induced levels were about an order of magnitude lower and the induction ratio was reduced to 3 to 5. In *Ps. aeruginosa* and *Ps. putida*, Tn951-specified *lac* expression was qualitatively similar to that in *P. mirabilis*. Possible reasons for anomalous expression in *Proteus* and *Pseudomonas* are discussed.

INTRODUCTION

Plasmids conferring upon normally Lac$^-$ species the ability to ferment lactose and utilize this sugar as sole carbon and energy source are familiar in the *Enterobacteriaceae*, e.g. *Salmonella* (Falkow & Baron, 1962; Synenki *et al.*, 1973; Le Minor *et al.*, 1974), *Proteus* (Sutter & Foecking, 1962; Falkow *et al.*, 1964; Le Minor & Coynault, 1976), *Yersinia* (Cornelis *et al.*, 1976) and others (see Guiso & Ullman, 1976). This phenomenon can be a nuisance in routine clinical identification. In addition, *lac* plasmids are found in the genus *Klebsiella* which also carries a chromosomal *lac* system (Reeve & Braithwaite, 1970; Brenchley & Magasanik, 1972). In at least one instance, plasmid-borne determinants responsible for lactose utilization are carried on a transposon (Cornelis *et al.*, 1978). This transposon, Tn951, was found on plasmid pGC1 in a clinical isolate of *Yersinia enterocolitica*. Previous reports (Cornelis *et al.*, 1978, 1979) have described its integration at various sites on the P1-group plasmid RP1, and have demonstrated that it contains a region homologous by heteroduplex analysis to the *lac* region of *Escherichia coli* K12, and of sufficient size just to accommodate the *lacI*, *Z* and *Y* genes but not *lacA*.

Observations on the expression of plasmid-borne genes in different bacterial species have shown that although in many cases there is little variation between one host and another, there are certain gene–host combinations in which expression is anomalous. Some striking instances involve *Proteus* hosts, in which the *E. coli* chromosomal *lac* genes and also those of the plasmid P*lac* (Falkow *et al.*, 1964; Wohlhieter *et al.*, 1964; Colby & Hu, 1968*a*, *b*, *c*; Stubbs *et al.*, 1973), the β-lactamase genes on a variety of conjugative R plasmids (Smith,

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1969; Dale & Smith, 1971) and genes controlling F-type sex pili (Baumberg & Dennison, 1975), are all poorly expressed. However, not all exogenous genes suffer regulatory anomalies in *Proteus*; Manson & Yanofsky (1976) reported that *E. coli* K12 *trp* genes on an F-prime show normal control in a wide variety of enterobacteria including *P. mirabilis*. Examples are known of poor expression in hosts other than *Proteus*. P1-group R plasmid-specified carbenicillin resistance is poorly expressed in *Rhizobium leguminosarum* by comparison with *E. coli* (Beringer, 1974). *Trp* genes of *Pseudomonas aeruginosa* translocated to R68.44 (Hedges *et al.*, 1977) and of *Rhizobium meliloti* translocated to R68.45 (Johnston *et al.*, 1978) are expressed weakly or not at all in *E. coli*, although the plasmids gave rise to derivatives which permitted increased expression. On the other hand, *E. coli* K12 *trp* genes cloned in RP4 and thereby transferred to *Ps. aeruginosa* or *R. leguminosarum* (Nagahari *et al.*, 1977, 1979) were expressed at the rate characteristic of the original host; and the *R. meliloti trp* genes carried on R-primes were expressed efficiently in *Ps. aeruginosa* as well as in *R. leguminosarum* (Johnston *et al.*, 1978).

With the advent of *in vitro* recombination techniques it is possible to study the expression of bacterial genes in a much wider variety of host species than hitherto. Not surprisingly, more examples of anomalous expression are becoming evident from this source, such as the failure of expression in *Bacillus subtilis* of some antibiotic resistance genes derived from Gram-negative plasmids (Ehrlich, 1978) and weak expression in *E. coli* of the *Bacillus licheniformis* β-lactamase gene cloned on a λ vector (Brammar, 1977). In none of these cases is the molecular basis for poor expression known.

Availability of an RP1::Tn951 plasmid such as pGC9114 facilitates further studies of *lac* expression in *Proteus* species, in which F*lac* is unstable, and permits extension of such studies to *Pseudomonas* species to which F*lac* cannot be transferred. We have accordingly introduced pGC9114 into *P. mirabilis*, *Ps. aeruginosa* and *Ps. putida* and have measured β-galactosidase activities therein with and without inducer. In *E. coli* and *P. mirabilis*, we were able to compare β-galactosidase activities specified by Tn951 with those specified by the *E. coli* chromosomal *lac* region introduced on an F-prime.

METHODS

Bacterial strains. Escherichia coli K12 derivatives were JC3272, F$^-$ *his lys trp lac*Δ*X74 rpsL*, X5097, F$^-$ Δ(*pro lac*) *X*111 *rpsL* (J. G. Scaife), and GL1490, F$^-$ Δ(*pro lac*) *X*111 (this laboratory). *Proteus mirabilis* strains (L. S. Baron) were WR11 and WR13 (*lac*$^+$) (Gemski *et al.*, 1967). *Pseudomonas aeruginosa* was PAO8, *met-28 ilv-202 str-1* (B. W. Holloway), and *Ps. putida* was PAW340, *trp str* (P. A. Williams). The F *proAB lac* plasmid was F128 (J. G. Scaife); the plasmid pGC9114 (= RP1::Tn951) has been described (Cornelis *et al.*, 1978).

Media, growth and harvesting of cells, and enzyme assays. Escherichia coli and *Proteus mirabilis* cultures were grown in Davis & Mingioli (1951) minimal salts supplemented with Casamino acids (Difco) to 0·5 % (w/v) as well as amino acids and vitamins specifically required. Glycerol, glucose or glucose 6-phosphate (Sigma) was added to 0·5 % (w/v), and (for inducing conditions) isopropyl β-D-thiogalactopyranoside (IPTG) to 1 mM (increase in concentration had no additional effect). Overnight cultures were diluted 20-fold into fresh medium and incubated with shaking at 37 °C; turbidity was read periodically on a Klett–Summerson colorimeter (no. 66 filter). Samples were taken at intervals during exponential growth, two drops of toluene were added and the mixture was incubated at 37 °C for 30 min. Portions (0·1 ml) of the toluene-treated sample or dilutions thereof were then assayed for β-galactosidase by the method of Pardee *et al.* (1959) as described by Miller (1972), activities being calculated on the basis of the unit as defined therein [it was assumed that Miller's value for the absorption coefficient of *o*-nitrophenol, 4500, is a misprint for the value of 7500 given by Pardee *et al.* (1959)]. Protein was assayed by the method of Lowry using cells centrifuged, resuspended in buffer and disrupted with an MSE 100 W ultrasonic disintegrator.

Pseudomonas aeruginosa and *Ps. putida* cultures were grown (at 37 °C and 30 °C, respectively) in minimal salts (Grinsted *et al.*, 1972) containing glycerol at 0·2 %, amino acids and thiamin as required, and (for inducing conditions) IPTG at 1 mM. Overnight cultures were diluted 10-fold in fresh medium and then incubated with shaking for 4 to 6 h until in late-exponential growth. An appropriate volume of culture was diluted in Z buffer (Miller, 1972) to give 1 ml, the cells were ultrasonically disrupted with a Branson sonifier (25 W), and β-galactosidase was assayed as described by Pardee *et al.* (1959). Also, a portion of the harvested

cells was washed twice in water and assayed for protein by the method of Lowry. Specific activities are given in units (mg protein)$^{-1}$ with the unit of activity as defined by Pardee *et al.* (1959).

Cultures to be assayed were checked for possible loss of plasmid and/or *lac*$^+$ determinant by plating on appropriate antibiotic and/or lactose indicator media.

The indicator medium for lactose fermentation by *Ps. aeruginosa* and *Ps. putida* contained 0·1 % (w/v) peptone, 1 % (w/v) lactose, 0·004 % (w/v) phenol red and 1·5 % (w/v) agar, at pH 7.

RESULTS AND DISCUSSION

Effect of E. coli K12 *strain background*

The three *E. coli* K12 (*lac* deletion) strains (JC3272, X5097 and GL1490) containing either F128 or pGC9114 were grown with glycerol, glucose or glucose 6-phosphate as carbon/energy source with or without inducer IPTG and their β-galactosidase activities were measured (Table 1). Clearly *lac* expression was quantitatively different between pGC9114 and F*lac*. Induced levels for the former were always lower, by factors of 1·5 to 7·7 depending on the background and carbon source, as also was the induction ratio, by factors of 7 to 52. In contrast, the uninduced levels for pGC9114 were invariably higher than for F*lac*, by factors of 2·9 to 17.

Such differences in regulation pattern between enterobacterial plasmid-borne *lac* operons and the *E. coli* K12 chromosomal one are not unprecedented; for instance, the maximal induced level of β-galactosidase and induction ratio are 2000 and 22, respectively, for a *lac* plasmid originally present in *Enterobacter liquefaciens* (Guiso & Ullman, 1976). It is not known whether the *lac* region of the latter, or indeed of any other naturally occurring plasmid, is transposon-linked.

Clear-cut effects of strain background (for cultures under otherwise identical conditions) included (i) lower F*lac*-determined activities in the X5097 than in the JC3272 or GL1490 backgrounds, (ii) lower induced pGC9114-determined activities in the JC3272 than in the GL1490 background, (iii) uninduced pGC9114-determined activities decreasing in the order GL1490 > JC3272 > X5097 and (iv) a higher induction ratio for pGC9114 in X5097 than in the other two backgrounds. Similar background effects may be noted elsewhere in the literature but their basis is unknown. Table 1 also demonstrates a reproducible though slight effect of catabolite repression on induction ratio such that the latter decreases as the former increases.

Expression of Tn951 *β-galactosidase in Proteus mirabilis, Pseudomonas aeruginosa and Ps. putida*

Specific activities of β-galactosidase in *P. mirabilis* WR13, which carries the *E. coli* K12 chromosomal *lac* operon introduced originally on F*lac* (Gemski *et al.*, 1967), and in WR11(pGC9114) are shown in Table 1. The induced levels are, in both cases, of the order of 10-fold less than for the same *lac* genes in *E. coli* K12. Moreover, the induction ratios were significantly reduced, to 2·3 to 4·8 for F*lac* and 3·2 to 5·4 for pGC9114. Activities showed little variation with the carbon/energy source provided, so that catabolite repression may not be operating.

Plasmid pGC9114 was transferred to *Ps. aeruginosa* PAO8 and *Ps. putida* PAW340 by conjugation (Cornelis *et al.*, 1976) selecting for its antibiotic resistance markers only. On streaking on peptone/phenol red/lactose agar, transconjugants acidified the medium more rapidly than did the *Pseudomonas* spp. controls: PAO8(pGC9114) did so after 2 d and PAW340(pGC9114) after 5 d, whereas PAO8 and PAW340 only acidified the medium after 8 d. The induced level of β-galactosidase in PAO8(pGC9114) was of the same order as that in WR11(pGC9114) (Table 1), the induction ratio also being similar or perhaps slightly greater. In PAW340(pGC9114), the induced level was even more depressed (Table 1), while the induction ratio was similar to that in WR11(pGC9114). It is interesting that PAO8

Table 1. *Expression of plasmid-borne lac in Escherichia coli, Proteus mirabilis, Pseudomonas aeruginosa and Ps. putida*

Cultures were grown in supplemented minimal salts medium with the additional carbon/energy source indicated and with or without isopropyl β-D-thiogalactopyranoside (IPTG) as inducer, sampled and assayed for β-galactosidase as described in Methods. Specific activities are expressed as nmol min^{-1} $(mg\ protein)^{-1}$.

		β-Galactosidase specific activity											
		Additional carbon/energy source: None			Glycerol			Glucose			Glucose 6-phosphate		
Strain	Plasmid	−IPTG	+IPTG	IR*	−IPTG	+IPTG	IR*	−IPTG	+IPTG	IR*	−IPTG	+IPTG	IR*
E. coli													
JC3272	F128				27	26000	930	9·9	8700	800	7·8	5800	740
	pGC9114				91	3800	41	34	1300	39	23	750	32
X5097	F128				5·9	15000	2600	3·1	2800	920	2·6	1900	730
	pGC9114				51	10000	200	11	1400	130	10	960	94
GL1490	F128				22	31000	1400	14	17000	1200	7·8	8200	1050
	pGC9114				320	8700	27	220	5300	24	130	2900	23
P. mirabilis													
WR11	None				ND	ND							
WR13	(*lac* genes originally from F*lac*)	700	2000	2·8	510	1800	3·5	420	2100	4·8	790	1800	2·3
WR11	pGC9114	210	680	3·2	86	340	3·9	89	490	5·4	190	680	3·6
Ps. aeruginosa													
PAO8	None				ND	7·3							
	pGC9114				58	390	6·8						
Ps. putida													
PAW340	None				ND	ND							
	pGC9114				29	80	2·9						

ND, Not detectable. * IR, Induction ratio.

appears to contain an IPTG-inducible *o*-nitrophenyl-β-D-galactopyranoside-hydrolysing enzyme, with, however, a very low activity.

Our results on the expression of *lacZ* (F*lac* and Tn951) in *P. mirabilis* confirm those of Colby & Hu (1968*a*, *b*, *c*) and Stubbs *et al.* (1973) in that induced levels of β-galactosidase are about an order of magnitude lower than in *E. coli* and induction is very slight. Possible explanations for anomalous expression of some 'foreign' genes in *Proteus* species (but not all: see, for example, Morgan & Kaplan, 1976) were discussed by Baumberg & Dennison (1975). Four possibilities, suggestive of further experiments, are: (i) lack of affinity of *P. mirabilis* RNA polymerase for the *lac* promoters; (ii) absence in *P. mirabilis* of cyclic AMP-mediated catabolite activator protein (CAP) stimulation of *lacZYA* transcription; (iii) lack of appropriate tRNAs in *P. mirabilis* for translation of *lac* transcripts; (iv) preferential degradation of *lac* mRNA and/or polypeptides in *P. mirabilis*. It is perhaps relevant that levels of λ*plac5*-hybridizable RNA correspond to β-galactosidase activities in *lac*$^+$ *P. mirabilis* much as in *E. coli* K12 (M. Roberts, unpublished results). Whereas (i), (iii) and (iv) account for both the low induced level of β-galactosidase and the low induction ratio, the former reflecting weak expression of *lacZYA*, and the latter weak expression of *lacI*, (ii) only accounts for the low induced level, and an additional hypothesis is needed to explain the low induction ratio.

Expression of *lacZ* on Tn951 in *Ps. aeruginosa* resembles that in *P. mirabilis*, while in *Ps. putida* the induced level of β-galactosidase is even lower. The same kinds of explanation suggested above for *P. mirabilis* could apply equally in these cases.

Insertions of Tn951 into wide host range P1-group plasmids may be useful for regulation and other studies. For instance, it appears that pGC9114 can be introduced into *Agrobacterium tumefaciens* and β-galactosidase expressed therein (N. Saeed, H. Gainer & G. R. K. Sastry, personal communication).

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