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Differential Regulation of Lambda p_L and p_R Promoters by a *c*I Repressor in a Broad-Host-Range Thermoregulated Plasmid Marker System

CRAIG WINSTANLEY,[1]* J. ALUN W. MORGAN,[1] ROGER W. PICKUP,[2] J. GWYNFRYN JONES,[2] AND JON R. SAUNDERS[1]

Department of Genetics and Microbiology, University of Liverpool, Liverpool L69 3BX,[1] and Freshwater Biological Association, Windermere Laboratories, Ambleside, Cumbria LA22 0LP,[2] United Kingdom

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Plasmid systems with unique markers were constructed to assess the fate of recombinant DNA and genetically manipulated bacteria in soil and freshwater model environments. On such constructs the marker gene, *xylE* (for catechol 2,3-dioxygenase), is expressed from the lambda promoter p_L or p_R, each of which is controlled by the temperature-sensitive lambda repressor *c*I857. Combinations of these elements were cloned into the broad-host-range plasmid pKT230 to form pLV1010 (p_L-*xylE*), pLV1011 (p_L-*xylE*-*c*I857), and pLV1013 (p_R-*xylE*-*c*I857). The recombinant plasmids were introduced into different gram-negative bacteria. The thermoregulated system of pLV1013 functioned well in a range of species, with *xylE* induction being readily achieved by elevation of the temperature from 28 to 37°C. There was a difference in the induction of catechol 2,3-dioxygenase activity, depending on whether *xylE* was expressed from p_L (pLV1011) or p_R (pLV1013). Our observations on testing the different systems in a number of hosts suggest that genes carried by the DNA of genetically engineered microorganisms may not be expressed in a predictable manner following transfer from the release host to other species.

Most accidental or deliberate releases of genetically engineered microorganisms (GEMs) are likely to involve runoff from soil into freshwater habitats. Little is known about the probable fate of GEMs or their recombinant DNAs should such releases occur. It is important, therefore, to study model systems to assess the possible hazards and environmental consequences involved.

The study of the fate of recombinant plasmids or the assessment of plasmid transfer in natural environments has generally relied upon experiments involving antibiotic resistance plasmids (5, 6, 18, 38). Any attempts to monitor the survival or transfer of such plasmids in aquatic systems are likely to be obscured by a high background of naturally occurring antibiotic-resistant bacteria (19, 20). The situation is further complicated by the fact that bacteria may enter a viable but nonculturable state in aquatic environments (4) which would preclude selection for resistance markers by plating techniques. Selection of a strain carrying antibiotic resistance markers on highly selective media can also reduce host pathogenicity or persistence (11). For these reasons it has been necessary to develop marker systems that do not rely upon antibiotic resistance (4, 7, 15, 29, 37).

We have developed a versatile marker system that can be applied in the assessment of the survival of GEMs in freshwater-sediment, soil, and other model systems. The basic test system involves detection of the *xylE* gene and its product (catechol 2,3-dioxygenase; C23O) of the TOL plasmid pWW0 (30, 42). On marker plasmids the *xylE* gene is expressed from the bacteriophage lambda p_L or p_R promoter under the control of the temperature-sensitive lambda repressor *c*I857. The potentially deleterious metabolic burden imposed on the cell by the high expression of *xylE*, caused by the derepression of lambda promoters, is countered by the presence of the *c*I857 repressor protein.

* Corresponding author.

We have cloned these constituent parts into the broad-host-range nonconjugative IncQ plasmid pKT230 (1), allowing assessment of the performance of our system in a range of gram-negative bacteria. In this paper we describe the expression of *xylE* from lambda promoters and significant differences in *c*I857 regulation depending on whether *xylE* is expressed from p_L or p_R.

MATERIALS AND METHODS

Bacterial strains and plasmids. The bacterial strains and plasmids used in this study are listed in Tables 1 and 2. Bacterial strains were routinely grown at 30°C in nutrient broth medium (lab/m, Bury, Lancashire, United Kingdom). Strains carrying recombinant plasmids were maintained in the presence of the relevant antibiotics at the appropriate concentrations.

DNA purification. pWW0 DNA was isolated from *Pseudomonas putida* by the rapid sucrose gradient method of Wheatcroft and Williams (41). Vector and recombinant plasmid DNAs were extracted from *Escherichia coli* by the cleared-lysate method (13) followed by cesium chloride-ethidium bromide centrifugation. The rapid boiling method of Holmes and Quigley (17) was used for small-scale plasmid isolations.

Restriction digestions and agarose gel electrophoresis. Restriction endonucleases were obtained from Boehringer Corporation Ltd. (Lewes, Sussex, United Kingdom), and digestions were performed in O'Farrell buffer (31) under the conditions recommended by the supplier. Agarose gel electrophoresis was carried out by standard procedures. Lambda DNA digested with *Hin*dIII was used as a standard, taking the sizes of the fragments to be those indicated by the supplier (Pharmacia Ltd., Milton Keynes, Buckinghamshire, United Kingdom).

DNA cloning and transformations. Reaction conditions for ligation with T4 DNA ligase and the production of blunt ends

TABLE 1. Bacterial strains

Strain	Description	Reference or source
Escherichia coli		
ED8654	Lac$^-$ Met$^-$ Thi$^-$ *hsd* r_K^- r_M^-	P. A. Williams
CA60(pNJ5000)	Thi$^-$ Leu$^-$ Thr$^-$ F$^-$ *supE44 hsdR hsdM recBC*	P. A. Williams
Klebsiella pneumoniae		
NCIB 418		9
K2819	Clinical isolate	N. G. Johnston
Serratia rubidaea	Freshwater isolate	This study
Aeromonas hydrophila NCTC 8049 (ATCC 7966)		36
Pseudomonas putida		
PRS2000 (ATCC 12633)		P. A. Williams
PaW1	Contains pWW0	42
PaW140	Plasmid-free derivative of PaW1	P. A. Williams
FBA11	Freshwater isolate	This study
Pseudomonas aeruginosa		
NCIB 8295 (ATCC 10145)		39
PAO1 (ATCC 15692)		16, P. A. Williams
Pseudomonas fluorescens FH1	Freshwater isolate	This study
Acinetobacter calcoaceticus ADP1		21, P. A. Williams

with T4 DNA polymerase I were as recommended by the supplier (Boehringer). Transformation into *E. coli* ED8654 was done by standard methods (3), and transformants were selected on nutrient agar containing the appropriate antibiotics at the following concentrations: streptomycin, 15 μg/ml; kanamycin, 15 μg/ml; and ampicillin, 30 μg/ml.

Mobilization of plasmids. Broad-host-range plasmid constructs were mobilized from *E. coli* ED8654 into other gram-negative hosts by means of a nonquantitative triparental mating procedure which involved the mobilization functions of pNJ5000, a plasmid which is only maintained in the presence of tetracycline at 15 μg/ml (12). Samples consisting of 10 μl of cells from overnight nutrient broth cultures of CA60(pNJ5000), ED8654 (containing the plasmid construct), and the recipient strain were successively overlaid on a nutrient agar plate and allowed to dry. Following overnight incubation at 30°C, cells were suspended in 0.5 ml of 100 mM phosphate buffer (pH 7.0), and dilutions were plated on minimal media with added succinate (10 mM) and the appropriate antibiotics at the following concentrations: streptomycin, 100 μg/ml; and kanamycin, 100 μg/ml.

TABLE 2. Plasmids

Plasmid	Genetic character(s)[a]	Reference or source
pPLc245	Ap^r p_L	32
pWW0	*xylE*	42
pKT230	Km^r Sm^r	1
pcI857	Km^r p_R *c*I857	33
pFBA10-1	Ap^r p_L *xylE*	This study
pFBA10-2	Km^r p_L *xylE c*I857	This study
pLV1010	Ap^r Sm^r p_L *xylE c*I857	This study
pLV1011	Sm^r p_L *xylE c*I857	This study
pLV1012	Km^r p_R *xylE c*I857	This study
pLV1013	Km^r Sm^r p_R *xylE c*I857	This study
pNJ5000	Tc^r	12

[a] Ap^r, Km^r, Sm^r, and Tc^r indicate resistance to ampicillin, kanamycin, streptomycin, and tetracycline, respectively.

Detection of *xylE*-expressing clones. Colonies expressing *xylE* (C23O$^+$) could be visualized by being sprayed with 1% (wt/vol) catechol solution. A positive reaction was indicated by the production of yellow coloration from the formation of 2-hydroxymuconicsemialdehyde.

Preparation of cell extracts and C23O assay. Cells were harvested, resuspended in 1 ml of 100 mM phosphate buffer (pH 7.0), and subjected to sonic disintegration on ice at an amplitude of 12 μm (peak to peak) for five periods of 15 s. The suspension was centrifuged at 25,000 × *g* for 15 min at 4°C. C23O was assayed by published procedures (34), and the specific activity was expressed as milliunits per milligram of protein. Protein concentrations were determined by the use of biuret reagent (Sigma Chemical Co., Poole, Dorset, United Kingdom) with bovine serum albumin as a standard.

SDS-PAGE. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis of cell extracts was carried out with 10% gels as described by Laemmli (23).

RESULTS

Construction of plasmids. The construction of the plasmids used in this study is outlined in Fig. 1. To enable *xylE* expression from p_L, the 2.4-kilobase *xylE*-containing *Xho*I fragment of the TOL plasmid pWW0 was excised and cloned into the *Sal*I site of pPLc245 to produce pFBA10-1. The formation of the unregulated p_L-*xylE* system of pLV1010 was achieved by the ligation of *Hin*dIII-digested pFBA10-1 into the *Hin*dIII site of the broad-host-range plasmid pKT230.

The *c*I857 control element was introduced into the p_L-*xylE* system by digestion of pcI857 and pFBA10-1 with *Pst*I followed by ligation to form pFBA10-2. Inducible C23O activity was observed in *E. coli* hosts containing pFBA10-2 after an increase in temperature from 28 to 42°C (data not shown). The p_L-*xylE*-*c*I857 region of pFBA10-2 was excised on a *Bgl*II-*Xba*I fragment which was subsequently treated with T4 DNA polymerase to produce blunt ends and ligated into the *Sma*I site of pKT230. This resulted in the formation of pLV1011, a broad-host-range plasmid containing a p_L-*xylE*-*c*I857 system.

To enable *xylE* expression from p_R, the *xylE* gene was excised from pFBA10-2 on a *Bam*HI-*Pst*I fragment which was subsequently rendered blunt ended with T4 DNA polymerase and ligated with *Bgl*II-digested pcI857 which had also been treated to produce blunt ends. The resulting plasmid, pLV1012, contained a p_R-*xylE*-*c*I857 region which was isolated on a *Pst*I-*Sma*I fragment and cloned into the *Bam*HI site of pKT230 with T4 DNA polymerase to produce blunt ends. This resulted in the formation of pLV1013, a broad-host-range plasmid containing a p_R-*xylE*-*c*I857 system. The gene arrangements of pLV1010, pLV1011, and pLV1013 were confirmed by restriction endonuclease digestion analysis.

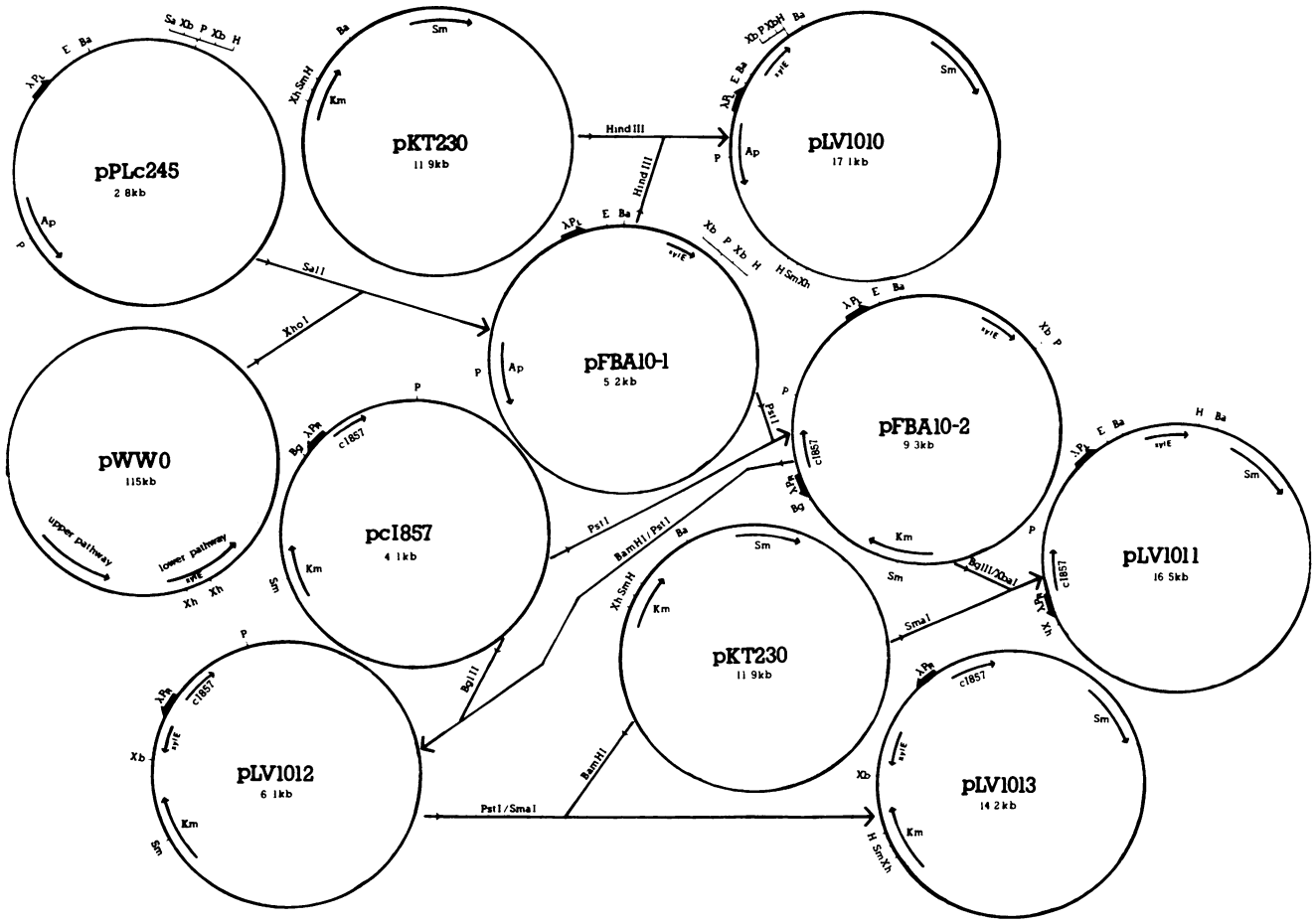

FIG. 1. Construction of marker plasmids. Abbreviations indicating cleavage sites for restriction enzymes are as follows: Ba, *Bam*HI; Bg, *Bgl*II; E, *Eco*RI; H, *Hin*dIII; P, *Pst*I; Sa, *Sal*I; Sm, *Sma*I; Xb, *Xba*I; Xh, *Xho*I. Ap, Km, and Sm indicate resistance to ampicillin, kanamycin, and streptomycin, respectively. Only restriction sites important for the construction of the plasmids are shown. kb, Kilobases.

Stability of plasmid constructs. Plasmids pLV1010, pLV1011, and pLV1013 were mobilized into a variety of gram-negative hosts to assess their stability. Table 3 shows a comparison of the stability of the three plasmids in different species of bacteria. Regulated plasmids pLV1011 and pLV1013 were both stable in *E. coli*, *Klebsiella pneumoniae*, and *Serratia rubidaea*, but pLV1011 was, in general, much less stable than pLV1013 in other species. Unregulated plasmid pLV1010 was considerably less stable than regulated plasmid pLV1013 in most cases. The exception to these observations was found in *Acinetobacter calcoaceticus* ADP1, in which all three marker plasmids were found to be highly unstable.

TABLE 3. Stability of plasmid constructs[a]

Host	Stability of:		
	pLV1013 (p_R-*xylE*-cI857)	pLV1011 (p_L-*xylE*-cI857)	pLV1010 (p_L-*xylE*)
E. coli ED8654	100	99	95
P. putida PRS2000	98	22	90
P. aeruginosa PAO1	90	18	51
A. hydrophila NCTC 8049	100	98	94
K. pneumoniae NCIB 418	100	99	55
S. rubidaea	100	100	67
A. calcoaceticus ADP1	11	30	4

[a] Stability was expressed as the percent retention of C23O$^+$ after overnight growth in nutrient broth at 30°C. Colonies were inoculated from plates containing the relevant antibiotics. Thus, in each case, all cells inoculated were C23O$^+$, with the exception of ADP1(pLV1010), for which antibiotic selection was not sufficient to maintain the C23O$^+$ phenotype (see the text). The average of two or more experimetns was taken.

Expression of *xylE* from marker plasmids. C23O activity was assayed for all three marker plasmids (pLV1010, pLV1011, and pLV1013) in various host bacteria, including some recently isolated from freshwater (Table 4). C23O assays of pLV1010-containing strains were carried out on cultures grown at 30°C. To assess the regulation of *xylE* expression from pLV1011 and pLV1013, we carried out time course studies of C23O activity in nutrient broth after incubation at elevated temperatures. Since incubation at 42°C had an adverse effect on some of the host strains, a temperature of 37°C was also used. Cultures were grown overnight at 28°C, after which the temperature of incubation was raised and 50-ml samples were removed for assay after 0, 2, 4, 6, 8, 10, 14, and 25 h. Relevant antibiotics were included in the media in cases in which, after overnight growth in nutrient broth, the plasmid present was less than 100% stable in the host being used. A graph showing the results of some time course studies for the activation of the pLV1013 system at 37°C in various hosts is presented in Fig. 2. Figure 3 shows the SDS-PAGE protein profile of various

TABLE 4. C23O activities of plasmid constructs

Host strain	C23O sp act (mU/mg of protein) in:								
	pLV1010	pLV1011 at:						pLV1013 at 37°C		
		37°C			42°C				
		Initial	Maximum	Fold increase	Initial	Maximum	Fold increase	Initial	Maximum	Fold increase
E. coli ED8654	24,000	0.9	44	49	1.9	7,100	3,700	7.1	4,800	680
P. putida										
PRS2000	52,000	72	330	4.6	190	200	1.1	87	5,000	57
PaW140	62,000	150	580	3.9	82	150	1.8	86	2,100	24
FBA11	5,500	150	580	3.9	220	260	1.2	130	3,200	25
P. aeruginosa										
PAO1	22,000	180	530	2.9	190	5,900	31	170	4,600	27
NCIB 8295	32,000	170	570	3.4	160	4,400	28	240	16,000	67
P. fluorescens FH1	5,800	180	740	4.1	280	280	1.0	290	5,200	18
A. hydrophila NCTC 8049	20,000	50	2,300	46	28	230	8.2	52	11,000	210
K. pneumoniae										
NCIB 418	22,000	18	340	19	4.4	2,600	590	31	6,300	200
K2819	14,000	37	400	11	23	1,200	52	11	3,300	300
S. rubidaea	9,500	6.6	36	5.5	4.7	120	26	8.9	2,300	260
A. calcoaceticus ADP1	5,600	2,000	2,000	1	1,900	1,900	1.0	560	3,400	6.1

pLV1013-containing strains grown at 30 and 37°C. To obtain a 37°C value for PRS2000(pLV1013), we grew the strain at 28°C and subsequently incubated it at 37°C overnight. A protein of the same molecular weight as C23O (35,000) could be seen at higher levels in the 37°C samples.

(i) *xylE* expression from pLV1010. High levels of C23O activity occurred in all hosts containing pLV1010, indicating the effectiveness of lambda promoters in a range of gram-negative bacteria. Specific activity measurements for pLV1010 in FBA11, FH1, and ADP1 were lower, probably because of the high instability of the $C23O^+$ phenotype in these hosts even in the presence of antibiotics. Stability tests for pLV1010 in these three hosts showed that there was more than a 90% loss of the $C23O^+$ phenotype after overnight culturing in nutrient broth containing ampicillin (100 μg/ml) and streptomycin (100 μg/ml). Analysis of $C23O^-$ colonies suggested that DNA had been deleted from pLV1010. It seems likely that the C23O activities recorded in these hosts were lower because of a high rate of loss of *xylE* from pLV1010.

(ii) *xylE* expression from pLV1011 at 37°C. In *E. coli xylE* expression increased 49-fold but still only rose to 44 mU/mg. A similar increase occurred in *Aeromonas hydrophila*, but a maximum activity of 2,300 mU/mg was obtained. In *K. pneumoniae* an increase of 10- to 20-fold was found, with a highest activity of 400 mU/mg, as compared with only a 5.5-fold increase leading to a highest activity of 36 mU/mg in *S. rubidaea*. In *Pseudomonas* species the initial C23O activity was much higher than that in the other species (72 to 180 mU/mg), with only small increases occurring (2.9- to 4.6-fold). The highest uninduced levels were found in *A. calcoaceticus*, in which the initial activity of 2,000 mU/mg was not increased by elevation of the temperature.

(iii) *xylE* expression from pLV1011 at 42°C. *xylE* expression in *E. coli* was increased 3,700-fold after incubation at 42°C to a maximum level of 7,100 mU/mg. An improvement in induction at 42°C over that at 37°C was also observed in *K. pneumoniae* (50- to 590-fold) and *S. rubidaea* (26-fold). There was also good induction in *Pseudomonas aeruginosa* (28- to 31-fold), with highest levels of 4,400 to 5,900 mU/mg. This result differed from that for the other *Pseudomonas* species, in which very little induction was found.

(iv) *xylE* expression from pLV1013 at 37°C. There was excellent induction of *xylE* from pLV1013 at 37°C in *E. coli* (680-fold), *K. pneumoniae* (200- to 300-fold), and *S. rubridaea* (260-fold) and good induction in all other species tested. As with pLV1011, the initial C230 activity was higher in *Pseudomonas* species and *A. calcoaceticus* than in other hosts. Unlike the p_L-*xylE*-*c*I857 system, at 37°C the pLV1013 system exhibited significant increases in *xylE* expression of 18- to 67-fold in *Pseudomonas* species and 6.1-fold in *A. calcoaceticus*. In addition, higher levels of C23O activity were found in all other species tested.

(v) *xylE* expression from pLV1013 at 42°C. *xylE* induction from pLV1013 at 42°C was similar to that at 37°C for all hosts with the exception of *P. putida*, *Pseudomonas fluorescens*, and *A. hydrophila*. In these hosts either very little increase was observed or initial increases were short-lived and followed by decreases (data not shown).

DISCUSSION

Thermoregulated expression systems have been developed with p_L in *E. coli* (32, 33) and *Erwinia* and *Serratia* species (25) and with p_R in *E. coli* (24) and *P. aeruginosa* (8). Comparisons of the two lambda promoters together with hybrid p_L-p_R promoters and their regulation by *c*I857 have been carried out in a number of enteric bacteria (28). In our search for an effective repressed-marker system to use for the detection of GEMs in natural environments, we compared the *c*I857 temperature-dependent regulation of expression of *xylE* from the two lambda promoters p_L and p_R. We

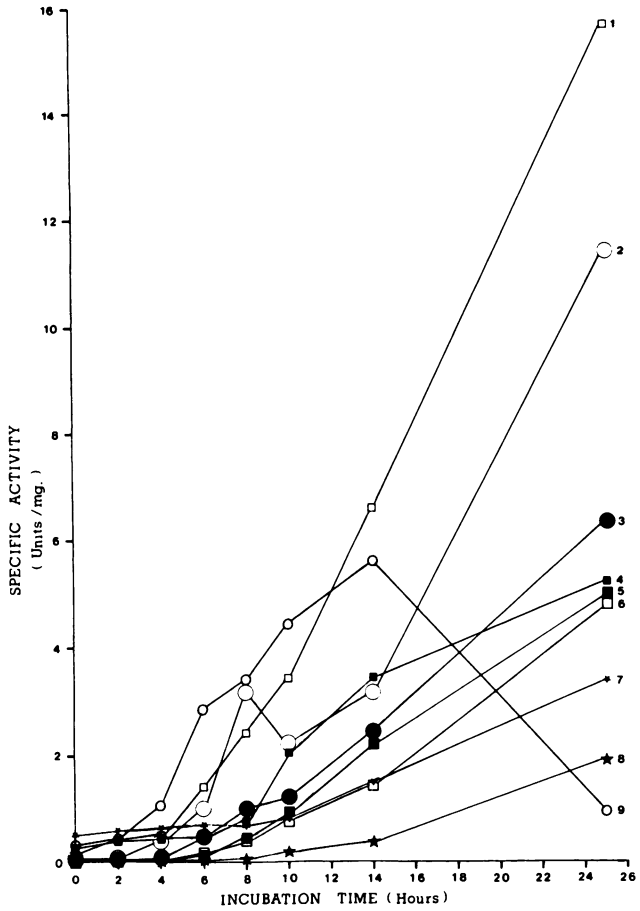

FIG. 2. Increase in C23O activity from pLV1013 at 37°C. Host strain: 1, NCIB 8295; 2, NCTC 8049; 3, NCIB 418; 4, FH1; 5, PRS2000; 6, ED8654; 7, ADP1; 8, *S. rubidaea*; 9, FBA11.

confirmed that both promoters are able to direct gene expression in a range of gram-negative bacteria. Our results also confirmed reported findings that expression from p_L and p_R is tightly regulated by *c*I857 in *Klebsiella* and *Serratia* species and the natural host *E. coli* (25, 28). We found, however, that uninduced C23O activity in both p_L-*xylE*-*c*I857 and p_R-*xylE*-*c*I857 systems was much higher in *Pseudomonas* and *Acinetobacter* hosts than in enteric bacteria, probably because of *Pseudomonas* promoter regions associated with *xylE* on the constructs. There was very little induction of either the p_L-*xylE*-*c*I857 system or the p_R-*xylE*-*c*I857 system in *P. putida*, *P. fluorescens*, and *A. hydrophila* at 42°C, probably because of harmful effects of the higher temperature on these species. Induction was found at 42°C in *P. aeruginosa*.

The most surprising difference between the p_R-*xylE*-*c*I857 system of pLV1013 and the p_L-*xylE*-*c*I857 system of pLV1011 was the superior induction of the p_R system at 37°C. An improvement of 10-fold or more was common, with the effect varying depending on the species. Thus, much more substantial induction of *xylE* was obtained in *P. putida* and *P. fluorescens* when the p_R system was operating. There was also some induction of the pLV1013 system but not the pLV1011 system in *A. calcoaceticus*. This result could be partially accounted for by the higher uninduced levels of *xylE* activity found with p_L. The difference between induction of the two promoters at 37°C, which varied depending on the host species, suggests that *c*I857 is not inactivated as effectively at 37°C for binding to the p_L operator as it is for binding to the p_R operator. This possibility could be due to species-specific effects that affect the thermostability and/or the relative efficiency of *c*I857 when bound to p_L but not p_R operator-promoter regions.

The p_R-*xylE*-*c*I857 system of pLV1013 proved to be far more stable than the p_L-*xylE*-*c*I857 system of pLV1011 in all hosts except *A. calcoaceticus* ADP1. This instability may have been due to the high uninduced level of *xylE* expression from pLV1013 in ADP1 as compared with other hosts. There is no obvious reason why the performance of the system should markedly differ in *A. calcoaceticus* and all the other species tested. Since p_L and p_R are both present on pLV1011, there is a possibility that the reduced induction of the pLV1011 system at 37°C was the result of titration of the *c*I857 repressor because of an interaction with both operators simultaneously. However, pPLc245 (p_L) was transformed into ED8654(pLV1013) (p_R) and found to have no effect in *trans* on the induction of *xylE* at 37°C, despite the higher copy number of the p_L-containing plasmid. The instability of the overexpressing construct, pLV1010, varied not only between but within bacterial species. In the freshwater isolates FBA11 and FH1 the C23O$^+$ phenotype was unstable even in the presence of streptomycin and ampicillin, whereas in the other *Pseudomonas* strains instability only occurred in the absence of antibiotics.

A number of groups have demonstrated the potential of soil and aquatic bacteria to receive recombinant DNA in genetic exchanges (2, 10, 22, 35, 38, 40, 43). There have also been reported cases of nonconjugative plasmids being mobilized by indigenous wastewater bacteria (26, 27). The kinds of effects which we have observed—differences in the thermoregulation of the two promoters, differences in plasmid stability depending on the promoter, species-specific differences in the regulation or stability of the marker systems—suggest that the DNA of released GEMs, if it transfers to other hosts, may not act in a predictable manner in the natural environment. This possibility adds to the importance of studying the fate of GEMs and recombinant DNA in model systems.

In our system, the presence of the released host and

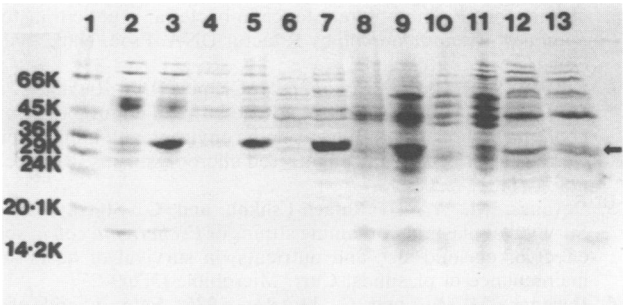

FIG. 3. SDS-PAGE of cellular products stained with Coomassie brilliant blue R250. Lanes: 1, molecular weight markers; 2, ED8654(pLV1013) grown at 30°C; 3, ED8654(pLV1013) grown at 37°C; 4, NCIB 418(pLV1013) grown at 30°C; 5, NCIB 418(pLV1013) grown at 37°C; 6, NCTC 8049(pLV1013) grown at 30°C; 7, NCTC 8049(pLV1013) grown at 37°C; 8, PAO1(pLV1013) grown at 30°C; 9, PAO1(pLV1013) grown at 37°C; 10, PRS2000(pLV1013) grown at 30°C; 11, PRS2000(pLV1013) incubated at 37°C; 12, *S. rubidaea*(pLV1013) grown at 30°C; 13, *S. rubidaea*(pLV1013) grown at 37°C. All cultures were grown in nutrient broth. The arrow indicates the position of the C23O protein.

plasmids transferred into other hosts, or organisms that have acquired the marker DNA by recombination, can be detected by a variety of biological and physical methods. These include detection of the enzyme activity (bioassay), the use of phenotypic markers carried by the plasmids, DNA hybridization with either whole *xylE* gene probes or oligonucleotide probes specific for *xylE* or *xylE* mRNA, and the use of polyclonal and monoclonal antibody probes which are specific for plasmid gene products or host-specific antigens. Most of these methods can be used to detect marked strains which have entered a viable but nonculturable state.

Our p_R-*xylE*-*c*I857 system was tested in a range of gram-negative bacteria and was stable in almost all cases. The system was temperature regulated in all the hosts tested. We have recently introduced our marker DNA into the broad-host-range IncP conjugative plasmid R68.45 (14). We will now be able to carry out a comparison of the fate of conjugative and nonconjugative mobilizable recombinant plasmids of two different incompatibility groups. We will also be able to compare survival in a number of different gram-negative hosts by using the various marker plasmids. Plasmids pLV1010 and pLV1011 can be utilized in release studies as unstable or unregulated controls to compare their behavior with that of our chosen system. This range of marker plasmids and hosts coupled with the use of a number of different methods of detection will provide a useful test system for studying the fate of GEMs and the transfer of recombinant DNA in various freshwater-sediment and soil systems.

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