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xylE Functions as an Efficient Reporter Gene in *Streptomyces* spp.: Use for the Study of *galP1*, a Catabolite-Controlled Promoter

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We describe the development of a convenient and sensitive reporter gene system for *Streptomyces* spp. based on the use of a promoterless copy of the *xylE* gene of *Pseudomonas putida*. The *xylE* gene product is a catechol dioxygenase, which converts the colorless substrate catechol to an intensely yellow hydroxymuconic semialdehyde. A promoterless copy of *xylE* was placed under the transcriptional control of *galP1*, a glucose-repressed and galactose-induced promoter from *Streptomyces lividans*, and its expression was examined in bacterial colonies on agar plates or in liquid cultures grown in the presence of glucose or galactose as the sole carbon source. On plates, colonies of bacteria grown on galactose turned bright yellow within a few minutes of being sprayed with a solution of catechol, whereas colonies on glucose-containing plates remained white or only slightly colored, even after extensive incubation. Activity of *galP1-xylE* fusions was conveniently measured in crude cell extracts with a simple colorimetric assay and was shown to faithfully reflect intracellular RNA levels, as determined by quantitative dot blots. Moreover, differences in expression levels of *xylE* fusions driven by mutant *galP1* promoters were readily apparent in color reactions on plates. The properties of *xylE* as a reporter gene thus make it suitable not only for quantitatively monitoring expression of regulated promoters in *Streptomyces* spp. but also for recovering mutations that alter the expression levels of promoters of interest.

The use of reporter genes, such as the *lacZ* gene of *Escherichia coli*, which encodes an easily assayed protein and allows a semiquantitative assessment of gene expression in colonies on agar plates (11, 35), has revolutionized the way genetic techniques can be applied to the analysis of gene regulation in both procaryotic and eucaryotic microorganisms. However, efforts to make use of *lacZ* as a reporter gene in *Streptomyces* species, which are of interest because of their unique morphology, their complex developmental life cycle, and the important compounds they make in secondary metabolism, have proved unsuccessful. All species examined thus far have significant endogenous β-galactosidase activity, and mutants lacking this activity were found to have pleiotropic defects, making them inappropriate for most gene regulation studies (13; Thomas Eckhardt, personal communication). In addition, the *lacZ* gene of *E. coli* is poorly expressed in *Streptomyces* species because of differences in codon usage (22; K. Chater, personal communication). Several other reporter gene systems have been investigated for use in *Streptomyces* spp. These include systems based on the *E. coli ampC* gene (a β-lactamase) (15); the *Streptomyces glaucescens tyr* gene (a tyrosinase) (18, 20); the *E. coli galK* (galactokinase) (8, 31), *neo* (an aminoglycoside phosphotransferase from Tn*5*) (4, 38), and *cat* (a chloramphenicol acetyltransferase) (4) genes; and the *Streptomyces vinaceus vph* gene (a phosphotransferase) (30). Although each of these has proved useful in some applications, all have disadvantages; most lack the sensitivity of *lacZ* (in *E. coli*) for detection of gene activity in colonies on plates, and there is no evidence that they accurately reflect the activity of promoters driving their expression. A system based on the *Vibrio harveyi luxAB* operon fragment is extremely sensitive and has proved to be very useful for investigating the spatial and temporal regulation of developmental genes in *Streptomyces coelicolor*, but it is inconvenient for screening large numbers of colonies (33).

In the present work, we have explored the use of *xylE*, a catechol dioxygenase gene from *Pseudomonas putida*, as a reporter gene in *Streptomyces* spp. to investigate the mechanism that mediates glucose catabolite control of the *S. coelicolor gal* operon. Catabolite control in all gram-positive bacteria is poorly understood and is of particular interest in *Streptomyces* species because of its influence on the expression of antibiotic biosynthesis genes (26) and extracellular enzymes (18, 37). The *Streptomyces lividans* and *S. coelicolor gal* operons are well-characterized transcription units that may provide a relatively simple model for defining the way glucose repression is accomplished in these species (14). Transcription of *gal* is controlled by two independently regulated promoters: *galP1*, which is responsible for glucose-sensitive, galactose-dependent transcription of the entire operon, and *galP2*, a weak, constitutive promoter internal to the operon that directs transcription of *galE* and *galK* (1, 14). We are interested in defining *cis*-acting sequences within or near *galP1* that are required for proper expression and regulation, and we are seeking to identify through mutational analysis genes encoding *trans*-acting factors that influence *galP1* utilization. These studies would be greatly facilitated by the availability of a sensitive reporter gene system that is easy to use and that reflects accurately subtle changes in expression from *galP1*.

The *xylE* gene product rapidly converts colorless catechol to an intensely yellow oxidation product and was first exploited as a reporter gene in *Bacillus subtilis* by Zukowski and colleagues (43). More recently, Moran and colleagues have successfully used *xylE-ctc* fusions in *B. subtilis* to recover second-site mutations that affected expression of the *ctc* promoter (28). When used as a histochemical indicator, catechol is sprayed onto a plate containing a field of colonies, and the reaction may be scored within a few minutes. Quantitative measures of specific activity can be carried out

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with a simple colorimetric assay. We demonstrate here that a plasmid-borne copy of *xylE* driven by *galP1* reproduces with excellent fidelity the effects of glucose and galactose on expression of the *gal* operon. These differences are easily visualized in *Streptomyces* colonies growing on agar plates. No special conditions of growth are necessary, and no special host strains are required, since there is no detectable background of endogenous catechol dioxygenase activity. Moreover, color reactions on plates were quite vivid, suggesting that this reporter system may be of general utility for recovering mutations that alter the expression of regulated *xylE* fusions.

MATERIALS AND METHODS

Bacterial strains and plasmids. *Streptomyces* strains used were *S. lividans* 1326 (19), *S. lividans* 12K (8), and *S. coelicolor* 2612 (19). *E. coli* TB1 (Focus 6:7, 1984) was used as a host for plasmid constructions and plasmid DNA preparation. To construct pXE4 (Fig. 1), plasmid pTG402 (43) was digested with *Hin*dIII and *Eco*RI to produce a 5.9-kilobase fragment that contained a promoterless *xylE* gene, an ampicillin resistance gene (*bla*), and a ColE1 origin of replication. This fragment was ligated to an 11.2-kilobase *Eco*RI-*Hin*dIII fragment generated from pMB158 that contained SCP2 replication and stability regions (5) and a gene conferring thiostrepton resistance (36). To construct pXE3 (Fig. 1), plasmid pTG402 was digested with *Bam*HI and *Hin*dIII and the larger fragment generated was ligated to a 250-base-pair (bp) *Bam*HI-*Hin*dIII fragment containing the *galP1* promoter. This *galP1* promoter-containing fragment included DNA sequences from −120 to +98 with respect to the apparent transcription start site (1). The resulting plasmid, pJW1, was digested with *Hin*dIII and *Eco*RI to generate a 5.9-kilobase fragment that was ligated to the 11.2-kilobase *Hin*dIII-*Eco*RI fragment from pMB158 (as for construction of pXE4). Plasmid pK21*galP2* has been described previously (14).

Plasmid isolation and transformation. Plasmid DNA was prepared from *E. coli* by alkaline lysis (6), followed by equilibrium centrifugation in cesium chloride-ethidium bromide gradients (9). Plasmid DNA was introduced into $CaCl_2$-treated *E. coli* cells as described by Cohen et al. (10), and transformants were grown on LB agar or in LB broth (3) containing 50 μg of ampicillin per ml. *Streptomyces* protoplasts were transformed as described by Thompson et al. (36), and transformants were selected on R2YE (19) supplemented with the appropriate amino acids and 10 μg of thiostrepton (a gift from E. R. Squibb and Sons, Inc.) per ml. *S. coelicolor* was transformed only with plasmid DNA isolated from *S. lividans*.

S1 nuclease protection analysis. RNA was isolated from *Streptomyces* spp., and S1 nuclease protection analysis was carried out as described previously (2, 40). The probe used in these experiments was a 410-bp *Bam*HI-*Bgl*II fragment containing *galP1*, labeled at the 5′ *Bgl*II end (Fig. 2).

Detection of catechol dioxygenase activity. For plate assays, *Streptomyces* transformants selected on R2YE-thiostrepton plates were replica plated or patched to modified minimal medium (8) containing either 1% glucose or 1% galactose. After incubation at 30°C for 2 days, these plates were sprayed with an aqueous solution of 0.5 M catechol. For liquid assays, *Streptomyces* cultures were grown at 30°C in 50 ml of SLAB medium (14) with 1% glycerol, 10% polyethylene glycol 8000, and 10 μg of thiostrepton per ml for 28 h (at this point the cultures were actively growing), with

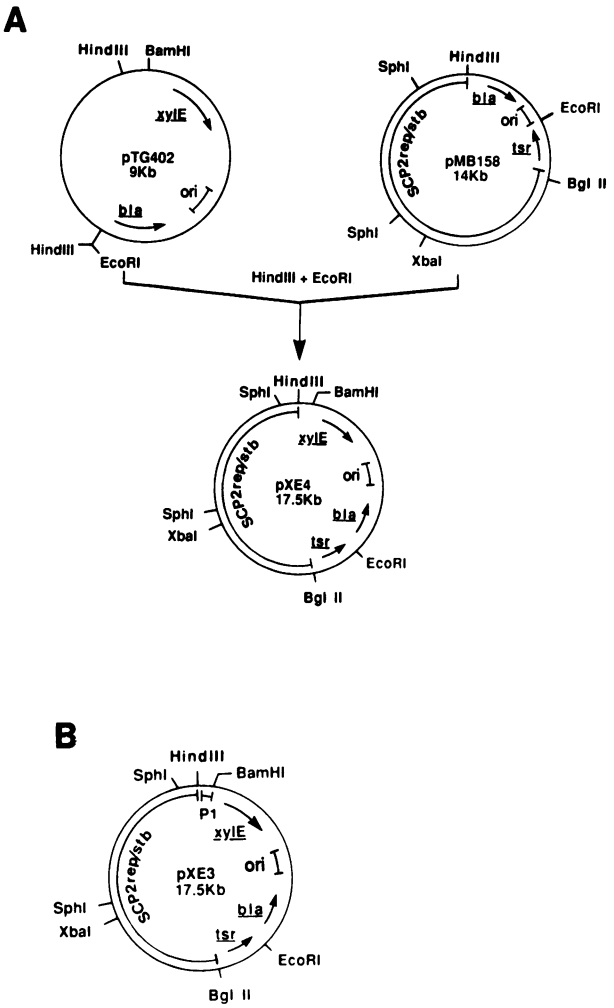

FIG. 1. Construction of plasmids pXE3 and pXE4. The following apply for all plasmids shown: *ori*, the ColE1 origin of replication; *tsr*, the thiostrepton resistance gene from *Streptomyces* spp.; *bla*, a β-lactamase gene from *E. coli*; SCP2 *rep*/*stb*, the replication and stability functions of *Streptomyces* plasmid SCP2*; *xylE*, a promoterless copy of the *xylE* gene from *P. putida* (39); P1, the *Streptomyces galP1* promoter (1). Plasmids pTG402 (39) and pMB158 (4) were described previously. In pXE3, expression of *xylE* is under the transcriptional control of *galP1*.

shaking at 200 rpm in baffled flasks; they were harvested by centrifugation and suspended in 5 ml of SLAB medium. Portions of this cell suspension were added to SLAB medium containing either 1% glucose or 1% galactose and were incubated for 2 h at 30°C. Cells were harvested by centrifugation, washed in 20 mM potassium phosphate, pH 7.2, and suspended to a final volume of 3 ml in sample buffer (100 mM potassium phosphate, pH 7.5, 20 mM EDTA, 10% acetone [vol/vol]). Cells were lysed by sonication for 1 min, 10 μl of 10% Triton X-100 per ml was added, and the extracts were placed on ice for 15 min. Cell debris was removed by centrifugation for 5 min in a microcentrifuge (model 235B; Fisher Scientific Co.) at 4°C. Catechol dioxygenase activities were determined spectrophotometrically as previously described (43). The reaction mixture consisted of assay buffer (100 mM potassium phosphate, pH 7.5, 0.2 mM catechol) and a quantity of cell extract adjusted to produce a linear change over the time of measurement. The assay buffer was

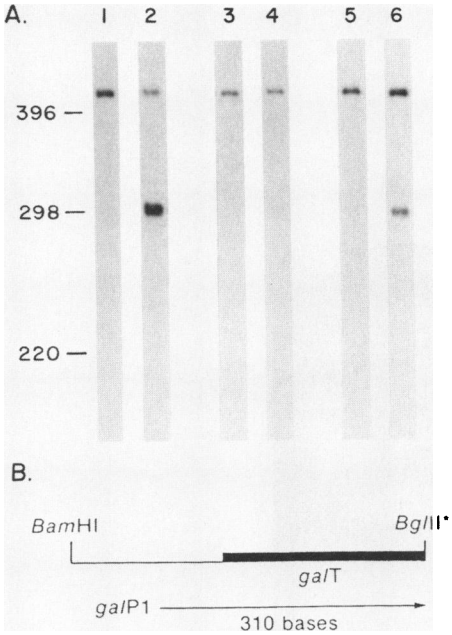

FIG. 2. S1 nuclease mapping experiments showing the presence or absence of *galP1*-specific RNA. (B) DNA fragment containing the *galP1* promoter used as probe. The apparent transcription start site of *galP1* is 310 bp from the 3′ (*Bgl*II) end. (A) Lanes: 1, RNA from glucose-grown wild-type *S. lividans* cells; 2, RNA from galactose-grown wild-type cells; 3, RNA from glucose-grown *galK* cells; 4, RNA from galactose-grown *galK* cells; 5, RNA from glucose-grown *galK* cells containing pK21*galP2* (which supplies galactokinase in *trans* under the control of *galP2*, a constitutive promoter); 6, RNA from galactose-grown *galK* cells containing pK21*galP2*.

preincubated for 1 min at 37°C, and the reactions were initiated by the addition of cell extracts. The optical density at 375 nm was measured over time (in most cases at 2, 4, and 6 min). Catechol dioxygenase activity was calculated as the rate of change in optical density at 375 nm per minute per milligram of protein and converted to milliunits per milligram (32). Protein concentrations were determined by the methods of Lowry et al. (25) or Bradford (7) by using bovine serum albumin as the protein standard.

3′-Deletion analysis. Restriction endonuclease recognition sites located 3′ of the apparent transcription start site of *galP1* at +98 (*Alu*I), +56 (*Fnu*4HI), +32 (*Hin*fI), +21 (*Aha*II), and +13 (*Hga*I) were used to construct *xylE* fusions. A 250-bp *Hin*dIII-*Bam*HI fragment containing sequences from −120 to +98 with respect to the apparent start site of *galP1* was isolated and used for subsequent digestion. The *Hga*I, *Hin*fI, and *Fnu*4HI deletions were generated by digestion with the respective restriction endonucleases, followed by treatment with the Klenow fragment of DNA polymerase I (39) and digestion with *Pst*I. These fragments were cloned into pUC18 via the *Hin*cII and *Pst*I sites. The *Aha*II deletion was generated by digestion with *Aha*II, followed by cloning into the *Hin*dIII and *Acc*I sites of pUC18 (42). Each deleted fragment was removed from the pUC18 derivative by digestion with *Hin*dIII and *Bam*HI and ligated to *Hin*dIII- and *Bam*HI-digested pXE4. These deletions of *galP1* were tested for both the amount of catechol dioxygenase activity and the amount of *xylE*-specific RNA present.

Detection of *galP1-xylE* transcript. RNA was isolated from *S. lividans* strains that had been grown in 50 ml of YEME medium containing either 1% glucose or 1% galactose (19). Cells were quick-cooled by addition of 50 ml of ice-cold 20 mM NaN_3, and RNA was isolated as described previously (19); this procedure was followed by treatment with 1 μg of RNase-free DNase (Worthington Diagnostics) per ml in 50 mM Tris hydrochloride, pH 7.5, and 10 mM $MgCl_2$ at room temperature for 20 min. After phenol-chloroform extraction, chloroform extraction, and ethanol precipitation, the RNA was suspended in diethyl pyrocarbonate-treated water. A 15-μg sample of RNA was denatured in formaldehyde as described by White and Bancroft (41), and 1, 2, and 4 μg of RNA were spotted in duplicate onto a nitrocellulose filter (BA45; 0.45 μm) and then baked in vacuo at 80°C for 2 h. The nitrocellulose filter was prehybridized in 50% formamide–5× SSPE (0.9 M NaCl, 50 mM $NaPO_4$, pH 7.7, 5 mM EDTA)–5× Denhardt solution (12)–100 μg of denatured salmon sperm DNA per ml–0.1% sodium dodecyl sulfate at 42°C for 12 h. The blot was hybridized to a nick-translated (29) probe in 50% formamide–5× SSPE–2× Denhardt solution–100 μg of denatured salmon sperm DNA per ml–0.1% sodium dodecyl sulfate at 42°C for 14 h. The blot was washed three times with 1× SSPE with 0.1% sodium dodecyl sulfate for 30 min at room temperature and then once in 0.1× SSPE with 0.1% sodium dodecyl sulfate for 30 min at 55°C. After visualization by autoradiography, the hybridization signals were measured in a scintillation counter (Beckman Instruments, Inc.). To prepare a probe, a 1.1-kilobase *Aha*II-*Sal*I fragment containing the entire *xylE* gene was cloned into pUC18 and a 590-bp *Bam*HI-*Sal*I fragment containing nucleotides −5 to +580, with respect to the translation start site of the *xylE* gene, was isolated and labeled.

RESULTS

Galactokinase activity is required for induction of *galP1*. In initial experiments, the *galK* gene product itself (galactokinase) was used to monitor expression from *galP1*. Indeed, the ease with which galactokinase specific activity can be measured was considered to be an advantage in studying regulation of the operon. However, preliminary results suggested that induction of *galP1* might actually depend upon galactokinase enzymatic activity (M. Brawner and J. Fornwald, unpublished results). To further investigate the effect of galactokinase on the induction of *galP1*, transcription from *galP1* was examined in wild-type *S. lividans*, *S. lividans* 12K (a *galK* mutant), and *S. lividans* 12K containing pK21*galP2*. The pK21*galP2* construction contains the *E. coli galK* gene under the transcriptional control of the *S. lividans galP2* promoter. Transcription from *galP2* is constitutive and independent of galactose or glucose regulation (14). Wild-type *S. lividans* 1326 and the 12K mutant with and without pK21*galP2* were grown on glucose or galactose, and samples were harvested to prepare RNA for determination of *galP1*-specific transcription by S1 nuclease protection. As expected, for *S. lividans* 1326, only RNA isolated from galactose-grown cells was observed to protect the *galP1*-containing probe (Fig. 2). In contrast, no protection was observed by RNA isolated from *S. lividans* 12K, even from cells grown on galactose. However, galactose-dependent transcription from *galP1* was restored when galactokinase was supplied in *trans* from pK21*galP2*. Similar results were obtained from three independently isolated *galK* mutants of *S. lividans* (data not shown). We conclude from these results that galactokinase is required for galactose induction of *galP1* transcription, thus complicating the interpretation of experiments in which *galK* is used as a reporter to monitor promoter activity.

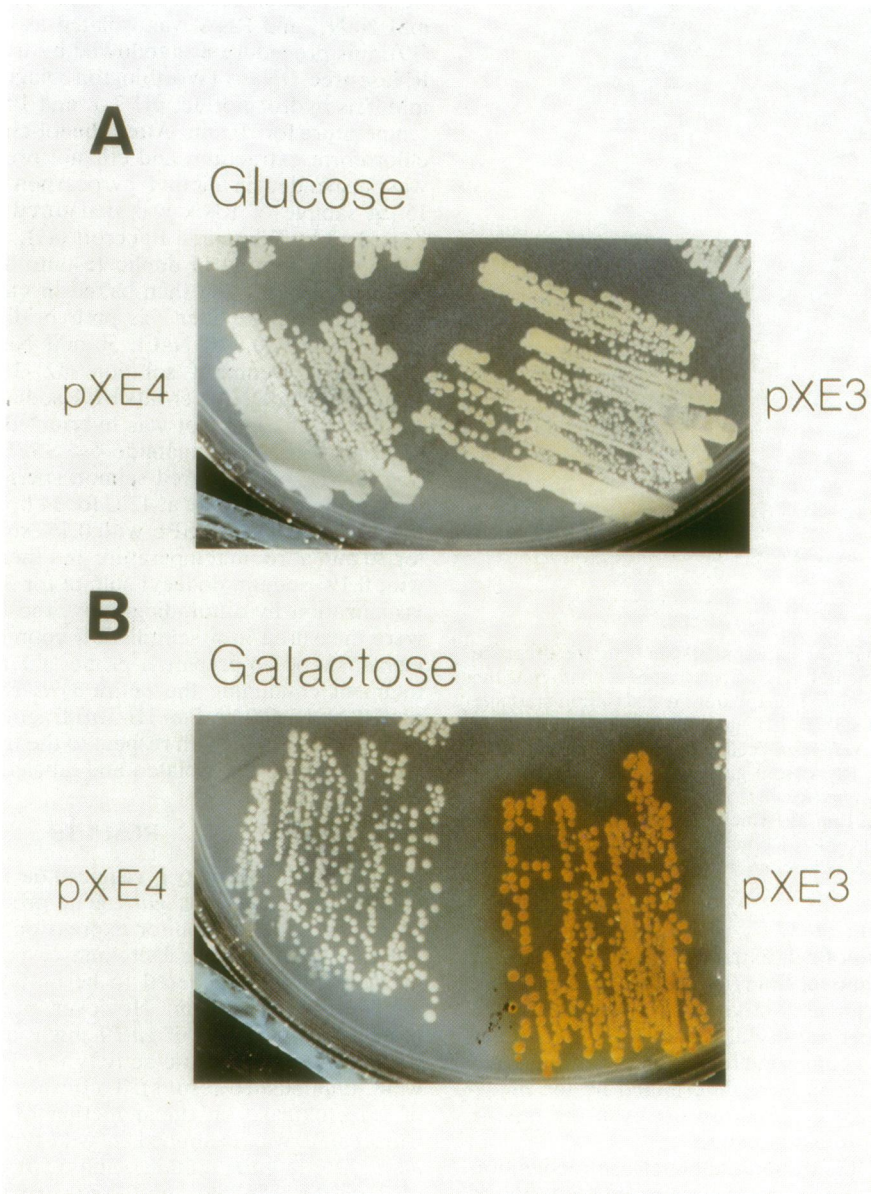

FIG. 3. Patches of *S. lividans* cells containing either pXE4 (which has a *xylE* gene with no promoter) or pXE3 (which has a *xylE* gene under the transcriptional control of *galP1*) grown on either glucose (A) or galactose (B) as carbon source, after exposure to catechol.

Regulated expression of the *xylE* gene in *Streptomyces* spp. The *xylE* gene of *P. putida* encodes a catechol 2,3-dioxygenase that converts catechol, a colorless compound, to 2-hydroxymuconic semialdehyde, which is intensely yellow. To determine whether this chromogenic reaction could be used to detect *galP1*-driven expression of *xylE*, *S. lividans* 1326 transformants containing pXE3 or pXE4 (Fig. 1) were replica plated or patched onto minimal medium containing either glucose (repressing conditions) or galactose (inducing conditions) as carbon source and the plates were sprayed with a 0.5 M solution of catechol after 2 days of growth. Color reactions are shown in Fig. 3. Colonies of pXE3-containing cells grown on glucose remained white (or turned a slight cream color) after exposure to catechol, while colonies of pXE3-containing cells grown on galactose were intensely yellow. Colonies of pXE4-containing cells grown on either glucose or galactose showed no color change with exposure to catechol, indicating little or no expression of *xylE*. Similar results were seen with colonies of *S. coelicolor* cells containing the pXE3 or pXE4 plasmid. No catechol dioxygenase was detected in colonies of either *S. lividans* or *S. coelicolor* in the absence of a *xylE*-containing plasmid. These results indicate that the *xylE* gene is expressed well in *Streptomyces* spp., that this expression is regulated appropriately by the *galP1* promoter, and that there is no detectable background of endogenous activity.

Quantitative measure of catechol dioxygenase activity. To determine whether color reactions observed on plates were

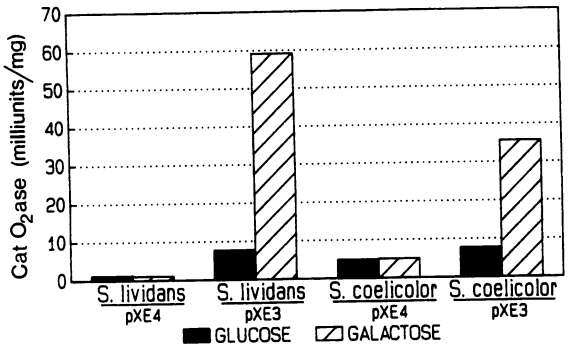

FIG. 4. Histogram showing results of quantitative catechol dioxygenase assays with *S. lividans* and *S. coelicolor* cells, containing either pXE3 or pXE4, after incubation in liquid culture for 2 h with either glucose or galactose as carbon source.

in agreement with quantitative assays of liquid cultures, *S. coelicolor* 2612 and *S. lividans* 1326 were grown in SLAB medium, incubated with either 1% glucose or 1% galactose for 2 h, and harvested for determination of catechol dioxygenase activity. In agreement with previous measurements of *galP1*-specific transcription by S1 nuclease protection analysis (14), quantitative assays of catechol dioxygenase activity showed induction of *xylE* expression from *galP1* in pXE3-containing cells grown on galactose, while glucose strongly repressed this expression (Fig. 4). Cultures of cells containing pXE4, which does not have a promoter inserted upstream of the *xylE* gene, showed a very low background level of expression. *S. coelicolor* and *S. lividans* cultures without either plasmid showed no detectable catechol dioxygenase activity. The SCP2 replicon used in our constructions was reported to have a copy number of two per cell (5). This was confirmed by Southern hybridization analysis (data not shown). Thus, the intensities of the color reactions and the sensitivities of the liquid assays are not artificially enhanced by high copy number.

Correlation between catechol dioxygenase activity and RNA level. To determine whether relative amounts of catechol dioxygenase specific activity produced by *galP1-xylE* fusions reflected accurately the relative levels of transcription directed by differentially active *galP1* promoters, we examined a series of *galP1-xylE* fusions in which *galP1* had suffered deletions extending toward the promoter from the 3′ direction. Deletions were constructed by taking advantage of several restriction endonuclease sites immediately downstream from the apparent transcription start site of *galP1* (Fig. 5). Bacteria containing *xylE* fusions driven by deletion

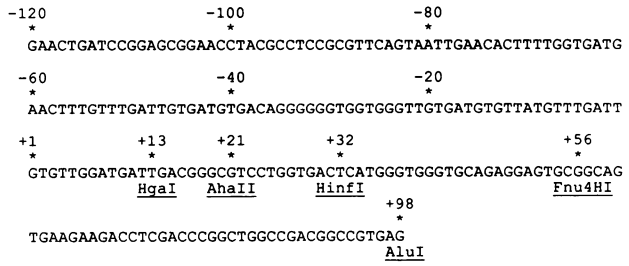

FIG. 5. DNA sequence of the *galP1* promoter region. Restriction endonuclease sites used for construction of 3′-deletion mutations are indicated. +1 indicates the apparent transcription start site.

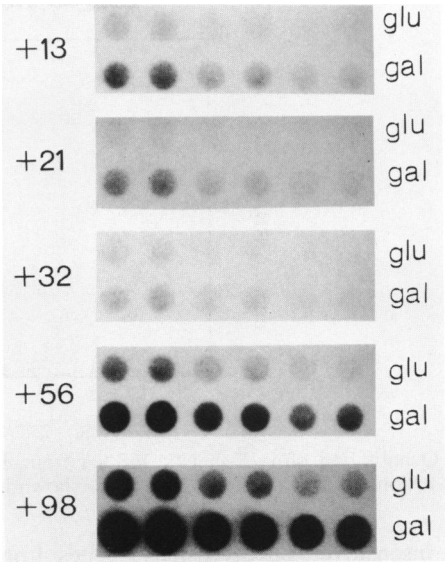

FIG. 6. Dot blots of *S. lividans* RNA extracted from cells containing various *galP1-xylE* fusions. +13, +21, +32, +56, and +98 refer to the number of nucleotides remaining after deletions were made 3′ with respect to the apparent transcription start site of *galP1*. Blots containing either 4, 2, or 1 μg of RNA (from left to right, in duplicate) were hybridized with a DNA probe (described in Materials and Methods) containing the *xylE* DNA sequence.

derivatives of *galP1* were grown on glucose or galactose, and culture samples were taken for catechol dioxygenase activity measurements and direct quantitation of *xylE*-specific transcript in dot blots (Fig. 6). The results (Fig. 7) indicate an excellent correlation between the amount of catechol dioxygenase activity measured and the amount of *xylE*-specific mRNA detected. No *xylE* RNA was detected in cells without a *xylE*-containing plasmid (data not shown). To determine whether subtle differences in expression levels could be detected in colonies on plates, colonies formed from cells containing the *galP1* deletions were sprayed with catechol. After growth on glucose, all of the cells containing the deletion derivatives remained white after exposure to catechol. Cells containing deletions to +13, +21, and +32 also remained white after growth on galactose. Cells containing the deletion to +56 were light yellow when grown on galactose, and cells containing the deletion to +98 were intensely yellow when grown on galactose (data not shown). This demonstrates that there is also an excellent correlation between the level of activity detected from *galP1* in quantitative assays and the color reaction seen on plates.

DISCUSSION

Effective reporter gene systems facilitate genetic analysis of regulated promoters in two ways: first, they provide an easy assay for changes in gene expression that result from deletions or point mutations introduced in vitro; second, if they provide a sensitive histochemical assay for expression levels in colonies on agar plates, they may allow the identification of genes encoding *trans*-acting factors through the isolation of extragenic mutations that increase or reduce expression. Although previous attempts have been made to develop such a system for *Streptomyces* species, these were only partially successful. Barriers to the effective use of *lacZ* have already been noted. While expression of the *E. coli ampC* gene can be detected in *Streptomyces* spp., assays are

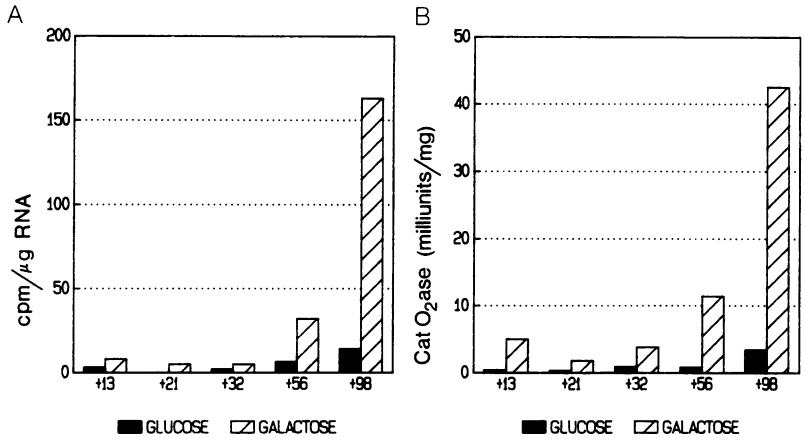

FIG. 7. Quantitation of *xylE*-specific mRNA from dot blot experiments (A) compared with catechol dioxygenase activity (B) from *galP1-xylE* fusions to the 3'-deletion mutations (shown in Fig. 5). *S. lividans* was used in these experiments.

relatively insensitive. The tyrosinase genes from several *Streptomyces* species have been cloned, and at least one has been extensively characterized (16); under appropriate conditions, expression of these genes can produce very strong colony pigmentation. In most cases, however, the cloned *tyr* genes have somewhat complex regulation of their own that complicates their use as reporter genes. Drug resistance genes have been used successfully in promoter-probe vectors and have allowed the identification of many *Streptomyces* promoters (4, 30, 38); however, their use requires selectable levels of expression during vegetative growth, and this limits their use for the study of developmentally regulated promoters. Horinouchi and Beppu (20) constructed a vector based on a gene from *S. coelicolor* that encodes a brown compound, but its use requires special hosts or growth conditions in which little or no interfering pigment is produced. Use of *galK* itself as a reporter gene has proved successful in some studies, and it offers the potential advantage of selection for both loss of function and gain of function (8, 31). Histochemical assays for *galK* expression are not very sensitive, however, and in our work, the direct involvement of galactokinase in the regulation of *galP1* rules out the use of *galK* in any event. The *luxAB* system developed by Schauer et al. (33) was perhaps the best previously available alternative, but its use for screening colonies involves either expensive, specialized electronic equipment or direct exposure of aldehyde-treated colonies to X-ray film.

We have shown here that the *xylE* gene of *P. putida* offers important advantages over existing alternative reporter genes available for use in *Streptomyces* spp. First, there is no detectable background of endogenous catechol dioxygenase activity (at least in *S. lividans* and *S. coelicolor*). Thus, no special host strains are needed. Second, the colorimetric assay for specific activity can be carried out by using standard, inexpensive, visible-wavelength spectrophotometers. Third, the histochemical substrate for the *xylE* gene product (catechol) is inexpensive, nontoxic to treated bacteria, and easy to use, and it produces an intense, rapidly developing visible reaction. In the case of *galP1-xylE* fusions, intense yellow staining developed within 5 min of exposure to catechol.

We have demonstrated the effectiveness of *xylE* as a reporter gene in *Streptomyces* spp. by using it to study the regulation of *galP1*, the glucose-repressed, galactose-induced promoter associated with the *gal* operons of *S. lividans* and *S. coelicolor*. This was motivated by two considerations. First, the mechanism by which glucose catabolite control is mediated in gram-positive bacteria remains almost completely obscure. We know only that the mechanism must be quite different from the cyclic AMP-modulated positive activation that plays a major role in the gram-negative enteric bacteria (27, 34), since cyclic AMP levels in *Streptomyces* species are not responsive to carbon source (17). Mutations in *S. coelicolor* that greatly reduce glucose kinase activity result in relief of glucose repression of utilization of some carbon sources (21). Second, glucose repression plays a well-established role in controlling the expression of at least some antibiotic biosynthesis genes, and since antibiotic biosynthesis is itself a developmentally regulated process, it is likely that glucose repression plays a role in the regulation of other developmental gene expression, such as morphological differentiation. By using *galP1-xylE* fusions, we have shown that the well-documented difference in expression of *galP1* in liquid cultures containing glucose or galactose as the primary carbon source (16) can be reproduced on agar plates and visualized readily after spraying the plates with catechol. We have shown that a series of deletion-impaired *galP1* promoters fused to *xylE* produce levels of catechol dioxygenase that reflect faithfully the levels of transcript produced. Differences in transcription levels produce easily visualized differences in histochemical staining of *Streptomyces* colonies producing catechol dioxygenase. This can be used to isolate mutants in which expression of *galP1* is altered. Thus, it is evident that *xylE* will be extremely useful in the further physical and functional analysis of *galP1* and, perhaps, can be similarly useful in the analysis of a wide range of other regulated promoters in *Streptomyces* species.

Finally, we have also shown that galactokinase is required for galactose induction of *galP1* transcription. The most likely explanation for this is that the product of the galactokinase-catalyzed reaction, galactose-1-phosphate, is the actual inducer of *galP1*. This would be reminiscent of the way the lactose and glycerol operons of *E. coli* are induced. In the case of the lactose operon, allolactose, produced in a reaction catalyzed by β-galactosidase, is the actual inducer (23). In the case of the glycerol operon, the actual inducer is glycerol-1-phosphate, produced in a reaction catalyzed by glycerol kinase (24). The advantage of such an arrangement, in the case of the *gal* operon, is presumably to establish a positive feedback loop that enhances induction and couples it with facilitated transport of galactose into the cell. A

practical implication for our studies is that galactokinase should be supplied in *trans* at a uniform level when induction of variously modified *galP1-xylE* fusions is examined.

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