Cloning of the Galactokinase Gene (*galK*) from *Streptomyces coelicolor* A3(2)

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Streptomyces coelicolor A3(2) and *Streptomyces lividans* 66 strains were shown to be sensitive to the galactose analogue 2-deoxy-D-galactose. Spontaneous resistant mutants were isolated that were Gal⁻ and lacked the enzyme galactokinase. The *galK* gene (structural gene for galactokinase) from *S. coelicolor* was cloned into *S. lividans* using the low copy number vector pIJ922. The resulting plasmid (pMT650), which contained a 14 kb insert, complemented *gal* mutations in both species. The presence of the *galK* gene on a 2.8 kb *EcoRI* fragment was confirmed by expressing it in *Escherichia coli* where it complemented a well characterized *galK* mutation.

INTRODUCTION

Recent advances in the molecular biology of *Streptomyces* have led to the isolation of genes involved in antibiotic synthesis (e.g. Hopwood *et al.*, 1983*a*). However, analysis of the regulation of such secondary metabolism genes is complicated because little is known about the genes of primary metabolism. The glycerol utilization genes of *Streptomyces coelicolor* have been cloned and are proving a useful model system to study control of primary metabolism (Seno *et al.*, 1984). We wanted to clone the galactokinase gene (*galK*) for a number of reasons.

(1) Hodgson (1982) investigated galactose uptake in *Streptomyces coelicolor* and the effect of glucose on galactose utilization. He did not assay the enzymes for galactose utilization, but concluded from indirect evidence that these enzymes were subject to repression by glucose and induction by galactose. It thus makes an interesting regulatory system.

(2) In *Escherichia coli* and *Salmonella*, *galK* mutants can be selected by their resistance to the galactose analogue 2-deoxy-D-galactose (2dGal) (Alper & Ames, 1975) and revertants to *galK*⁺ can be selected by their ability to utilize galactose as a carbon source. This system was used in *E. coli* for the isolation and characterization of insertion sequences (ISs) (for a recent review, see Cullum, 1985). We wanted to isolate ISs in *Streptomyces* and needed an alternative to the replicon fusion system used by Chater *et al.* (1985) because it is likely that some interesting transposable elements will not produce replicon fusions (e.g. like Tn10 in *E. coli*, Bender & Kleckner, 1986).

(3) Galactokinase enzyme activity is easily assayed and the *galK* gene could be developed as a quantitative measurement system for *in vivo* promoter strength as in *E. coli* (McKenney *et al.*, 1981). The possibility of selecting both *galK* and *galK*⁺ alleles may allow isolation of various classes of regulatory mutants affecting cloned promoters.

In this paper we demonstrate that *galK* mutants can be selected in *S. coelicolor* A3(2) and *Streptomyces lividans* 66. Further, we cloned the *galK* gene from *S. coelicolor* and demonstrated its expression in *E. coli*.

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Abbreviation: 2dGal, 2-deoxy-D-galactose.

METHODS

Media and growth conditions. Media and conditions for *Streptomyces* transformation were as described by Kendall & Cullum (1984). *E. coli* transformation was as described by Altenbuchner & Cullum (1985). 2dGal^R mutants were selected on MM (minimal medium of Hopwood, 1967) supplemented with the appropriate amino acids, 20 mM glycerol as a carbon source and 20 mM 2dGal (Sigma). 'Suppressed' minimal medium (SM) was developed in this study to distinguish Gal⁺ and Gal⁻ strains: the basic medium (per litre: 0.5 g K₂HPO₄, 5.3 g (NH₄)₂HPO₄, 15 g Difco Bacto agar) was autoclaved for 30 min (121 °C). The following sterile additions were made to the molten agar: 0.5 ml trace element solution (Chater *et al.*, 1982), 1 ml MgSO₄·7H₂O (40 mg ml⁻¹), 1% (w/v) galactose, appropriate amino acid supplements (100 µg ml⁻¹). MacConkey-galactose agar was prepared by adding 1% galactose to MacConkey agar base (Difco).

Strains and plasmids. The following *Streptomyces* strains were used: *S. coelicolor* A3(2) strains M130 (*hisA1 uraA1 strA1 SCP1-SCP2*⁻; Bibb & Hopwood, 1981) and J802 (*agaA7* NF; Hodgson & Chater, 1981), *S. lividans* 66 strain TK64 (*pro-2 str-6 SLP2-SLP3*⁻; Hopwood *et al.*, 1983b). *E. coli* strain HB101 has the genotype F⁻*hsdS20 recA13 ara-14 proA2 lacY1 galK2 rpsL20 xyl-5 mtl-1 supE44* (Boyer & Rouland-Dussoix, 1969) and AB257 has the genotype Hfr *metB1 rel-1* (Bachmann, 1972).

The *Streptomyces* vectors pIJ350 (Kieser *et al.*, 1982), pIJ702 (Katz *et al.*, 1983) and pIJ922 (Lydiat *et al.*, 1985) were kindly supplied by David Hopwood. The *E. coli* vector pEMBL8 and its host strain 71/18 are described by Dente *et al.* (1983).

DNA manipulations. Preparation of total DNA from *Streptomyces* strains, plasmid preparation, restriction digests, alkaline phosphatase treatment of DNA, ligation and labelling of DNA by nick translation and Southern blottings were all as described previously by Kendall & Cullum (1984). Total DNA from strain J802 was partially digested with restriction enzyme *Sau3A* as described by Kendall & Cullum (1984), except that the average fragment size was 10 kb. This partial digest was ligated with alkaline-phosphatase-treated, *Bam*HI-digested DNA of plasmid pIJ922. The ligation mixture was transformed into the Gal⁻ *S. lividans* strain K561 and a Gal⁺ transformant carried the plasmid pMT650 (see Results and Discussion). Plasmids pMT651 and pMT652 were constructed by ligating *Eco*RI digests of DNA of pMT650 together with *Eco*RI-digested DNA of the *E. coli* vector pEMBL8. The ligation mixture was transformed into strain 71/18 and clones containing inserts were identified as white colonies as described by Altenbuchner & Cullum (1985). The required clones were identified by restriction analysis of rapid plasmid preparations and the resulting plasmids (pMT651 and pMT652) were transformed into HB101 to test their ability to complement an *E. coli galK* mutation.

Galactokinase assays. Assays of *E. coli* strains were as described by McKenney *et al.* (1981) except the cells were grown in L-broth (Lennox, 1955). *Streptomyces* strains were assayed after growth in L-broth using the same method except that cell lysis was achieved by sonication (MSE Soniprep 150, 6 cycles of 30 s on/45 s off). Protein concentration was measured as described by Kendall & Cullum (1984).

RESULTS AND DISCUSSION

Isolation of Gal⁻ mutants

S. coelicolor A3(2) strains M130 and J802 and a *S. lividans* 66 strain (TK64) were sensitive to 20 mM 2dGal when grown on MM with glycerol as a carbon source. Spontaneous 2dGal^R mutants arose at a frequency of about 10⁻⁶ per spore and these were tested, after purification on 2dGal-containing agar, for growth using galactose as a carbon source on SM agar plates. It was necessary to use SM agar, because it gave less background growth in the absence of an added carbon source than MM. SM medium became darkened in colour on autoclaving and we suspect that this leads to destruction of free low Mr compounds that would allow background growth or, in the case of the agarase-producing strain M130 (Hodgson & Chater, 1981), induction of agar-utilizing enzymes. The majority of mutants (about 70%) from both species were Gal⁻. The Gal⁻ mutants were tested for reversion frequency and one mutant from each species showing low reversion frequency (10⁻⁹ per spore) was chosen; these were K521 (from M130) and K561 (from TK64).

K521 and K561 were both potential hosts for selection of cloned *gal* genes by complementation of a *gal* mutation. We showed that both strains could be transformed with plasmid DNA at similar frequencies to their parent strains, thus allowing their use as hosts for shotgun cloning experiments. We also tested K521 and K561 for galactokinase activity. They both had very low levels of activity compared to their Gal⁺ parent strains (Table 1). We interpreted these results as showing that K521 and K561 lacked galactokinase activity, as the

Table 1. Assays of galactokinase activity

Species	Strain	Gal phenotype	Galactokinase specific activity*
<i>S. coelicolor</i> A3(2)	M130	+	25.0
<i>S. coelicolor</i> A3(2)	K521	—	1.2
<i>S. lividans</i> 66	TK64	+	10.4
<i>S. lividans</i> 66	K561	—	1.1
<i>S. lividans</i> 66	K561(pMT650)	+	6.8
<i>E. coli</i> K12	HB101	—	1.3
<i>E. coli</i> K12	HB101(pMT651)	+	32.4
<i>E. coli</i> K12	AB257	+	31.5

* μmol galactose converted to galactose 1-phosphate min^{-1} (mg protein) $^{-1}$.

levels measured were comparable to the background level shown by a well characterized *E. coli galK*[−] strain, HB101 (Table 1).

Cloning of DNA from *S. coelicolor* that complements galactokinase-negative mutants

Preliminary attempts to clone *gal* genes from *S. coelicolor* using the multicopy vectors pIJ350 (Kieser *et al.*, 1982) and pIJ702 (Katz *et al.*, 1983) were unsuccessful, although about 100 000 recombinant plasmids were generated in several experiments. We therefore used the low copy number vector pIJ922 (Lydiat *et al.*, 1985) for cloning. *S. lividans* was used as a host strain for cloning because it is easier to use for transformation than *S. coelicolor* and it was expected that sequence divergence between the two species would reduce the probability of recombination occurring between cloned sequences and the host chromosome.

Total DNA from the Gal⁺ *S. coelicolor* strain J802 was partially digested with *Sau*3A to give an average fragment size of 10 kb. This was ligated to *Bam*HI-cleaved, phosphatase-treated pIJ922 DNA and the ligated DNA was used for transforming the Gal[−] strain K561; about 7000 recombinant clones were obtained. The clones were replicated onto galactose-containing SM medium and 12 potential Gal⁺ clones were identified. Plasmid DNA was prepared from the 12 candidates and checked for its ability to transform K561 to Gal⁺. This yielded one positive clone and the plasmid it harboured was named pMT650.

pMT650 has a 14 kb insert whose restriction map is shown in Fig. 1. K561(pMT650) showed higher galactokinase activity than the Gal[−] strain K561 (Table 1). The data in Table 1 show a clear qualitative difference between strains having galactokinase activity and those lacking it (K521, K561 and HB101). As growth was on L-broth rather than minimal medium and no attempts were made to determine inducers of the enzyme activity, the data should not be interpreted quantitatively. pMT650 was introduced into the *S. coelicolor* strain K521 where it complemented the *gal* mutation. As it is possible that the 14 kb insert in pMT650 codes for several *gal* genes it can not be concluded that K521 and K561 carry mutations in the same gene.

Complementation of a *galK* mutation of *E. coli*

It seemed likely that pMT650 carried the structural gene for galactokinase (*galK*), but it could not be ruled out that it coded for a positive control gene. However, in *E. coli* there are well characterized *galK* mutants. We therefore attempted to obtain the expression of sequences from pMT650 in *E. coli* to complement the *galK* mutation in strain HB101 (Boyer & Rouland-Dussoix, 1969). We cloned various subfragments into the polylinker of the multicopy plasmid pEMBL8 (Dente *et al.*, 1983); this puts the sequences adjacent to a *lac* promoter. When the 2.8 kb *Eco*RI fragment of pMT650 (Fig. 1) was cloned into the *Eco*RI site of pEMBL8, the resulting transformant strain, HB101(pMT651), grew as pale pink colonies on MacConkey-galactose agar, i.e. the strain has a weak Gal⁺ phenotype. HB101(pMT651) showed a galactokinase activity comparable to that of a Gal⁺ *E. coli* strain AB257 (Table 1); as the strains were grown on L-broth, this is the basal galactokinase level in *E. coli* rather than an induced level.

Fig. 1. Restriction map of the 14 kb DNA insert in pMT650. The boxed region shows the 2.8 kb *EcoRI* fragment that contains the *galK* gene. The arrow shows the probable direction of transcription of the *galK* gene.

pMT651 could retransform HB101 to a weak Gal⁺ phenotype, showing that the *galK* gene was plasmid-borne. pMT651 was used as a hybridization probe against Southern transfers of total DNA from strains M130 and TK64 digested with *EcoRI* (data not shown). In both species, there was a single band of 2.8 kb hybridizing to the probe. We therefore conclude that the 2.8 kb *EcoRI* fragment of pMT651 carries the *galK* gene from *S. coelicolor* A3(2).

When the 2.8 kb *EcoRI* fragment was in the opposite orientation in pEMBL8, the resulting plasmid (pMT652) did not complement the HB101 *galK* mutation. This suggests that transcription of the *galK* gene occurs from a promoter on the plasmid rather than from a *Streptomyces* promoter internal to the *EcoRI* fragment. From the construction of pEMBL8, the transcription into the polylinker sequence occurs from the *lac* promoter (Dente *et al.*, 1983). If this is indeed responsible for expressing the *galK* gene, then the direction of transcription of *galK* must be that shown in Fig. 1.

We have now cloned the *galK* gene from *S. coelicolor*. This will be useful for studies of the structure and control of the *gal* genes of *Streptomyces*. It will also enable characterization of DNA rearrangements that cause spontaneous *gal* mutations and probably the isolation of insertion sequences. Construction of *galK*-derived promoter probe vectors will also be possible. Such work is at present being undertaken in this laboratory.

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