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., 1983a). 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). 2dGalR 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- hisD20 recA14 ara-14 proA2 lacY1 galK2 rpsL20 xyl-5 mtl-1 supE44 (Boyer & Rouland-Dussox, 1969) and AB257 has the genotype F' metB1 rel-1 (Bachmann, 1972).

The Streptomyces vectors pIJ350 (Kieser et al., 1982), pIJ702 (Katz et al., 1983) and pIJ922 (Lydiate 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, BamHI-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 EcoRI digests of DNA of pMT650 together with EcoRI-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 01-1/45 s). 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 2dGalR 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. 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

<table>
<thead>
<tr>
<th>Species</th>
<th>Strain</th>
<th>Gal phenotype</th>
<th>Galactokinase specific activity*</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. coelicolor</td>
<td>M130</td>
<td>+</td>
<td>25.0</td>
</tr>
<tr>
<td>S. coelicolor</td>
<td>K521</td>
<td>-</td>
<td>1.0</td>
</tr>
<tr>
<td>S. lividans 66</td>
<td>TK64</td>
<td>+</td>
<td>1.0</td>
</tr>
<tr>
<td>S. lividans 66</td>
<td>K561</td>
<td>-</td>
<td>1.0</td>
</tr>
<tr>
<td>S. lividans 66</td>
<td>K561(pMT650)</td>
<td>+</td>
<td>6.8</td>
</tr>
<tr>
<td>E. coli K12</td>
<td>HB101</td>
<td>-</td>
<td>1.0</td>
</tr>
<tr>
<td>E. coli K12</td>
<td>HB101(pMT651)</td>
<td>+</td>
<td>12.4</td>
</tr>
<tr>
<td>E. coli K12</td>
<td>AB257</td>
<td>+</td>
<td>31.5</td>
</tr>
</tbody>
</table>

*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 (Lydiate 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 Sau3A to give an average fragment size of 10 kb. This was ligated to BamHI-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 cannot 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 EcoRI fragment of pMT650 (Fig. 1) was cloned into the EcoRI 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.
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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**REFERENCES**


