Natural and synthetic tetracycline-inducible promoters for use in the antibiotic-producing bacteria *Streptomyces*

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ABSTRACT

Bacteria in the genus *Streptomyces* are major producers of antibiotics and other pharmacologically active compounds. Genetic and physiological manipulations of these bacteria are important for new drug discovery and production development. An essential part of any ‘genetic toolkit’ is the availability of regulatable promoters. We have adapted the tetracycline (Tc) repressor/operator (TetR/tetO) regulatable system from transposon Tn10 for use in *Streptomyces*. The synthetic Tc controllable promoter (tcp), tcp830, was active in a wide range of *Streptomyces* species, and varying levels of induction were observed after the addition of 1–100 ng/ml of anhydrotetracycline (aTc). *Streptomyces coelicolor* contained an innate Tc-controllable promoter regulated by a TetR homologue (SCO0253). Both natural and synthetic promoters were active and inducible throughout growth. Using the *luxAB* genes expressing luciferase as a reporter system, we showed that induction factors of up to 270 could be obtained for tcp830. The effect of inducers on the growth of *S. coelicolor* was determined; addition of aTc at concentrations where induction is optimal, i.e. 0.1–1 μg/ml, ranged from no effect on growth rate to a small increase in the lag period compared with cultures with no inducer.

INTRODUCTION

Unnatural control of gene expression is an essential tool in genetic analysis and requires the exploitation of controllable promoters to be used in recombinant constructs. A suitable promoter should, ideally, be completely off when repressed, tunable to different strengths when induced, and the inducer should have no pleiotropic effects on general growth. Currently, the most widely used promoter for regulated gene expression in *Streptomyces* spp. is the thiostrepton inducible promoter, *ptipA* (1). This promoter has provided reliable and controllable gene expression under many circumstances, but thiostrepton induces a regulon of proteins, is dependent on the presence of an activator, TipAL, and a resistance gene, *tsr*, and the uninduced level of promoter activity is sometimes significant (1–3). Other controllable expression systems developed for use in *Streptomyces* or related genera include the *Rhodococcus rhodochrous* nitralase promoter, *Par* regulated by Nitr (4) and the *Streptomyces coelicolor* gylP promoter, *glyP1/P2* glycerol-inducible system (5,6). While the *Par* system appears to be excellent for protein overproduction from high copy number plasmids, its not clear at this time whether it will also be a promoter of choice for ectopic, controlled expression in routine genetic analysis. Furthermore, the glycerol-inducible system is of restricted use as the addition of glycerol may be undesirable for many physiological investigations. There is clearly a need for alternative regulatable promoters.

The tetracycline (Tc)-inducible repressor (tetR)-operator (tetO) interaction from the *Escherichia coli* transposon Tn10 has been successfully adapted for use as a tool for regulating gene expression in many organisms (7,8). The tetO–TetR interaction is very strong (*Kd* ~ 10⁻¹¹ M⁻¹), explaining the observed high-level repression in the absence of Tc. When Tc is present, the affinity of the repressor for tetO is reduced by nine orders of magnitude, resulting in very high induction factors (IFs), i.e. the level of expression in the induced state compared with the expression in the repressed state.

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(9,10). Several members of the Tc family can be used as inducers at sub-inhibitory levels of antibiotic (9). Anhydrotetracycline (aTc) is a more active inducer than Tc and has a higher minimum inhibitory concentration (9,11). Here, we describe synthetic Tc-controllable promoters (tcps), a tetR allele derived from the Tn10 tetR/tetO system adapted for use in Streptomyces, and an endogenous Tc-responsive repress/operator system in S. coelicolor.

MATERIALS AND METHODS

Bacterial strains

E.coli DH5α was used as a general cloning host and was grown and maintained according to the standard methods (12). S. coelicolor strain, J1929 (13), a derivative of M145, was used as a recipient to assay the synthetic and natural promoters. Other Streptomyces strains used in this study were Streptomyces avermitiliis, Streptomyces lividans 66 strain TK24, Streptomyces ambofaciens BES2268, Streptomyces griseus ATCC 12475, Streptomyces roseosporus ATCC 31568 and Streptomyces venezuelae ATCC 15439. All Streptomyces strains were grown and maintained according to the standard procedures (6).

The minimal inhibitory concentrations (MICs) conferred by S. coelicolor J1929 strains containing tcp-neo fusions were assayed by spotting 5 x 10⁵ spores suspended in 10 μl of water on SMMS (supplemented minimal medium, solid) (6) agar plates containing increasing amounts of kanamycin and supplemented with either 0 μg/ml aTc or 1.5 μg/ml aTc. The MIC was scored as the level of kanamycin required to inhibit the lawn of growth, i.e. the appearance of single colonies within the inoculated area.

Plasmid and strain constructions

DNA manipulations were performed using standard procedures (12). The promoter–reporter gene fusions were integrated into the Streptomyces chromosome at the φC31 attB site (Figures 1 and 2). Here, we describe an overview of their construction. Detailed information on all DNA manipulations is provided in the Supplementary Material.

To modify tetR for expression in Streptomyces, we used the synthetic derivative of the tetR gene, tTA2, from pUHT61-2 (14), kindly provided by Prof. H. Bujard and Prof. W. Hillen. The SV40 activation domain was replaced by the natural stop codon, the codons at the 5’ end were optimized for expression in Streptomyces and a ribosome binding site (rbs) was introduced. This modified tetR gene was renamed tTA2 for tetR, adapted for expression in Streptomyces. A strong constitutive promoter from the Streptomyces ghanaensis phage I19 (15), SF14, was used to drive expression of tetRIS.

Plasmids containing synthetic tcp-neo fusions were constructed as follows (Figure 1). A plasmid, pPC700, was constructed containing, located between two transcription terminators, tmmr and tfd (16), one of the synthetic promoters, tcp700, fused to a promoterless neo gene, and the tetRIS gene in the integrating vector, pSET152 (17). Plasmids pPC808A pPC808T, pPC830, pPC840A, pPC840T, pPC850 and pPC861 are derivatives of pPC700 containing modified tcps in place of tcp700 (Figure 1A). A promoterless derivative of pPC700, pAR840, was constructed. To provide constitutive controls, the tetRIS gene from each of these plasmids was disrupted to produce pPC706, pPS808A, pPS808T, pPS830, pPS840A, pPS840T, pPS850, pPS861 and pAR850, respectively.

Plasmids containing the tcp830–luxAB fusions were constructed as follows (Figure 2). Initially, the neo gene downstream of tcp830 in pPC830 was replaced with a promoterless luxAB (obtained from pND18; Dr Paul Herron) to form pAR860. pND18 contains the modified luxAB genes from M13-1201 (K. Chater, unpublished data), in which all the TTA codons have been removed and the rare N-terminal codons from luxA and luxB have been replaced by more commonly used versions. The ΔtetRIS derivative of pAR860 was pAR870. These fusions were subsequently modified to produce pAR933 by incorporating an rbs upstream of luxAB. The strategy for the construction of pAR933 permitted the fragment encoding tmmr, tcp830, rbs, luxAB to be inserted in two orientations, a and b. pAR933a is directly comparable in plasmid organization with those such as pPC830 containing the neo fusions while pAR933b had the fragment encoding tmmr, tcp830, rbs, luxAB inverted.

To assay the innate tcp from S. coelicolor (Figure 2), PCR was used to amplify fragments encoding the regulatory region between SCO0252 and SCO0253, itcp0252 (for innate tcp upstream of SCO0252), and itcp0252 plus SCO0253. These fragments were inserted with the tcp0252 promoter reading into the luxAB genes to form pAR913 and pAR911, respectively. The divergent nature of this endogenous promoter–operator/repressor region was maintained during the constructions.

To activate SCO0253, the REDIRECT method developed by Gust et al. (18) was used. The S. coelicolor J1929 recombinants were screened by PCR for SCO0253 replaced with the aada-oriT cassette derived from pIJ778 (2722 bp) and the mutant was named S. coelicolor D32.

Growth parameters and expression of luxAB

This method was based on that of Ali et al. (2). An aliquot of 0.3 ml of molten SMMS with or without inducer was added to each of the 96 wells of a black opti-plate and left to set. For all the S. coelicolor experiments, each well was inoculated with 10⁵ spores in 4 μl of water. Fewer spores were used for other Streptomyces species. Two biological replicates for each construct were tested. Growth at 30°C was determined using the photometric capacity (OD₄₉₂) of the Anthos Lucy 1 luminometer and measured (in triplicate) before incubation and immediately before assaying luminescence. The luciferase substrate, n-decanal, 2 ml, was impregnated into 3MM paper placed inside the opti-plate lid for 60 s at room temperature and luminescence readings (30°C, 0.1 s of integration time) started 50 s later. The specific luciferase activity (SLA) was calculated as Lucy units divided by growth. SLA values at particular growth intervals were pooled to calculate the mean SLA and the 95% confidence intervals (CIs) as determined by the Student t-test. The IF is the ratio of SLAs from the induced/uninduced conditions.

To determine the growth parameters, microtitre plates, prepared as described above, were incubated continuously in the Lucy machine, and the OD₄₉₂ was measured for each well
every 15 min for 51 h. We obtained 288 datasets from 96 well cultures on three replicated plates. The OD492 at time = 0 was subtracted from each subsequent reading (correcting to 0 for any negative values), and these values were then multiplied by 200 and converted to log10. Visual inspection of the curves log(OD492 × 200) versus time indicated the linear region of each plot, and this was used to calculate a regression line using the Marquardt–Levenberg algorithm implemented in the SigmaPlot program. The growth rates (μ) were calculated from the slopes of the regression lines and averaged (μc). Extrapolation of the regression lines to the x-axis gave the lag times (λ), and these were averaged (λc).
RESULTS

Construction of synthetic Tc-inducible promoters for use in *Streptomyces*

Few *Streptomyces* promoters have been subjected to a structure–function analysis. One promoter that is used widely for the expression of heterologous genes in *Streptomyces*, *ermEp1*, is comparatively strong, constitutive and relatively well characterized (19). Several promoters (tcp) were constructed containing the promoter elements from *ermEp1* and two or three operators using both the tetO1 and tetO2 sequences (Figure 1). The tetR gene from Tn10 contains 18 TTA

Figure 2. Regulation of luxAB expression by tcp830 and the innate Tc-inducible promoter, tcp0252. (A and B) Plasmid organization of the tcp830–luxAB fusion (A) and the tcp0252–luxAB fusion (B). pAR933a encodes tcp830 reading towards the luxAB genes and contains an rbs inserted upstream of the start codon for luxA to optimize expression. pAR933b is similar to pAR933a, except that the fragment containing tmmr, tcp850, luxAB is inverted compared with pAR933a. pAR870 is similar to pAR933a, except that it is tetRiS and lacks the rbs upstream of luxAB. pAR911a and pAR911b encode the innate promoter–operator, tcp0252, and repressor, SCO0253. They are different only in the orientations of the fragment containing tmmr, SCO0253, tcp0252, luxAB. pAR911a and pAR911b differ with pAR911a and pAR911b, respectively, as they lack the SCO0253 repressor gene. (C) Sequence of tcp0252 is compared with the Tn10 tetO1 and tetO2 sequences. (D and E) Regulation of luciferase driven by tcp830 in *S.coelicolor* pAR933a (D) and tcp0252 in *S.coelicolor* pAR911b (E) during a growth course. The black filled symbols and the open symbols indicate the levels of luciferase expression in induced cultures with 1 μg/ml aTc, and in uninduced cultures (0 μg/ml aTc), respectively. The data [277 measurements for both null and induced conditions in (D), and 206 and 187 for the null and induced conditions, respectively, in (E)] were grouped by growth intervals and the means of these and 95% CIs are shown. (F) The IF versus growth was calculated using the data from (D). Above the curve in (D–F) is a growth phase indicator showing how the OD492 values correlate with the rapid growth phase (Growth I), the transition phase (TrI and TrII) and the second growth phase (Growth II). Transition phase was divided into TrI and TrII at the point where the growth curve undergoes an inflection.
leucine codons. This is a rarely used codon in vegetative genes in *Streptomyces* and implied that the Tn10 tetR gene was unlikely to be efficiently expressed (20). The synthetic tetR derivative, tTA-2 (14), was designed for use in higher eukaryotes but, fortuitously, it also suits the highly biased codons in streptomycetes. Thus, tTA-2 was adapted as described in Materials and Methods for expression in *Streptomyces* and was renamed ‘tetRiS’ for tetR adapted for use in *Streptomyces*.

The tcps were fused to a promoterless neo gene, conferring kanamycin resistance (Figure 1). These plasmids were constructed with or without a tetRiS gene (the latter to obtain levels of kanamycin resistance in the genetically depressed controls) in an integrating vector and introduced into *S.coelicolor*. The regulation was assayed by determining MICs to kanamycin in the presence and absence of aTc (Figure 1). The tcps showed different levels of expression in the repressed and depressed states. tcp808A and tcp840A had very low promoter activity (conferring resistance to <10 μg/ml kanamycin; data not shown), whereas the activities of tcp808T and tcp840T (with the A at −15 replaced with a T) were much higher, indicating that the ermEp1 promoter belongs to the extended −10 type promoters as suggested by others (19,21). tcp850 and tcp861 despite not having a T at −15, had moderate promoter activity, possibly due to a more consensus-like −35 sequence. tcp700 showed the strongest promoter activity, but was not as highly repressed as tcp808T, tcp830 and tcp840. tcp830 showed the biggest difference in the levels of kanamycin resistance between induced and uninduced cultures and, when induced, was among the strongest tested.

*S.coelicolor* contains an innate Tc-inducible repressor/promoter operator

Studies using the tcp-neo fusions indicated that, with the exception of the negative control, the level of resistance to kanamycin conferred by the tcps was always higher in the presence of aTc than in its absence, even in the absence of the tetRiS gene (Figure 1). These data suggested that *S.coelicolor* may encode an innate Tc-responsive repressor that can interact with the tetO sequences in the synthetic tcps. Bioinformatic analysis of the *S.coelicolor* genome was performed to identify a candidate gene. A BLASTP search of the *S.coelicolor* proteins identified SCO0253 as the closest match to Tn10 TetR. This gene is divergent from SCO0252, a gene that encodes a putative monoxygenase (22). In the promoter–operator region between SCO0253 and SCO0252, which we named tcp0252 (innate tetracycline controllable promoter upstream of SCO0252) sequences with similarity to the tetO sequences from Tn10 were detected (Figure 2). A search of the entire set of intergenic regions of *S.coelicolor* genome was performed to look for further tetO-like sequences. We used the online Patser program (23,24) that scans sequences against position-specific scoring matrices (PSSMs). We built two PSSMs: first, one from the alignment matrix comprising both tetO operators and the two putative SCO0253 tetO-like sequences; second, a PSSM was made collecting the repression change data after saturation mutagenesis of the tetO1 operator (25). Both PSSMs gave similar results: only two significant sequences were found and these were in the intergenic region SCO0253–SCO0252. The separation between the tetO-like sequences was 12 nt, 1 nt more than in the Tn10 tetO region.

To test whether SCO0253 is indeed a Tc-inducible repressor acting on the synthetic tcp, we created a knockout of SCO0253 using the REDIRECT system (18). The knockout strain, *S.coelicolor* D32, was indistinguishable in growth characteristics from the parent strain J1929. To assay the activity of the tcp830 promoter in both J1929 and D32, we constructed a plasmid, pAR870, containing a tcp830–luxAB fusion (see legend to Figure 2). The use of luxAB as a reporter system enabled simultaneous measurement of promoter activities in many strains with multiple independent cultures. The ratio of mean SLAs in the induced versus the uninduced state is given as the IF (Table 1). Although the levels of lux expression were low (most likely due to the absence of an rbs upstream of lux in pAR870), it was clear that tcp830 was repressed in J1929 but not in D32 in the absence of aTc (Table 1). Thus, we concluded that SCO0253 is indeed a Tc-responsive repressor.

We introduced pAR870 into six other *Streptomyces* strains to perform a similar test and to see whether tcp830 is active in other strain backgrounds. The level of luciferase activity in the absence of aTc varied from 0.042 luciferase units (with the A at −15 replaced with a T) to 13.4 U (*S.lividans*) to 13.4 U (*S.roseosporus*) (Table 1). After the addition of aTc, three strains showed induction of the promoter, while the remaining three showed no induction. We concluded that the promoter is generally active in most *Streptomyces* species and that three of these, *S.avermitilis*, *S.bambofaciens* and *S.lividans*, contain a functional homologue of SCO0253 whose product cross-reacts with the tcp830 promoter.

To test whether the promoter for SCO0252, tcp0252, was Tc inducible, the DNA region between the two initiation codons for SCO0252 and SCO0253 was amplified by PCR and inserted upstream of luxAB to form pAR913 (see legend to Figure 2). In addition, a fragment containing tcp0252 and all

| Strain         | Null n* | Growth (OD492) | SLA     | aTc (1 μg/ml) n | Growth (OD492) | SLA     | IFb |
|----------------|---------|----------------|---------|----------------|----------------|---------|-----|
| *S.coelicolor* J1929 | 23      | 0.593 ± 0.010  | 0.419 ± 0.073 | 12              | 0.601 ± 0.018  | 1.27 ± 0.307 | 3.03|
| *S.coelicolor* D32    | 14      | 0.601 ± 0.013  | 2.14 ± 0.393 | 23              | 0.578 ± 0.009  | 1.54 ± 0.204 | 0.72|
| *S.bambofaciens*      | 5       | 0.526 ± 0.015  | 0.132 ± 0.058 | 12              | 0.569 ± 0.011  | 2.23 ± 0.353 | 16.9|
| *S.avermitilis*       | 24      | 0.675 ± 0.014  | 0.405 ± 0.041 | 14              | 0.646 ± 0.045  | 1.91 ± 0.377 | 4.72|
| *S.lividans*          | 6       | 0.733 ± 0.044  | 0.042 ± 0.028 | 11              | 0.699 ± 0.026  | 0.256 ± 0.106 | 6.12|
| *S.roseosporus*       | 5       | 0.753 ± 0.029  | 13.4 ± 1.18  | 6               | 0.757 ± 0.034  | 12.1 ± 1.59  | 0.90|
| *S.griseus*           | 12      | 0.697 ± 0.065  | 0.376 ± 0.068 | 12              | 0.672 ± 0.089  | 0.223 ± 0.058 | 0.59|
| *S.venezuelae*        | 11      | 0.491 ± 0.022  | 1.34 ± 0.793 | 12              | 0.505 ± 0.027  | 1.26 ± 0.801 | 0.94|

*a*Number of cultures used to calculate growth and SLA.

*b*Bold indicates inducible luciferase expression.

![Image](https://academic.oup.com/nar/article/33/9/e87/2401590)
of SCO0253 was inserted upstream of luxAB to form pAR911 (Figure 2). As these constructs contain the rbs for SCO0252 positioned just upstream of the initiation codon for luxAB, the levels of luciferase expression were much higher than those observed with pAR870. In the constructs containing pAR913, particularly pAR913b (in which the fragment encoding tmnr, itcp0252, luxAB is inverted compared with pAR913a), luciferase expression was clearly repressed in the absence of aTc or Tc, presumably by the chromosomal copy of SCO0253 (Table 2). To boost this regulation, a fragment containing tcp830 was inserted upstream of luxAB to form pAR911a and pAR911b (Figure 2). S.coelicolor containing these plasmids showed higher IFs; 6.26 when pAR911a was present and 18.8 when pAR911b was present (Table 2). Surprisingly, this increase in IF was largely due to increases in luciferase activity in the induced state, rather than decreases in activity in the repressed state. In brief, the tcp0252 promoter is clearly inducible by aTc and Tc but seems to be variable, perhaps dependent on the sequence context. This contrasts with the data obtained with the synthetic, tcp830–luxAB constructs.

**Tc-inducible control of gene expression throughout the Streptomyces growth cycle**

We compared the Tc-inducible expression from the synthetic tcp830 promoter with the innate tcp0252 promoter throughout growth of S.coelicolor. For the innate promoter construct, we used J1929 containing pAR911b. For the synthetic promoter construct, we modified pAR860 (to make pAR933a and pAR933b) to contain an rbs upstream of luxAB, so that the expression of luciferase from the natural and synthetic promoters was comparable. Thus, S.coelicolor containing pAR933a and pAR933b (different by the orientations of insertion of the fragment encoding tmnr, tcp830, rbs, luxAB) or pAR911b were assayed with and without the inducer aTc during the growth time-course (Figure 2).

Luciferase levels in the induced cultures containing the tcp830–luxAB fusion (S.coelicolor pAR933a) increased rapidly during the first (exponential) growth phase to reach a maximum during the transition phase. Expression continued at a lower level during the second growth phase up to 65 h post-inoculation (Figure 2D). The levels of uninduced luciferase expression were also maximal during the transition phase. IFs were generally in the order of 20–40 until growth phase II when they rapidly increased to ~270 (Figure 2F). These experiments were repeated for the S.coelicolor pAR933b construct and similar data were obtained (data not shown). S.coelicolor pAR911b containing the itcp0252 promoter showed variable levels of the luciferase expression during early growth and then similar levels to that obtained with the tcp830 promoter (Figure 2E). As the levels of luciferase expression in the uninduced cultures with itcp0252 were slightly higher than with tcp830, the IF values for itcp0252 were generally lower (data not shown). We conclude that both promoters could be employed for regulated expression up to, and possibly beyond, 65 h of growth, although the synthetic promoter tcp830 is more tightly repressed in the uninduced condition.

**Global effects of Tc and aTc in Streptomyces**

If Tc and/or aTc are to be optimal as inducers, the general effects of these chemicals on the growth and physiology of Streptomyces should be minimal. To address this, the lowest level of inducer added to the medium at the start of growth that would provide maximal induction was determined (Figure 3). The IF increased 10-fold when aTc was increased from 1 to 10 ng/ml, suggesting that the aTc concentration could be used to ‘tune’ expression. Above 100 ng/ml aTc the IF barely changed, suggesting that at this concentration the promoter was fully induced. The growth rates of S.coelicolor in the presence and absence of aTc or Tc were then assayed (Figure 4). aTc at 1 or 0.1 µg/ml and Tc at 0.1 µg/ml did not affect the growth rate. However, 1 µg/ml of Tc or doxycycline...
null promoter generally for the regulation of transcription in Streptomyces. Using the luxAB operon as a reporter, we were able to assay easily and quantitatively the level of enzyme activity on microtitre plates, with many replicates, enabling a robust statistical analysis of the data. This promoter also proved to be functional in a broad range of Streptomyces species. IFs obtained with tcp830 ranged from 17 to 40 during early, rapid growth and up to 270 during the late stages of growth. These ratios compare with ratios that have been obtained previously with the tipIA promoter, i.e. ~60-fold using xylE as a reporter gene, (26) and ~15-fold using luxAB (2). With the P\textsubscript{nitA}NitR system, the reporter gene products were assayed after 120 h of growth with inducer added at 96 h; only in one construct could the IF be calculated (~23-fold) as in most cases the uninduced levels of enzyme activity were undetectable (4).

A high priority in this work was placed on whether the inducers, Tc or aTc, would have a pleiotropic effect on general growth and physiology of Streptomyces that might interfere with the interpretation of data when comparing induced and uninduced states. We observed minor effects on growth in the presence of aTc at levels that could be used for maximal induction of tcp830. We have also performed microarray analysis on global gene expression in the presence and absence of aTc (1 \textmu g/ml) and only 1.11% of genes significantly change their expression levels as a result of the presence of aTc (A. Rodríguez-García, R. Pérez-Redondo and M. C. M. Smith, manuscript in preparation).

During the course of these studies, we discovered an innate Tc-inducible promoter, tcp0252, regulated by a tetR homologue, SCO0253. SCO0253 is expressed divergently from SCO0252, which encodes a putative monooxygenase. Although a protein, TetX, with a monooxygenase motif has previously been implicated in conferring resistance to tetracyclines (27), SCO0252 is more similar to putative monooxygenases that are thought to catabolise other aromatic compounds. Between these two open reading frames is a region containing sequences that are similar to tetO from Tn10 (Figure 2). Using a spectrophotometric assay for Tc or aTc, no degradation of drug was detected in culture supernatants (data not shown). While this negative result did not provide information on the function of SCO0252, the lack of degradation of Tc and aTc in the culture indicated that the level of inducer of the tcp is not reduced during normal growth. A search of the S.\textit{coelicolor} genome indicated that there are 27 tetR-like genes located adjacent to putative oxidoreductases or monooxygenases, and 20 of these gene pairs are in a divergent arrangement. Possibly each one of these gene pairs is involved in detoxification.

### Table 3. Growth parameters for \textit{S.coelicolor} in the presence or absence of inducers

| Condition | \( \mu_\text{e} \) (h\(^{-1}\)) 95% CI | t-Test P-value | \( \lambda_\text{e} \) (h) 95% CI | t-Test P-value | Difference (h) with null |
|-----------|---------------------------------|----------------|---------------------------------|----------------|------------------------|
| Null      | 0.397 ± 0.0235                  | Reference      | 0.72 ± 0.71                     | Reference      | —                      |
| aTc 0.1  | 0.401 ± 0.0224                  | 0.177          | 0.72 ± 0.72                     | 0.068          | 0.9                    |
| Tc 0.1   | 0.383 ± 0.0240                  | 0.823          | 0.72 ± 0.84                     | 0.054          | 1.1                    |
| aTc 1    | 0.378 ± 0.0189                  | 0.938          | 0.72 ± 0.72                     | 0.010          | 1.3                    |
| Tc 1     | 0.354 ± 0.0194                  | 0.080          | 0.97 ± 0.72                     | <0.001         | 2.4                    |
| Dc 1     | 0.351 ± 0.0179                  | 0.054          | 0.71 ± 0.72                     | <0.001         | 11.7                   |

(Dc) slowed the growth rate by small amounts (12 and 7%, respectively; Table 3). All the inducers caused an increase in the lag time ranging from 0.9 h for 0.1 \textmu g/ml aTc to 11.7 h for Dc (Table 3 and Figure 4). As we showed that 0.1 \textmu g/ml of aTc is sufficient to induce the tcp830, conditions can be used where growth is almost unaffected by the addition of inducer.

### DISCUSSION

Data presented here demonstrate the utility of both synthetic and natural promoters that are regulated by Tc and aTc for use in \textit{Streptomyces}. Of the synthetic promoters, tcp830 proved to be the most effective for providing strong promoter activity when induced and efficient repression. As this was true for both reporter genes used in this study, i.e. \textit{neo} and luxAB, it is likely that tcp830 will prove to be an effective and
In brief, the tcp830 promoter provides a much-needed alternative to the existing promoters for use in Streptomyces and possibly also in other actinomycetes to regulate gene expression. Vectors compatible with the REDIRECT PCR targeting system developed by Gust et al. (18), and an integrating vector that contains tcp830 reading towards a multiple cloning site into which a gene of interest can be inserted, have been constructed (Supplementary Material).

SUPPLEMENTARY MATERIAL
Supplementary Material is available at NAR Online.

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