

## The Level of a Transcript Required for Production of a *Streptomyces coelicolor* Antibiotic Is Conditionally Dependent on a tRNA Gene

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In *Streptomyces coelicolor* A3(2), *bldA* mutants are conditionally defective in aerial mycelium formation and fail to synthesize all four antibiotics produced by *bldA*<sup>+</sup> strains. Previous studies showed that *bldA* specifies the tRNA for the rarely used leucine codon UUA. Here we describe experiments examining the abundance in a *bldA* mutant of a transcript involved in antibiotic production. With use of a bacteriophage-based integrative vector, a promoterless *xylE* reporter gene was inserted into a previously undescribed gene for an early step in biosynthesis of the red antibiotic undecylprodigiosin, located in the *red* gene cluster. With this transcriptional fusion present at unit copy number in the chromosome, *xylE* expression in a *bldA*<sup>+</sup> strain was maximal late in growth in a liquid production medium and was virtually absent in a *bldA* mutant. On plates of a different medium, the *bldA* mutant was able to produce undecylprodigiosin and to express the *red::xylE* fusion, but both abilities were repressed by increasing the concentration of phosphate in the medium. These experiments showed that the undecylprodigiosin deficiency of *bldA* mutants cannot be accounted for by the presence of TTA codons in the *red* structural genes, but rather that *bldA* influences *red* gene mRNA abundance. In low-phosphate conditions, an alternative regulatory pathway can lead to *red* gene expression.

Streptomycetes are mycelial gram-positive soil bacteria well known for their morphological complexity, which involves the production of chains of spores on specialized aerial hyphae, and for their preeminence as producers of antibiotics. In *Streptomyces coelicolor* A3(2) (genetically the most-studied representative of the genus), *bldA* mutants are one of several classes of *bld* mutants defective in the formation of normal aerial mycelium and spores (2–5, 18). This phenotype in *bldA* (and some other *bld*) mutants is carbon source dependent; it is seen on minimal agar medium containing glucose or cellobiose as a carbon source, whereas aerial mycelium development and sporulation appear to occur normally on other carbon sources, such as mannitol or maltose (18). In addition, production of four different antibiotics, actinorhodin, undecylprodigiosin, methylenomycin, and the calcium-dependent antibiotic (the first two of which are pigmented), is abolished in *bldA* (and some other *bld*) mutants. Growth on carbon sources that permit normal aerial mycelium and spores to form does not restore antibiotic production (2, 18).

The *bldA* gene has been cloned (21) and shown to encode a leucine tRNA for the codon UUA, which is rare in *Streptomyces* spp. (14; B. K. Leskiw, E. J. Lawlor, J. M. Abalos, and K. F. Chater, unpublished data). Expression of several genes containing TTA codons is abolished or reduced in a *bldA* mutant (Leskiw et al., unpublished data). Accumulation of the *bldA*-encoded tRNA occurs on minimal agar medium at about the time when aerial mycelium is being produced (14). Usually, all four antibiotics appear to be produced in the wild-type *S. coelicolor* at or after this time.

Complete gene sets for the production of actinorhodin (16), undecylprodigiosin (9, 10; F. Malpartida, J. Niemi, R.

Navarrete, and D. A. Hopwood, Gene, in press), and methylenomycin (8; L. Woodburn and K. F. Chater, unpublished results) have been cloned from *S. coelicolor*, making it possible to assess transcription of antibiotic production genes in a *bldA* mutant. These gene sets are about 20 kb or more in size and appear to be transcriptionally complex. It is therefore a nontrivial task to study their transcriptional organization and regulation by direct RNA analysis. In addition, there appear to be technical difficulties with RNA isolation from antibiotic-producing cultures, leading (at least in our hands) to poorly reproducible results with RNA dot blot and low-resolution S1 nuclease protection analyses of the *red* gene cluster (for undecylprodigiosin synthesis). We have therefore investigated the applicability of transcriptional fusion of a reporter gene to a chromosomally located (and previously undescribed) *red* gene and have used this system to assess the effect of *bldA* mutation on *red* gene transcription. The reporter gene chosen was *xylE* (13, 24), which contains no TTA codons (24) and should therefore be transcriptionally independent of *bldA*. This gene has been successfully used in *Streptomyces lividans*, in which convincing correlations of the activity of catechol 2,3-dioxygenase (the *xylE* gene product) with mRNA levels were demonstrated for the *gal* operon (13). Our results suggest that the abundance of at least one *red* transcript is greatly reduced in a *bldA* mutant.

### MATERIALS AND METHODS

**Bacterial strains and culture conditions.** *Streptomyces* strains used in this study are listed in Table 1. Standard agar media (R2 medium, without or with [R2YE] yeast extract, and minimal medium) and culture conditions for *Streptomyces* strains were as described by Hopwood et al. (12). SY liquid medium (20), in which *S. coelicolor* produces undecylprodigiosin, was used to study the expression of *red::xylE* fusions in relation to growth. Either spores or mycelial

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TABLE 1. *Streptomyces* strains

Species	John Innes strain no.	Genotype <sup>a</sup>	Reference
<i>S. coelicolor</i> A3(2)	J1501	<i>hisA1 uraA1 strA1</i> SCP1 <sup>-b</sup> SCP2 <sup>-</sup> Pg1 <sup>-</sup>	12
	J1700	<i>bldA39 hisA1 uraA1 strA1</i> SCP1 <sup>-</sup> SCP2 <sup>-</sup> Pg1 <sup>-</sup>	E. J. Lawlor, personal communication
	TK16	<i>argA1 guaA1 act(IV)117 redA59</i> SCP1 <sup>-</sup> SCP2 <sup>-</sup>	9
	JF2	<i>argA1 guaA1 act(II)105 redB59</i> SCP1 <sup>-</sup> SCP2 <sup>-</sup>	9
	TK17	<i>proA1 argA1 cysD18 strA1 act(I)118 redC1</i> SCP1 <sup>-</sup> SCP2 <sup>-</sup>	9
	JF1	<i>argA1 guaA1 act(II)177 redD42</i> SCP1 <sup>-</sup> SCP2 <sup>-</sup>	9
	JF4	<i>proA1 argA1 uraA1 strA1 act(V)109 redE60</i> SCP1 <sup>-</sup> SCP2 <sup>-</sup>	9
	EG9	<i>bldA39 hisA1 uraA1 strA1 pwb-9</i> SCP1 <sup>-</sup> SCP2 <sup>-</sup> Pg1 <sup>-</sup>	E. P. Guthrie, unpublished data
	J1501	Wild type	15
<i>S. lividans</i> 66	1326	Wild type	15

<sup>a</sup> SCP1 and SCP2 are plasmids of *S. coelicolor* A3(2), and Pg1<sup>-</sup> strains allow efficient propagation of phage  $\phi$ C31.

<sup>b</sup> Erroneous SCP1 status noted in references 12.

fragments were first inoculated as a starter culture into 10 ml of SY with added histidine, uracil, thiostrepton (5  $\mu$ g/ml), and antifoam (0.015% [vol/vol] Rhodorsil-426R; Rhone-Poulenc) and grown with aeration for 30 h at 30°C. The mycelium was subsequently homogenized in a Potter homogenizer; the culture was added to 100 ml of SY medium with the same additions but without thiostrepton in a 500-ml flask baffled with a coiled spring (10) to give an optical density at 450 nm of between 0.1 and 0.2 and was grown with shaking at 30°C.

**Bacteriophages.** KC860, an *att*-deleted derivative of the temperate phage  $\phi$ C31 (C. J. Bruton, E. P. Guthrie, and K. F. Chater, unpublished data), carries a promoterless *xylE* gene together with genes for resistance to the antibiotics thiostrepton and viomycin (Fig. 1). Phage techniques were those described by Hopwood et al. (12). To generate lysogens, master plates containing plaques were replicated to a lawn of J1501 spores. After sporulation, the plates were replicated onto R2YE containing thiostrepton (50  $\mu$ g/ml), on which only the lysogens could grow.

**Plasmids.** Plasmid pIJ2341 (pBR329 with 13 kb of *S.*

*coelicolor* DNA which complements the *redC* and *redD* mutations; Fig. 1; Malpartida et al., in press) was the source of *red* DNA used in this work.

**Isolation and in vitro manipulation of DNA.** Plasmid DNA was isolated from *Escherichia coli* as described by Maniatis et al. (17). *Streptomyces* phage DNA was made by the small- or large-scale methods (12). Total DNA of *Streptomyces* was isolated by the method of Hopwood et al. (12). Restriction endonucleases (Bethesda Research Laboratories, Inc., or Boehringer-Mannheim) and T4 ligase (Bethesda Research Laboratories) were used as recommended by the manufacturers. Agarose gel electrophoresis, nick translation, Southern blots, hybridization, and washes of Southern blots were performed essentially as described previously (12). Isolation of DNA fragments from agarose gels was done by using DEAE-paper (23).

**Cosynthesis tests.** Spores of *S. coelicolor red* mutants (Table 1) and lysogens of EG9, a mutant with a Red<sup>+</sup> Act<sup>-</sup> phenotype (see Results), were streaked close together, but without contact, on R2YE plates and incubated at 30°C for 4 days.

**Activity of *xylE*.** Expression of the *xylE* gene was monitored by spraying 3-day-old colonies on R2YE or minimal medium plates with an aqueous solution of 0.5 M catechol (24). Yellow color (the Ylo<sup>+</sup> phenotype), associated with production of 2-hydroxymuconic semialdehyde, developed over a period of 2 to 4 h. The catechol dioxygenase-specific activity of the bacteria grown in SY liquid medium was measured by assaying sonicated cell extracts (13, 24). Protein concentrations were determined by the Bio-Rad Microassay procedure (1).

## RESULTS

**Construction of a transcriptional fusion of the *xylE* reporter gene to a transcription unit from the *red* biosynthetic gene cluster.** We found that a 1.4-kb *Bam*HI fragment from pIJ2341 (Fig. 1) could be used in phage-mediated gene disruption experiments (7) to eliminate undecylprodigiosin synthesis (the Red<sup>-</sup> phenotype). This fragment (from a region of the cloned DNA that had not previously been analyzed functionally) was therefore internal to a *red* transcription unit and could potentially be used to construct transcriptional fusions of a reporter gene to that transcription unit in its chromosomal location (Fig. 1). A suitable integrating phage vector, KC860 (Fig. 1), has recently been constructed to allow such fusions with the *xylE* reporter gene (Bruton et al., unpublished data). To construct the *red::xylE* fusion, the mixture of DNA resulting from ligation of the

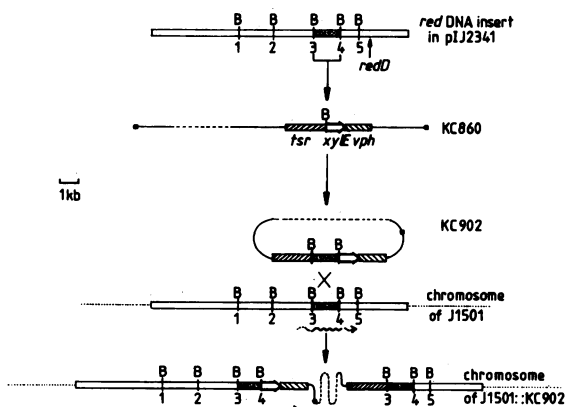


FIG. 1. Fusion of *xylE* to a chromosomal *red* gene. A *Bam*HI fragment (■) from the *red* DNA of pIJ2341 was introduced into the *Bam*HI site of KC860 to give KC902 (or, for the opposite orientation, KC903). The arrow in *xylE* shows the correct transcriptional orientation. KC902 DNA (circularized through joining of its cohesive ends; ■) could then insert into the chromosome of J1501 by homologous recombination. Abbreviations and symbols: *tsr*, thiostrepton resistance gene; *vph*, viomycin resistance gene; *xylE*, a *Pseudomonas* gene encoding catechol 2,3-dioxygenase; B, *Bam*HI site; ~~, presumed transcript; ---, omitted parts of the  $\phi$ C31 genome; ···, J1501 chromosome (flanking the region homologous to pIJ2341). The position of the *redD* regulatory gene (19) is indicated.

gel-purified 1.4-kb *Bam*HI fragment into the *Bam*HI site of KC860 was used to transfect *S. lividans* 1326. Phages which carried the insert were identified by their ability to lysogenize *S. coelicolor*: because the parental phage, KC860, is AttP<sup>-</sup>, only phages with the cloned insert could establish stable lysogeny. Lysogens were checked for *xylE* expression (the Ylo<sup>+</sup> phenotype) and for their Red phenotype. All were Red<sup>-</sup>, but some were Ylo<sup>+</sup> and others were Ylo<sup>-</sup>. Restriction analysis of DNA isolated from phages released from the lysogens, coupled with Southern blotting of *Eco*RI, *Kpn*I, and *Xho*I digests of total DNA isolated from the lysogens, showed that their Ylo<sup>+</sup> or Ylo<sup>-</sup> phenotype was determined by the orientation of the 1.4-kb insert in phage KC860. Representative phages of the two types were designated KC902 and KC903, respectively. The orientation dependence for *xylE* expression shows that the mutagenic *Bam*HI fragment is transcribed left to right on the map shown in Fig. 1.

**The *red::xylE* fusion in wild-type *S. coelicolor* is expressed after the most rapid growth is completed in batch cultures.** To determine at what stage of growth the *red* gene fused to *xylE* was transcribed, liquid batch cultures were set up by using the two different lysogens J1501::KC902 and J1501::KC903. The medium used, SY, had been observed previously to give strong production of undecylprodigiosin (10). At different time points, samples of the culture were removed and used to prepare cell extracts for the assay of catechol dioxygenase activity. A sharp peak in activity was observed when the doubling time of the J1501::KC902 cultures lengthened to more than 4 h, as they approached stationary phase (Fig. 2). During the earlier, more rapid growth phase (doubling time, <3 h), there was little or no activity. No such increase in *xylE* expression was detected in J1501::KC903 (Fig. 2). (Note that the range of activities detected in our work was about 10 times lower than the range obtained with plasmid-borne fusions of *xylE* to the *gal* operon [13] and is probably close to the lower limit for the reliable use of *xylE* as a reporter; below this range, incubation times in the spectrophotometric assay would need to be inordinately long to obtain significant differences from negative controls.)

**The *red::xylE* fusion is to a *red* structural gene rather than to a pathway-wide regulatory gene.** The Red<sup>-</sup> phenotype of J1501::KC902 and J1501::KC903 lysogens could be due to disruption of structural or regulatory genes, especially since pIJ2341 (the source of the mutagenic fragment) contains a putative regulatory gene, *redD* (Fig. 1; 19, 22; Malpartida et al., in press). Loss of a pathway-wide regulatory gene would be expected to cause failure to synthesize any pathway-specific enzymes, whereas structural gene mutants should produce most of these enzymes. A simple test of this is cosynthesis: late-blocked mutants often secrete intermediates that can be converted to the final product by early-blocked mutants. For undecylprodigiosin, these tests must be done in a strain that is not producing the red-blue indicator pigment actinorhodin, which masks the red pigment. We therefore used KC902 and KC903 to lysogenize strain EG9, a derivative of the *bldA* mutant J1700. In EG9, actinorhodin is not produced because of the *bldA39* mutation, but undecylprodigiosin is made because of a so-called *pwb* mutation located close to or in the *red* region of the chromosome (E. P. Guthrie and K. F. Chater, unpublished data). The EG9 lysogens were both Red<sup>-</sup>, but they could produce red pigment when grown close to mutants for the late genes *redB* and *redE* (strains JF2 and JF4). No cosynthesis was seen with test strains blocked at early (TK16, *redA*) or intermediate (TK17, *redC*) steps or with a pleiotro-

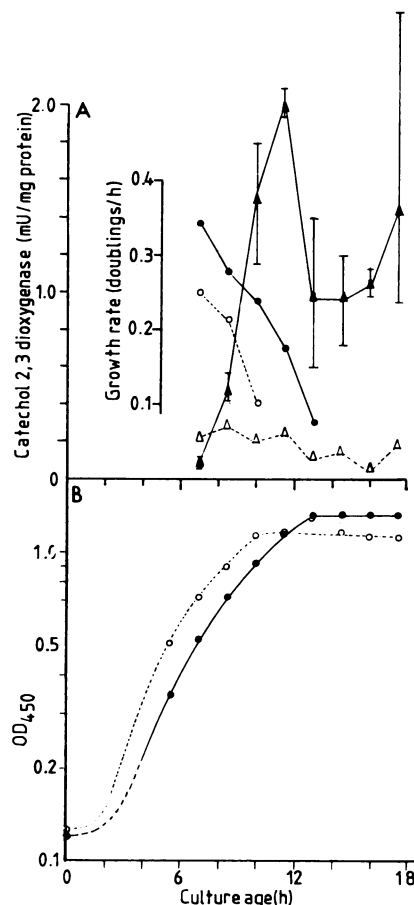


FIG. 2. Catechol 2,3-dioxygenase activity during batch culture of a strain (J1501::KC902) in which *xylE* is fused to a *red* gene. (A) Specific activity of catechol 2,3-dioxygenase (showing the range of three measurements [I] and the mean [▲] for each point) and growth rate (doublings per hour; ●) estimated by the slope of tangents to the growth curve shown in panel B (●). Note that only panel B has been plotted exponentially. The equivalent sets of data for a strain (J1501::KC903) in which *xylE* is incorrectly oriented in the same chromosomal location are shown by open symbols and broken lines.

pic regulatory defect (JF1, *redD*). It follows that the enzymes that act late in the pathway were active in the lysogens. The Red<sup>-</sup> phenotype of the lysogens therefore resulted from the inactivation of one or more of the early structural genes. (Further aspects of the *red::xylE* fusions in *pwb* mutants will be described elsewhere.)

**Expression of the *red::xylE* fusion is greatly reduced in a *bldA* mutant.** To help to determine whether the block on undecylprodigiosin biosynthesis in a *bldA* mutant of *S. coelicolor* was at the level of mRNA abundance, lysogens of a *bldA39* mutant, J1700, were made with the phages KC902 and KC903. With both lysogens, *xylE* activity was virtually undetectable on R2YE or minimal medium test plates (Table 2) and barely measurable in each of four liquid culture experiments in SY medium (about 0.3 mU of catechol oxygenase per mg of protein, compared with 2.1 mU/mg for a *bldA*<sup>+</sup> isogenic strain). By themselves, these results do not allow discrimination between models that explain *bldA* dependence by invoking either effects on the initiation of transcription or mRNA stability or translational effects from possible upstream UUA codons. However, further studies



TABLE 2. Effects of a *bldA* mutation and growth medium on transcription of a *red::xylE* fusion and production of red pigment

Medium	J1501 ( <i>bldA</i> <sup>+</sup> ) <sup>a</sup>			J1700 ( <i>bldA39</i> )		
	Red phenotype (no insert)	Ylo phenotype <sup>b</sup>		Red phenotype (no insert)	Ylo phenotype	
		KC902 lysogen	KC903 lysogen		KC902 lysogen	KC903 lysogen
MM <sup>c</sup> (4 mM PO <sub>4</sub> )	Red <sup>+</sup>	Ylo <sup>+</sup>	Ylo <sup>-</sup>	Red <sup>-</sup>	Ylo <sup>-</sup>	Ylo <sup>-</sup>
R2YE	Red <sup>+</sup>	Ylo <sup>++</sup>	Ylo <sup>-</sup>	Red <sup>-</sup>	Ylo <sup>±</sup>	Ylo <sup>-</sup>
R2	Red <sup>+</sup>	Ylo <sup>++</sup>	Ylo <sup>-</sup>	Red <sup>+</sup>	Ylo <sup>++</sup>	Ylo <sup>-</sup>
MM (0.04 mM PO <sub>4</sub> )	Red <sup>+</sup>			Red <sup>+</sup>	Ylo <sup>+</sup>	Ylo <sup>-</sup>

<sup>a</sup> In J1501, it was usually difficult to score the Red and Ylo phenotypes because blue actinorhodin was also produced.

<sup>b</sup> Ylo<sup>-</sup>, No detectable yellow color; Ylo<sup>±</sup>, faintly detectable yellow coloration confined to mycelium; Ylo<sup>+</sup>, weak production of yellow pigment in the medium; Ylo<sup>++</sup>, conspicuous production of yellow pigment in the medium.

<sup>c</sup> MM, Minimal medium.

(next section) make translational effects an unlikely explanation.

**In low-phosphate conditions, undecylprodigiosin synthesis and expression of the *red::xylE* fusions are not dependent on *bldA*.** Originally, *bldA* mutants were described as being unable to produce any of the four antibiotics normally produced in wild-type *S. coelicolor* (11, 18). In the course of this work, we noticed that J1700, when grown on R2 agar medium lacking yeast extract, produced red pigment but no actinorhodin or aerial mycelium. Further investigation, to find out why red pigment was observed on R2 but not on minimal medium, revealed that lowering the phosphate concentration in minimal medium from the normal 4 mM to 0.04 mM (as in R2) gave rise to red pigment, but (again) no actinorhodin or aerial mycelium (Table 2). (We did not study methylenomycin or the calcium-dependent antibiotic production because these conditions do not favor their production and because J1700, lacking SCP1, does not contain methylenomycin production genes.) J1700::KC902 colonies were Ylo<sup>+</sup> on the low-phosphate minimal medium but Ylo<sup>-</sup> on normal minimal medium. *bldA* dependence for translation of UUA codons in other mRNA species in *S. coelicolor* was not overcome by reducing the phosphate level in minimal medium (Leskiw et al., unpublished data). The strong expression of the *red::xylE* fusion, and of undecylprodigiosin synthesis, in J1700 growing on low-phosphate medium therefore implies an absence of UUA codons in any of the *red* transcripts. Thus, the amount, rather than the translation, of *red* mRNA is probably the means by which *bldA* affects undecylprodigiosin synthesis, and the phosphate effect is also mediated at the level of mRNA abundance.

## DISCUSSION

**The delayed onset of undecylprodigiosin synthesis is regulated at the level of mRNA abundance.** The time course for *xylE* expression during batch culture (Fig. 2) shows that in our laboratory conditions, the mRNA for an early *red* gene (or genes) is maximally abundant rather briefly and only after the most rapid growth phase is over. After this time, mRNA levels are maintained at least for several hours at about half the maximal level. This time course does not distinguish between nutrient limitation and growth rate reduction as the possible cause for the accumulation of *red* mRNA. Nevertheless, it coincides with the broad expectation for the expression of antibiotic production genes and with observations on the time of appearance of an *O*-methyltransferase (the product of the *redE* and *redF* genes) involved at a late step in undecylprodigiosin biosynthesis (10). Hence, the prophage is sufficiently stably integrated to permit its use for

physiological studies. The use of *xylE* fusions to chromosomally located antibiotic production genes may be a valuable aid to fundamental and applied studies. Further support for the potential value of such fusions has been obtained with a fusion of *xylE* to an actinorhodin biosynthetic transcription unit (Bruton et al., unpublished data).

**Abundance of *red* gene mRNA is subject to at least two regulatory influences.** We have shown that accumulation of a *red* transcript encoding at least one enzyme acting early in the biosynthesis of undecylprodigiosin depends on *bldA* during growth on either of two agar media (R2YE or minimal medium) and in SY liquid medium. This finding is compatible with a model(s) in which the *bldA* gene product, tRNA<sup>Leu</sup><sub>UUA</sub>, is required for the translation of a hypothetical UUA-codon-containing mRNA whose protein product participates in the general transcriptional activation of antibiotic production genes (6). The nature of this participation is not clear; several other genes, whose products remain unidentified, are dispensable for growth but pleiotropically necessary for antibiotic production (3, 6). Among these may be the gene(s) directly dependent on *bldA* for translation.

On minimal medium with low (0.04 mM) phosphate (and on R2 agar medium lacking yeast extract), the *red* genes are expressed even in a *bldA* mutant. Thus, in addition to the pleiotropic *bldA*-dependent regulatory system, there appears to be a second route to activation, which is inactivated by phosphate. (Inhibition of this system by yeast extract [0.5% in R2YE] could perhaps be caused by phosphate in the yeast extract.) Since phosphate repression is not seen in a *bldA*<sup>+</sup> strain, *bldA*-dependent activation of *red* genes overrides the phosphate effect. It is interesting that the phosphate-sensitive activation shows more pathway specificity than does *bldA*-dependent activation, in that it does not affect actinorhodin synthesis. This specificity, coupled with the observation that the dependence of TTA-containing genes on *bldA* is not alleviated by reducing the phosphate in minimal medium (Leskiw et al., unpublished data), makes it unlikely that expression of an alternative tRNA<sup>Leu</sup><sub>UUA</sub> can explain *red* activation on low phosphate. Further analysis of these systems and their interplay with *pwb* mutations and the pathway-specific *redD* positive regulatory gene (19, 22) is in progress (Guthrie and Chater, unpublished data).

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#### LITERATURE CITED

1. **Bradford, M.** 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **72**:248–254.
2. **Champness, W. C.** 1988. New loci required for *Streptomyces coelicolor* morphological and physiological differentiation. *J. Bacteriol.* **170**:1168–1174.
3. **Champness, W. C., T. Adamidis, and P. Riggle.** 1989. Developmental genes and antibiotic synthesis, p. 108–112. *In* C. L. Hershberger, S. W. Queener, and G. Hegeman (ed.), *Genetics and molecular biology of industrial microorganisms*. American Society for Microbiology, Washington, D.C.
4. **Chater, K. F.** 1984. Morphological and physiological differentiation in *Streptomyces*, p. 89–115. *In* R. Losick and L. Shapiro (ed.), *Microbial development*. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
5. **Chater, K. F.** 1989. Sporulation in *Streptomyces* species, p. 277–299. *In* I. Smith, R. Slepecky, and P. Setlow (ed.), *Regulation of prokaryotic development*. American Society for Microbiology, Washington, D.C.
6. **Chater, K. F.** 1990. The improving prospects for yield increase by genetic engineering in antibiotic-producing streptomycetes. *Bio/Technology* **8**:115–121.
7. **Chater, K. F., and C. J. Bruton.** 1983. Mutational cloning and the isolation of antibiotic production genes. *Gene* **26**:67–78.
8. **Chater, K. F., and C. J. Bruton.** 1985. Resistance, regulatory and production genes for the antibiotic methylenomycin are clustered. *EMBO J.* **4**:1893–1897.
9. **Feitelson, J. S., and D. A. Hopwood.** 1983. Cloning of a *Streptomyces* gene for an O-methyltransferase involved in antibiotic biosynthesis. *Mol. Gen. Genet.* **190**:394–398.
10. **Feitelson, J. S., F. Malpartida, and D. A. Hopwood.** 1985. Genetic and biochemical characterisation of the *red* gene cluster of *Streptomyces coelicolor* A3(2). *J. Gen. Microbiol.* **131**:2431–2441.
11. **Hopwood, D. A., and H. M. Wright.** 1983. CDA is a new chromosomally-determined antibiotic from *Streptomyces coelicolor* A3(2). *J. Gen. Microbiol.* **129**:3565–3573.
12. **Hopwood, D. A., M. J. Bibb, K. F. Chater, T. Kieser, C. J. Bruton, H. M. Kieser, D. J. Lydiate, C. P. Smith, J. M. Ward, and H. Schrempf.** 1985. *Genetic manipulation of Streptomyces: a laboratory manual*. John Innes Foundation, Norwich, United Kingdom.
13. **Ingram, C., M. Brawner, P. Youngman, and J. Westpheling.** 1989. *xylE* functions as an efficient reporter gene in *Streptomyces* spp.: use for the study of *galP1*, a catabolite-controlled promoter. *J. Bacteriol.* **171**:6617–6624.
14. **Lawlor, E. J., H. A. Baylis, and K. F. Chater.** 1987. Pleiotropic morphological and antibiotic deficiencies result from mutations in a gene encoding a tRNA-like product in *Streptomyces coelicolor* A3(2). *Genes Dev.* **2**:1305–1310.
15. **Lomovskaya, N. D., N. M. Mkrtumian, N. L. Gostimskaya, and V. N. Danilenko.** 1972. Characterization of a temperate actinophage  $\phi$ C31 isolated from *Streptomyces coelicolor* A3(2). *J. Virol.* **9**:258–262.
16. **Malpartida, F., and D. A. Hopwood.** 1984. Molecular cloning of the whole biosynthetic pathway of a *Streptomyces* antibiotic and its expression in a heterologous host. *Nature (London)* **309**:462–464.
17. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. *Molecular cloning: a laboratory manual*. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
18. **Merrick, M. J.** 1976. A morphological and genetic mapping study of bald colony mutants of *Streptomyces coelicolor*. *J. Gen. Microbiol.* **96**:299–315.
19. **Narva, K. E., and J. S. Feitelson.** 1990. Nucleotide sequence and transcriptional analysis of the *redD* locus of *Streptomyces coelicolor* A3(2). *J. Bacteriol.* **172**:326–333.
20. **Oki, T., Y. Matsuzawa, K. Kiyoshima, and A. Yoshimoto.** 1981. New anthracyclines, feudomycins produced by the mutant from *Streptomyces coeruleorubidus* ME130-A4. *J. Antibiot.* **34**:783–790.
21. **Piret, J. M., and K. F. Chater.** 1985. Phage-mediated cloning of *bldA*, a region involved in *Streptomyces coelicolor* morphological development, and its analysis by genetic complementation. *J. Bacteriol.* **163**:965–972.
22. **Rudd, B. A. M., and D. A. Hopwood.** 1980. A pigmented mycelial antibiotic in *Streptomyces coelicolor*: control by a chromosomal cluster. *J. Gen. Microbiol.* **119**:333–340.
23. **Winberg, G., and M.-L. Hammarskjold.** 1980. Isolation of DNA from agarose gels using DEAE paper. Application to restriction site mapping of adenovirus type 16 DNA. *Nucleic Acids Res.* **8**:253–264.
24. **Zukowski, M. M., D. F. Gaffney, D. Speck, M. Kauffmann, A. Findeli, A. Wisecup, and J. Lecocq.** 1983. Chromogenic identification of genetic regulatory signals in *Bacillus subtilis* based on expression of a cloned *Pseudomonas* gene. *Proc. Natl. Acad. Sci. USA* **80**:1101–1105.