Genetic Analysis of absB, a *Streptomyces coelicolor* Locus Involved in Global Antibiotic Regulation

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The filamentous soil bacterium *Streptomyces coelicolor* is known to produce four antibiotics which are genetically and structurally distinct. An extensive search for antibiotic regulatory mutants led to the discovery of absB mutants, which are antibiotic deficient but sporulation proficient. Genetic analysis of the absB mutants has resulted in definition of the absB locus at 5 o’clock on the genetic map. Multiple cloned copies of the actII-ORF4 gene, an activator of synthesis of the antibiotic actinorhodin, restore actinorhodin biosynthetic capability to the absB mutants. These results are interpreted to mean that the failure of absB mutants to produce antibiotics results from decreased expression of the antibiotic genes. The absB gene is proposed to be involved in global regulation of antibiotic synthesis.

Bacteria of the order Actinomycetales are capable of producing a diverse array of medically useful secondary metabolites. The streptomycetes have been the major focus of antibiotic research, and the genetically best-characterized species, *Streptomyces coelicolor*, is known to produce four structurally and genetically distinct antibiotics: actinorhodin, undecylprodigiosin, methylenomycin, and calcium-dependent antibiotic.

Genetic studies on *S. coelicolor*’s four antibiotics have progressed to the point where all of the biosynthetic gene clusters have been mutationally defined and mapped (18, 22, 32, 33). Three gene clusters have been cloned: act (26, 27), red (9, 28), and mny (7). Regulatory studies have progressed farthest with the act and red genes. The 22-kbp act gene cluster encodes many open reading frames, expressed in at least six transcripts (10, 27). The actII-ORF4 gene encodes a gene product required for transcription of the act biosynthetic genes (reviewed in reference 10). The red cluster (9, 28) is also large (35 kbp) and complex and also includes a gene, redD, which plays a positive role in expression of the red biosynthetic genes (30).

In a plate-grown *Streptomyces* culture, growing hyphae initially form a dense, matted mycelium. Later, aerially directed hyphae form on the colony surface and develop into chains of spores. Antibiotic synthesis is developmentally regulated and is generally found to coincide with sporulation (6). In liquid culture, most streptomycetes do not sporulate, but antibiotic production is delayed until the culture enters the stationary phase.

Evidence that sporulation and production of the four *S. coelicolor* antibiotics are subject to a common genetic control derives from the isolation of single mutations that block both processes—the bld mutations (5, 29, 34, 37). The best-characterized bld gene, bldA, encodes a leucyl-tRNA capable of translating the codon UUA (23). UUA has thus far been found primarily in genes involved in development, including several antibiotic resistance genes (reviewed in reference 24), a gene involved in *Streptomyces griseus* sporulation (2), and the actII-ORF4 gene (10). Therefore,

one aspect of actinorhodin regulation involves a translational requirement for the bldA tRNA (10).

Single mutations that completely block production of the four *S. coelicolor* antibiotics but allow abundant sporulation (Abs+ phenotype) [1] define the previously discovered absA locus (1). The Abs+ phenotype suggests the existence of a global regulatory mechanism that is, at least in part, specific to antibiotic synthesis and distinct from sporulation control.

Here, we report on the discovery and characterization of an additional locus, absB, which can also cause an Abs− phenotype by mutation.

**MATERIALS AND METHODS**

**Bacterial strains and phages.** The strains used for genetic analysis were derivatives of *S. coelicolor* A3(2) (Table 1). *Streptomyces lividans* 1326 was used for phage propagation (25). Procedures for phage propagation were as described by Hopwood et al. (15). Lysogen formation was accomplished as described previously (1, 5).

**Media and culture techniques.** Minimal plate medium for genetic analysis, nutrient agar, and media R5 and R2 were as described by Hopwood et al. (15). YEG (30) contained 1% yeast extract and 1% glucose; SY (12) contained 0.3% yeast extract and 1% starch. R5, YEG, and SY were supplemented with histidine and uracil at 50 and 7.5 μg/ml, respectively. Thiostrepton (gift of S. J. Lucania, E. R. Squibb and Sons, Inc., or Sigma Chemical Co.) was used at a concentration of 50 μg/ml.

**Antibiotic assays.** The assays used for methylenomycin and calcium-dependent antibiotic were those described previously (1). For actinorhodin and undecylprodigiosin quantitations, the tested strains were streaked onto cellulose-acetate filters on R5 medium. After 5 to 6 days, the mycelia were scraped off and weighed. Approximately 20 mg was extracted with 0.5 ml of chloroform for 30 min at room temperature, with shaking. Then, 0.5 ml of 1 N NaOH was added, and the tubes were vortexed and then spun in a microcentrifuge for 15 s. The aqueous phase contained actinorhodin, which is blue at alkaline pH. The A490 of the aqueous phase was determined. The chloroform phase contained the undecylprodigiosin, which was yellow. For absorbance measurements of undecylprodigiosin, the chlo-

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TABLE 1. Strains, phages, and plasmids used

<table>
<thead>
<tr>
<th>Strain, phage, or plasmid</th>
<th>Relevant characteristics</th>
<th>Source or reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>S. coelicolor</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M124</td>
<td>cysD18 argA1 proA1 SCP1 − SCP2 −</td>
<td>D. Hopwood (15)</td>
</tr>
<tr>
<td>J650</td>
<td>cysD18 mthB2 aagA1 NF SCP2 +</td>
<td>K. Chater (29)</td>
</tr>
<tr>
<td>J1501</td>
<td>hisA1 uraA1 strA1 SCP1 − SCP2 − Pgl −</td>
<td>K. Chater (7, 15)</td>
</tr>
<tr>
<td>BH5</td>
<td>cysD18 proA1 argA1 aasB SCP2 +</td>
<td>S. Horinouchi (14)</td>
</tr>
<tr>
<td>BH51b</td>
<td>Same as BH5 but NF</td>
<td>This work</td>
</tr>
<tr>
<td>CI20</td>
<td>hisA1 uraA1 strA1 abs-120 SCP1 − SCP2 − Pgl −</td>
<td>This work</td>
</tr>
<tr>
<td>CI201b</td>
<td>Same as CI20 but NF</td>
<td>This work</td>
</tr>
<tr>
<td>CI170</td>
<td>hisA1 uraA1 strA1 abs-170 SCP1 − SCP2 − Pgl +</td>
<td>This work</td>
</tr>
<tr>
<td>CI1701b</td>
<td>Same as CI170 but NF</td>
<td>This work</td>
</tr>
<tr>
<td>CI75</td>
<td>hisA1 uraA1 strA1 abs-175 SCP1 − SCP2 − Pgl +</td>
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</tr>
<tr>
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<td>proA1 cysA15 argA1 abs-175 NF</td>
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<td>CI752b</td>
<td>hisA1 uraA1 strA1 abs-175 NF</td>
<td>This work</td>
</tr>
<tr>
<td>C246</td>
<td>hisA1 uraA1 strA1 abs-246 SCP1 − SCP2 − Pgl −</td>
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</tr>
<tr>
<td>C252</td>
<td>hisA1 uraA1 strA1 abs-252 SCP1 − SCP2 − Pgl −</td>
<td>This work</td>
</tr>
<tr>
<td>C576b</td>
<td>hisA1 uraA1 strA1 abs-576 SCP1 − SCP2 − Pgl +</td>
<td>This work</td>
</tr>
<tr>
<td>C5761b</td>
<td>hisA1 uraA1 strA1 abs-576 NF</td>
<td>This work</td>
</tr>
<tr>
<td>C604f</td>
<td>hisA1 uraA1 strA1 act red SCP1 − SCP2 − Pgl +</td>
<td>This work</td>
</tr>
</tbody>
</table>

| **Phages**               |                         |                     |
| φC31 KC900               | c + battP actI insert, Thio' Vio' | K. Chater (4) |
| φC31 KC902               | c + battP red insert, Thio' Vio' | K. Chater (12) |

| **Plasmids**             |                         |                     |
| pIJ702                   | Thio' Mel +             | 21                  |
| pAT107                   | pIJ702 (21) and 2.7-kb SphI actII-ORF4 insert, Thio' | This work |

* Abbreviations: SCP1, S. coelicolor plasmid 1 (35); SCP2, S. coelicolor plasmid 2 (3); NF, SCP1 is integrated into the chromosome at 9 o'clock (reviewed in reference 16); Pgl +, φC31 sensitive (8); Vio', vioycin resistant; Thio', thiostrepton resistant; Mel +, melanin production. NF derivatives were obtained as described previously (1, 5).

b Recombinant from a cross with J650.

* Recombinant from a cross with 1514.

f The unpigmented strain C604 was demonstrated to carry act and red mutations by genetic mapping (data not shown).

roform layer was acidified with HCl. The A530 of the now-red chloroform phase was then determined.

For visual assessment of actinorhodin and undecylprodigiosin production on plate medium, strains with disruptions of either the act or red genetic pathway were used to facilitate observation of each pigment. The act and red clusters were disrupted by insert-directed integration of the previously described phage KC900 (4) or KC902 (12), respectively. Both phages also carry the ylxE gene (catechol 2,3-dioxygenase [20]), and transcriptional fusions to the actI or red promoter are formed in the integrants, but this feature of the phages is not relevant to their use in these experiments.

**Mutagenesis and mutant isolation.** Mutagenesis and mutant isolation were carried out as described previously (1).

**Genetic mapping techniques.** Crosses and data analysis were done as described previously (1, 5). Chromosomal recombination was mediated primarily by the plasmid SCP1, integrated at 9 o’clock on the genetic map to give the NF fertility type (17). For the crosses shown in Fig. 3B and C, NF absB derivatives were obtained from NF × SCP1 − crosses; these were identified by their Dag − and methylenomycin-resistant phenotypes, as described previously (1).

**Recombinant DNA techniques.** DNA isolations for plasmid and chromosomal DNA were done as described by Hopwood et al. (15). SphI-digested total DNA from M124 was ligated with SphI-digested, dephosphorylated pIJ702 DNA and used to transform A120 protoplasts, with selection in thiostrepton (50 µg/ml). Protoplast manipulations and transformations were done as described by Hopwood et al. (15).

**RESULTS**

**Isolation and characterization of AbsB − mutants.** A two-step screen for sporulation-proficient, antibiotic-deficient mutants was devised; well-sporulating colonies visibly lacking the antibiotic pigments actinorhodin and undecylprodigiosin were isolated and then tested for loss of calcium-dependent antibiotic and methylenomycin. An initial screen for mutants lacking any detectable antibiotic led to the isolation of absA mutants (1). Double act red mutants such as C604 were also isolated (Table 1). In the course of that screen, mutants with a leaky Abs − phenotype were also observed, but only one was isolated. Such mutants could define additional genes involved in antibiotic regulation, so a second mutant hunt was undertaken with the goal of isolating mutants with a strong but not absolute Abs − phenotype.

In this study, six Abs − mutants were isolated, C576 by UV mutagenesis, and the others (Table 1) by N-methyl-N'-nitro-N-nitosoguanidine mutagenesis. The frequency was about 1 in 10,000 survivors of mutagenesis. The phenotypes of all six were very similar. This screen of approximately 120,000 colonies did not yield any additional absA mutants, a result that was not surprising in light of the previously observed absA isolation frequency of 1 in 200,000 colonies, although that was obtained by UV mutagenesis (1). The mutant colonies sporulated as well as the parent strain on media such as R5 and glucose minimal medium, R2, SY, YEG, and maltose minimal medium. Table 2 shows that the representative mutant strain C120 was unable to produce actinorhodin or undecylprodigiosin on a variety of complex
TABLE 2. Observation of actinorhodin and undecylprodigiosin pigments in absB mutant strains

<table>
<thead>
<tr>
<th>Strain</th>
<th>Lysogen</th>
<th>Antibiotic observed</th>
<th>Pigment* in medium:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>R2</td>
</tr>
<tr>
<td>J1501</td>
<td>KC902</td>
<td>Actinorhodin</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>KC900</td>
<td>Undecylprodigiosin</td>
<td>+</td>
</tr>
<tr>
<td>C120</td>
<td>KC902</td>
<td>Actinorhodin</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>KC900</td>
<td>Undecylprodigiosin</td>
<td>–</td>
</tr>
</tbody>
</table>

* All media are described in Materials and Methods; MM+G and MM+M are minimal medium with glucose or maltose, respectively. To facilitate observation of the actinorhodin and undecylprodigiosin pigments, strains with Abs or act gene disruptions, respectively, were used. Phage KC902 inactivated the undecylprodigiosin pathway, and phage KC900 inactivated the actinorhodin pathway (see Materials and Methods). Observations were made after 6 days of incubation at 30°C.

and minimal media. Actinorhodin and undecylprodigiosin production was quantitated on R5 medium (Table 3), as described in Materials and Methods. The AbsB phenotype (i.e., uncoupling of sporulation from antibiotic synthesis) was leaky in two conditions: (i) on thin R5 plates, the mutant strains sporulated slightly less well than J1501, and (ii) isolated colonies on R5 produced detectable but greatly reduced amounts of actinorhodin and undecylprodigiosin.

Guthrie and Chater (12) have proposed the existence of a phosphate-sensitive, bldA-independent mechanism for activation of undecylprodigiosin synthesis, since they had observed that a bldA mutant could produce undecylprodigiosin on low-phosphate (0.04 mM) glucose minimal medium (or R2, which lacks yeast extract). Accordingly, the Abs- strains were tested on these media. Undecylprodigiosin production in the J1501 parental strain responded to lowering of the phosphate concentration in glucose minimal medium. However, the Abs- mutant strain produced no undecylprodigiosin on the low-phosphate medium than on other media (Table 2).

The level of the third known antibiotic, calcium-dependent antibiotic, was also greatly reduced in the absB isolates, as shown for the representative strain C120 in Fig. 1.

Because the methylenomycin production and resistance genes are carried on the SCP1 plasmid, SCP1+ derivatives of absB mutants were constructed as described in Materials and Methods. The SCP1- parent strain, J1501, was strongly inhibited by methylenomycin produced by its SCP1-carrying derivative (Fig. 2, intersection of A and B). However, the SCP1+ absB mutant strain produced much less antibiotic (Fig. 2, intersection of A and D). Although the C120 strain was impaired in methylenomycin production, it did exhibit methylenomycin resistance comparable to that of J1501 NF (data not shown).

**Genetic analysis of AbsB mutants.** An initial cross between an AbsB+ isolate, C576, and a mapping strain, J650, is shown in Fig. 3A. The recombinant progeny sorted into only two phenotypic classes, Abs- and Abs+, suggesting that a single mutant locus was responsible for the Abs- phenotype. The frequency at which Abs- progeny occurred suggested that the mutant locus was either in the uraA-strA interval or in the mthB-hisA interval. The frequency of cosegregation of the putative absB mutant allele was higher with the mthB+ allele than with the uraA allele. Because the Abs- mutant strain was SCP1-, this type of cross with an SCP1+ (NF) strain resulted in complex allele gradients (36; reviewed in reference 16) among the progeny. Therefore, for more definitive mapping, SCP1+ NF absB mutant derivatives were isolated from crosses with strains 1514 and J650 (see Materials and Methods). The absB NF strain C576 was then crossed against J650 (Fig. 3B) to confirm absB segregation with the mthB locus. In this cross, the gradient of allele frequencies and clear segregation of the absB allele with the mthB+ allele led us to assign a position for the absB mutant locus at approximately 5 o'clock, counterclockwise of mthB. Segregation of the absB allele with the hisA1 allele in this

FIG. 1. Calcium-dependent antibiotic assay of AbsB+ mutant strains. Plugs were cut out of 2-day-old Oxoid nutrient agar plates and placed onto plates of Oxoid nutrient agar with (right plate) and without (left plate) 12 mM Ca(NO₃)₂. Soft agar with and without calcium was seeded with S. aureus. Because actinorhodin also kills S. aureus, an act red double mutant, C604, was included to ensure that the assay conditions were testing calcium-dependent antibiotic activity. Plug A, C604 (Abs+ act red); plug B, J1501 (Abs+ parent); plug C, C120 (Abs-).

### TABLE 3. Measurements of actinorhodin and undecylprodigiosin production in absB mutant and actII-ORF4-stimulated strains

<table>
<thead>
<tr>
<th>Strain</th>
<th>A₅₉₀</th>
<th>A₃₅₀</th>
</tr>
</thead>
<tbody>
<tr>
<td>J1501</td>
<td>0.46</td>
<td>1.1</td>
</tr>
<tr>
<td>C604</td>
<td>0.03</td>
<td>0.02</td>
</tr>
<tr>
<td>C576</td>
<td>0.03</td>
<td>0.09</td>
</tr>
<tr>
<td>C120</td>
<td>0.01</td>
<td>0.17</td>
</tr>
<tr>
<td>C120(pAT107)</td>
<td>2.68</td>
<td>0.03</td>
</tr>
</tbody>
</table>

* Values represent the average of duplicates, extracted from 20 mg of mycelia grown on R5 plates as described in Materials and Methods. Absorbance at 590 nm was the maximum for actinorhodin. Absorbance at 530 nm was the maximum for undecylprodigiosin.
were performed. The mutualities yielded 235 prodigiosin and 576 between data arg+.

The affected locus, possibly related, for the JS501 strain, was responsible for the mutant phenotype. An additional cross was performed to locate the absB mutation abs-120 with respect to the cysD locus, counter-clockwise of mthB. Strain C1201 (Table 1) was crossed with strain J650. Selection for 262 his+ strA1 recombinants yielded 235 mth+, 215 abs-120, and 187 cys+ recombinants. These data indicate that the relative order is mthB-absB-cysD, in the 5 o’clock map location.

Many of the AbsB− mutant strains were also crossed against each other, in a series of pairwise crosses. For example, C1701 (NF abs-170 uraA1 hisA1 strA1) was crossed with C1751 (abs-175 argA1 cysA15 proA1 strA1). Two selective conditions were used to effect reciprocal selections: selection for ura+ arg+ recombinants (cross I) and selection for his+ pro+ recombinants (cross II). Abs+ recombinants occurred at a frequency of 1% for cross I and 0.4% for cross II. Similar crosses yielding similar results were performed with strains carrying the abs-120 and abs-576 mutations. The low recombination frequencies between the abs mutations strongly suggested that they all affected the same locus. Accordingly, we have named the locus absB.

The position of the absB locus was similar to that of another, possibly related, locus, afsB (14). Mutants carrying mutations of the afsB locus were reported to sporulate but produce reduced amounts of the actinorhodin and undecylprodigiosin pigments (14). We compared an afsB mutant strain, BH5, with two absB mutant strains, C120 and C576. BHK and an SCP1+ BHK derivative, BH51 (Table 1), were tested for calcium-dependent antibiotic and methylenomycin production, respectively, in plate tests and were found to produce as much antibiotic as JS501 or JS501 NF (data not shown). Although BH5 was unpigmented on minimal medium with glucose, it produced abundant actinorhodin on RS; undecylprodigiosin production was not tested. In addition, genetic crosses between BH5 and C1201, C5761, and C1752 (Table 1) were performed. With selection for ura+ strA1 recombinants, the progeny were 67, 42, and 45% Pgm+ (pigmented), respectively. These substantial recombination frequencies indicated that absB and afsB were different loci. Because of the plasmid status (Table 1) of the strains in these crosses, they yielded complex allele gradients and were not useful for ordering absB and afsB with respect to each other.

**Effect of multiple copies of the regulatory gene actII-ORF4 on actinorhodin synthesis.** In the course of experiments intended to clone the absB allele (unpublished data), we isolated a plasmid, pAT107, that stimulated copious actinorhodin but not undecylprodigiosin production in strain C120 (Table 3). The plasmid carried a 2.7-kbp SphI insert. The same insert was found in 17 of approximately 20,000 transformants and occurred in both orientations. Restriction mapping of the SphI fragment with PstI and BamHI indicated that it corresponded to a portion of the actinorhodin gene cluster (13, 27) and included the actII-ORF4 promoter and open reading frame (10). The plasmid vector pJL702 (21) replicates to a copy number of 30 to 100 copies per genome. Thus, the multiple copies of actII-ORF4 present in C120 (pAT107) were able to bypass the absB block to actinorhodin synthesis.

**DISCUSSION**

An extensive search for *S. coelicolor* mutants that are sporulation proficient and antibiotic deficient has resulted in identification of a new abs locus at 5 o’clock, absB. Mutations at the absB locus cause a phenotype similar to the previously described absA mutant phenotype (1). Like absA mutations, which map to 10 o’clock (1), the absB mutations affect production of the four known *S. coelicolor* antibiotics. These antibiotics, undecylprodigiosin, actinorhodin, methylenomycin, and calcium-dependent antibiotic, are produced by gene products encoded in gene clusters located at 5, 6, 9, and 11 o’clock, respectively (15).

The absA and absB mutants can be phenotypically distinguished in two ways: absB mutants are somewhat leakier for antibiotic production on some complex media and do not accumulate spontaneous suppressive mutations, as do absA mutants (31a).

Evidence that the absB mutants fail to produce antibiotics because they are defective in antibiotic gene expression comes from the observation that multiple cloned copies of the actII-ORF4 gene bypass the block to actinorhodin synthesis. Because actII-ORF4 is a regulatory gene for act expression (reviewed in reference 10), this result argues that the absB mutants are metabolically and biosynthetically competent to produce actinorhodin but fail to do so because the absB mutation prevents adequate expression of the actII-ORF4 gene. A similar bypass effect of multicopy actII-ORF4 in bldA, bldB, bldD, bldG, and bldH mutants has been described (31).

The frequency at which absB mutations occurred is similar to the frequency for occurrence of loss-of-function mutations in other *S. coelicolor* genes. In addition, the six mutant strains all showed very similar phenotypes. These

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**FIG. 2.** Methylenomycin assays of AbsB− mutant strain. See Materials and Methods for strain construction and growth conditions. Streaks: A, JS501; B, JS501 NF; C, C120; D, C1201 NF.
FIG. 3. Mapping of *abs*-576. (A) Strain C576 was crossed with strain J650, with selection as indicated by triangles; 52% of the his+ strA1 recombinants were abs-576. Allele frequencies among the recombinants are indicated, and segregation of abs-576 with uraA and mthB is tabulated. (B) Strain C5761 was crossed with J650, with selection for ura+ strA1 recombinants; 76% were abs-576. Segregation of abs-576 with hisA and mthB is tabulated. Although *cysD18* was not tested in this cross, a later cross involved the *cysD* locus (see text). (C) Strain C5762 was crossed with 1514, with selection for his+ strA1 recombinants; 83% were abs-576. Segregation of abs-576 with uraA, cysA, and argA is tabulated. Two recombinants were not scored for arg.
observations suggest that the \textit{absB} mutants have suffered a loss or reduction of \textit{absB}\textsuperscript{*} activity and the \textit{absB}\textsuperscript{*} allele is therefore likely to encode a required function for normal antibiotic gene expression.

The results discussed here lead us to propose a working model for regulation of the four known \textit{S. coelicolor} antibiotics. In this model, the four antibiotic gene clusters are members of a regulon that is subject to at least two levels of regulation. The phenotype of \textit{bld}\textsuperscript{d} mutants suggests that one aspect of regulation involves the \textit{bld} genes in coordinate regulation of antibiotic genes with early sporulation genes. The second aspect of regulation would involve specific regulation of the putative antibiotic gene regulon by genes such as \textit{absA} and \textit{absB}. At least for \textit{absB}\textsuperscript{*}, such regulation would be at the level of accumulation of at least some of the antibiotic gene transcripts. \textit{absB} regulation might be mediated through regulation of \textit{actII-ORF4} for \textit{act} genes and through \textit{redD} for \textit{red} genes. Pathway-specific regulatory genes for calcium-dependent antibiotic and methylenomycin, comparable to \textit{actII-ORF4} and \textit{redD}, may also exist and be regulated by \textit{absB}. At least three additional genes have been implicated in antibiotic regulation, but in contrast to the \textit{abs} mutations, mutations of these additional genes do not exert strong effects on all four \textit{S. coelicolor} antibiotics. Mutations of the \textit{afsB} locus caused a reduction in actinorhodin and undecylprodigiosin production (14), in a medium-dependent fashion. Mutant alleles of the \textit{afsR} gene (19), which resulted from disruption (but not deletion) of the \textit{afsR} open reading frame, caused reduction but not elimination of actinorhodin production (19); effects on the other antibiotics were not described. Mutations of the \textit{abaA} gene abrogate actinorhodin production and strongly decrease undecylprodigiosin and calcium-dependent antibiotic production but do not significantly affect methylenomycin production (11).

Further examination of the mechanisms by which the \textit{abs}, \textit{aba}, and \textit{afs} genes exert their regulatory effects, and clarification of the conditions under which coordinate genetic regulation of \textit{S. coelicolor}'s antibiotics occurs, should contribute to definition of the antibiotic regulatory network in \textit{S. coelicolor} and lead to increased understanding of antibiotic regulation in other members of the \textit{Actinomycetes}.

ACKNOWLEDGMENTS

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REFERENCES


