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New Loci Required for *Streptomyces coelicolor* Morphological and Physiological Differentiation†

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Streptomyces coelicolor **colonies differentiate both morphologically, producing aerial spore chains, and physiologically, producing antibiotics as secondary metabolites. Single mutations, which block both aspects of differentiation, define *bld* (bald colony) genes. To identify new *bld* genes, mutagenized colonies were screened for blocks in the earliest stage of sporulation, the formation of aerial mycelia, and blocks in antibiotic synthesis. The mutations in 12 mutants were mapped; in each strain, the pleiotropic phenotype was due to a single mutation. Seven of the strains contained mutations in known *bld* loci, *bldA* and *bldB*. Three strains contained mutations in a new locus, *bldG*, and two contained mutations in another new locus, *bldH*. Like the previously defined *bldA* mutants, the *bldG* and *bldH* mutants were developmentally blocked on glucose. On a variety of carbon sources whose utilization was subject to glucose repression, the developmental blocks were partially relieved for *bldG* (and *bldA*) mutants and fully relieved for *bldH* mutants. These results are compatible with an hypothesis which suggests that there are two alternative controls on *S. coelicolor* differentiation, one of which is glucose repressible.**

Streptomycetes, mycelial soil bacteria, possess two characteristics which make them attractive procaryotes for study: they develop into complex, multicellular, sporulating structures and they produce a vast array of medically and agriculturally important secondary metabolites, such as antibacterial, antitumor, antifungal, and antiparasitic drugs. The latter characteristic has made streptomycetes invaluable commercially. However, the regulatory influences governing morphological and physiological differentiation remain very poorly understood. Recently, answers to basic questions about streptomycete developmental regulation have become accessible to molecular genetic analysis (3).

When observed on solid media, the growth of a streptomycete colony is an example of a primitive multicellular developmental cycle. Early in the life of a streptomycete colony, growth occurs as expansion at hyphal tips (1). The colony continues its branching hyphal growth for approximately 3 days at 30°C. Then, aerial branches develop and secondary metabolism (the production of antibiotics from the intermediates of primary metabolism) commences. The synchrony with which these two processes occur is striking; one can first observe pigmentation (several antibiotics are pigmented) just when aerial mycelia appear. Each aerial hypha grows into a multinucleoid filament which eventually subdivides into haploid spores.

In *Streptomyces coelicolor*, mutants have been isolated which fail to form aerial mycelia. These mutants carried mutations named *bld* (for bald) because the colonies are smoother than the wild-type velvety colonies (17). These *bld* mutants were also pleiotropically blocked for antibiotic synthesis. Thus, the Bld$^-$ phenotype offers a convenient visual selection for mutants blocked early in differentiation.

Fourteen *bld* mutants have been mapped to four loci named *bldA*, *bldB*, *bldC*, and *bldD* (3, 17). Alleles of *bldA* respond to variations in carbon source; they are bald when grown on glucose but form sporulating aerial mycelia when grown on mannitol or a variety of other carbon sources (17). Wild-type *S. coelicolor* sporulates normally on glucose medium. In these mutants, antibiotic production is deficient on either carbon source. Alleles of *bldB* are unconditionally blocked, with respect to carbon source, in both their morphological and physiological development. However, *bldB* mutants are capable of some antibiotic production when grown on complex media (3). A *bldC* allele responds similarly to *bldA* with respect to aerial mycelium formation but fails to produce only one of the normal four antibiotics, and a *bldD* allele has a phenotype similar to that of *bldA*. Both the *bldA* gene and the *bldB* gene have been cloned (18; J. Piret, personal communication).

Further isolation and genetic analysis of mutants blocked early in differentiation promises to contribute to understanding of the regulation of the transition from the vegetative to the differentiated colony. In this study, new mutants blocked at an early stage in differentiation were isolated. The mutations in 12 of these were mapped; and 5 were found to help define two new loci which affect both sporulation and antibiotic production.

MATERIALS AND METHODS

Bacterial strains. All strains used in the genetic analysis were derivatives of *S. coelicolor* A3(2) (Table 1). *Streptomyces lividans* 66 was used for phage propagation (15).

Phages. Complementation tests were carried out by using derivatives of the temperate phage ϕC31 (Table 1). ϕC31 KC603 carries a cloned 5.6-kilobase insert which includes the *bldA*$^+$ allele (18); ϕC31 KC628 carries a cloned 4-kilobase insert which includes the *bldB*$^+$ allele (J. Piret, personal communication).

Media and culture techniques. R5 medium, a complex buffered medium containing 10% sucrose, 1% glucose, 0.5% yeast extract, and 0.01% Casamino Acids (Difco Laboratories, Detroit, Mich.) (10) was used for crosses; minimal medium (10) was used for analyzing nutritional markers. For phenotypic analysis of *bld* mutants, glucose was replaced by

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TABLE 1. Strains of *S. coelicolor* and phage used in this study

Strain	Relevant genotype[a]	Source
Bacteria		
J650	*cysD18 mthB2* NF	K. Chater
J668	*cysD18 mthB2 bldA39* NF	K. Chater (17)
J669	*cysD18 mthB2 bldB43* NF	K. Chater (17)
1514	*nicA1 proA1 cysA1 argA1 uraA1* NF	K. Chater
1258	*argA1 cysC3 hisC9 proA1 strA1 uraA1* NF	K. Chater
J1501	*hisA1 uraA1 strA1* SCP1$^-$ SCP2$^-$ Pgl$^-$	K. Chater (5)
C101J	*cysD18 mthB2 bld-101J* NF	This work
C103	*hisA1 uraA1 strA1* SCP1$^-$ SCP2$^-$ Pgl$^-$ *bld-103*	This work
C107	*hisA1 uraA1 strA1* SCP1$^-$ SCP2$^-$ Pgl$^-$ *bld-107*	This work
C109	*hisA1 uraA1 strA1* SCP1$^-$ SCP2$^-$ Pgl$^-$ *bld-109*	This work
C112	*hisA1 uraA1 strA1* SCP1$^-$ SCP2$^-$ Pgl$^-$ *bld-112*	This work
C181	*hisA1 uraA1 strA1* SCP1$^-$ SCP2$^-$ Pgl$^-$ *bld-181*	This work
C186	*hisA1 uraA1 strA1* SCP1$^-$ SCP2$^-$ Pgl$^-$ *bld-186*	This work
C209	*hisA1 uraA1 strA1* SCP1$^-$ SCP2$^-$ Pgl$^-$ *bld-209*	This work
C216	*hisA1 uraA1 strA1* SCP1$^-$ SCP2$^-$ Pgl$^-$ *bld-216*	This work
C249	*hisA1 uraA1 strA1* SCP1$^-$ SCP2$^-$ Pgl$^-$ *bld-249*	This work
C301	*hisA1 uraA1 strA1* SCP1$^-$ SCP2$^-$ Pgl$^-$ *bld-301*	This work
C309	*hisA1 uraA1 strA1* SCP1$^-$ SCP2$^-$ Pgl$^-$ *bld-309*	This work
Phage		
φC31 KC603	c^+ Δ*attP* and *bldA*$^+$ insert, Vio^r	K. Chater (18)
φC31 KC628	c^+ Δ*attP* and *bldB*$^+$ insert, Vio^r	J. Piret

[a] Abbreviations: SCP1, *S. coelicolor* plasmid 1 (22); SCP2, *S. coelicolor* plasmid 2 (2); NF, SCP1 is integrated into the chromosome (see text); Pgl$^-$, φC31 sensitive (5); Vio^r, viomycin resistance.

other carbon sources at a final concentration of 1.0%. For transductions, suspensions of mycelial fragments were grown in yeast extract-malt extract medium supplemented with 34% sucrose and 5 mM $MgCl_2$ (10) and the appropriate auxotrophic requirements. Cultures were shaken in baffled flasks (50 ml in 250-ml flask) for 5 days at 30°C and then concentrated 10 times by centrifugation. Streptomycin was used at a concentration of 10 μg/ml; viomycin (gift of The Upjohn Co., Kalamazoo, Mich.) was used at a final concentration of 30 μg/ml. Culture growth and spore preparations were as described by Hopwood et al. (10).

Mutagenesis and mutant isolation. Spores of J650 and J1501 (Table 1) were irradiated with UV light (254 nm) to a survival rate of 0.1 to 1% (10) and then plated at 1,000 colonies per plate, on R5, glucose minimal, or maltose minimal medium. After incubation at 30°C for 5 days, colonies were visually screened for mutant phenotypes. Under these growth conditions, wild-type *S. coelicolor* colonies are abundantly covered with gray aerial mycelia and the substrate mycelia and surrounding agar are deep blue due to production of the pigmented antibiotic actinorhodin. An additional antibiotic, the red-pigmented undecylprodigiosin, is also produced in the substrate mycelium (20). Colonies which failed to either develop pigmentation or develop aerial mycelia, remaining bald, arose at a frequency of about 10^{-4} and were isolated and purified. *bld-101J*, *-107*, *-109*, and *-112* were isolated from R5 medium; *bld-103* and *-181* were isolated from glucose minimal medium; and *bld-186*, *-209*, *-216*, *-249*, *-301*, and *-309* were isolated from maltose minimal medium. Screenings on maltose medium were carried out in an attempt to find mutants that were blocked in the development on all carbon sources.

Microscopy. Impression preparations of colony surfaces were examined by phase-contrast microscopy (4).

Genetic mapping techniques. Crosses and data analysis were carried out as described by Hopwood and Chater (9). Chromosomal transfer was mediated primarily by the plasmid SCP1. The designation NF refers to the integrated state of SCP1, at 9 o'clock on the genetic map. In an NF × SCP1$^-$ cross (for example strain J650 × J1501 or a J1501 derivative), all of the recombinants inherit the integrated plasmid (9). The frequency of other markers donated by the NF parent falls off, in both directions, with increasing distance from the SCP1 integration site. For example, in the cross shown in Fig. 1, 1514 NF × C301 SCP1$^-$, greater than 90% of the spore progeny will be *hisA1 strA1* if the parental cultures are well mixed. In practice, I plated such crosses on streptomycin-containing media to exclude any 1514 parental genomes. Parental *bld* genomes were excluded because spore progeny were analyzed; because *bld* strains do not sporulate, *bld* spores will arise only from the mating of mycelia. We also analyzed *hisA*$^+$ *strA1* recombinants from NF × SCP1$^-$ crosses. For example, in the cross shown in Fig. 2, both parental genomes were excluded and recombination in the lower arc could be assessed. Some crosses were between two NF strains, for example the C101J NF × 1258 NF cross shown in Fig. 3A. In this type of cross, all regions of the chromosome are donated with equal frequency (9).

Transductions and complementation tests. Spot transductions of *bld* mutants with KC603 and KC628 were carried out as described previously (18) to test for restoration of the Bld$^+$ phenotype. For allelism tests, phages carrying putative new *bldA* or *bldB* alleles were obtained from Bld$^-$ homogenotic KC603 or KC628 transductants. These phages were tested for failure to complement a standard *bldA* mutant (strain J668) or *bldB* mutant (strain J669), essentially as described previously (18).

RESULTS

Isolation and phenotypic characterization of Bld mutants. A collection of mutants which failed to develop normal aerial mycelia was isolated as described above. These were characterized as to their ability to produce antibiotics. Twelve mutants which were severely blocked in both processes were chosen for further analysis. As this pleiotropic phenotype was similar to that of previously isolated *S. coelicolor* developmental mutants, which carried mutations given the name *bld* (17), these new mutations were also named *bld*. These new *bld* mutants were grouped into four distinct phenotypic classes (Table 2).

S. coelicolor A3(2) normally produces four antibiotics: actinorhodin (19, 24), undecylprodigiosin (20), methylenomycin (13, 23), and calcium-dependent antibiotic (11, 14). These 12 mutants were all pleiotropically blocked for synthesis of at least the first three of these antibiotics (calcium-dependent antibiotic synthesis was not assessed). Antibiotic synthesis was also assessed on media containing glucose or

TABLE 2. Phenotypes of *bld* mutants

Class	*bld* mutation	Antibiotic synthesis[a]	Aerial mycelium development[b]	Surface morphology[c]
I	*bld-209* *bld-216* *bld-301* *bld-309*	Absent	Carbon source dependent[d]	Soft, sculptured, yellowish
II	*bld-112* *bld-186* *bld-249*	Absent	Absent	Hard, smooth
III	*bld-101J* *bld-103* *bld-107*	Absent	Carbon source dependent[d]	Soft, smooth
IV	*bld-109* *bld-181*	Carbon source dependent[d]	Carbon source dependent[d]	Hard, fragmenting

[a] Colonies failing to produce either actinorhodin or undecylprodigiosin lacked pigmentation. Methylenomycin synthesis and resistance genes are all located on the plasmid SCP1 (13). Methylenomycin synthesis was assayed by testing for antibiotic activity in SCP1$^+$ *bld* mutants when cross-streaked against SCP1$^-$ J1501.

[b] Aerial hyphae were easily visible with the naked eye or with a stereo microscope. Mature spores were visualized by phase-contrast microscopy (see text).

[c] Hard colonies did not break up easily with an inoculating needle.

[d] Carbon source dependence means colonies failed to develop normally on glucose or cellobiose but did develop on arabinose, galactose, glycerol, maltose, or mannitol.

the alternative carbon sources listed in footnote *d* of Table 2. Mutants of classes I, II, and III failed to synthesize antibiotics on any of the carbon sources tested. However, mutants of class IV were restored to normal antibiotic production on carbon sources such as arabinose, galactose, glycerol, mannitol, or maltose.

Aerial mycelium formation normally began after 3 days of incubation at 30°C, at the same time antibiotic synthesis began. The immature aerial hyphae were white and turned gray as the spore chains matured. All of the 12 *bld* mutants failed to initiate aerial hyphal growth. However, this aspect of colony differentiation, like antibiotic synthesis, was also carbon source dependent in many of the mutants. Mutants in classes I, III, and IV developed normally sporulating aerial mycelia on the carbon sources other than glucose or cellobiose.

The phenotypes of the class I mutants were essentially identical to the reported *bldA* phenotype (17). Colonies of these mutants were somewhat larger than wild-type colonies and developed a very wrinkled surface on glucose media. When grown on carbon sources such as maltose, mannitol, and galactose, sporulating aerial hyphae formed, although these were much sparser than in the wild type. The *bldA* mutants are not completely blocked for aerial hyphal development when grown on glucose media; rather, they produce hyphae which are deficient in cell wall galactose and which lie prostrate on the colony surface (3).

Class II mutants had a phenotype very similar to that of the *bldB* mutants (17). Like *bldB* mutants, their developmental blocks were unconditional with respect to carbon source.

The class III mutant phenotype was similar to the class I phenotype, with respect to carbon source-dependent differentiation. However, these colonies were of normal size and did not develop the extremely wrinkled surface characteristic of class I mutants. When grown on glucose minimal medium, these colonies developed very sparse aerial mycelium. When colony impression preparations were viewed by phase-contrast microscopy, no spores were visible.

The class IV mutant phenotype was novel; both morphological and physiological differentiations were deficient on glucose or cellobiose but normal on the alternative carbon sources.

Mapping of *bld* mutants. (i) Class I. Because the class I mutants all had phenotypes very similar to those reported for *bldA* mutants, mapping experiments were performed to determine whether the responsible mutations mapped to the *bldA* locus, which is close to and counterclockwise of *cysA* (17) (Fig. 5). In Fig. 1 is presented the mapping scheme for *bld-301*. Based on the allele frequencies, *bld-301* was close to the 9 o'clock position, either clockwise or counterclockwise of SCP1. Because the *bld-301* allele segregated with the *cysA*$^+$ allele, the position near *cysA* was chosen. Other crosses were performed to test segregation of *bld-301* with the *uraA1* allele; segregation was independent (data not shown). The other class I mutants, *bld-209*, *-216*, and *-309* were also crossed with 1514, and similar data and map locations were obtained (see Table 3). Therefore, based on phenotypic similarities and map position, it seems likely that the class I mutants are new alleles of *bldA*.

To determine whether these class I mutants were indeed new *bldA* alleles, they were tested for complementation with a *bldA*$^+$ allele cloned (18) into a vector based on the temperate phage φC31. The phage strain KC603 (Table 1) was used to transduce *bld-301*, *bld-209*, *bld-216*, and *bld-309* to Vio^r as described above. In all four mutants, KC603 restored not only normal aerial mycelium formation and subsequent sporulation but also pigmented antibiotic production. Therefore, these mutants are very likely alleles of *bldA*. To determine whether these mutants were alleles of *bldA* or of a gene neighboring *bldA* on the cloned insert, allelism tests were performed by the method described by Piret and Chater (18), as described above. Derivatives of KC603 carrying each of the *bldA* alleles were obtained and

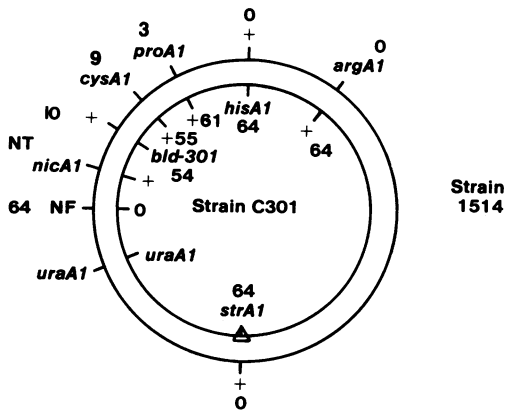


FIG. 1. Mapping of *bld-301*. Strain C301 (inner circle) was crossed with strain 1514 (outer circle). Selection for *strA1* (triangle) excluded *bld*$^+$ parental genomes. Numbers around the circles indicate allele frequencies among the 64 recombinants scored. Segregation of the *bld* allele with respect to the *cysA*$^+$ allele is tabulated. NT, Not tested.

TABLE 3. Segregation of *bld* mutations with auxotrophic markers

Class and mutation	% *strA1 his*$^+$ recombinants which were *bld*[a]	Probability of independent segregation with unselected alleles[b]:			
		uraA[c]	*mthB*[c]	*argA*[d]	*cysA*[d]
Class I					
bld-209	52	NT[e]	>0.5	NT	<0.001
bld-216	26	>0.5	>0.5	NT	<0.001
bld-301	25	NT	>0.5	>0.5	<0.001
bld-309	16	0.15	>0.5	NT	<0.001
Class II					
bld-112	94	>0.5	<0.001	NT	NT
bld-186	95	>0.5	<0.001	NT	NT
bld-249	93	>0.5	<0.001	NT	NT
Class III					
bld-101J	67	<0.001	>0.5	0.03	NT
bld-103	49	<0.001	>0.5	0.3	>0.5
bld-107	20	<0.001	>0.5	>0.5	>0.5
Class IV					
bld-109	96	0.16	<0.001	0.01	NT
bld-181	97	0.1	<0.001	0.08	NT

[a] Selections are explained in the text.
[b] χ^2 was calculated by the method described by Hopwood et al. (10) from tabulations of genotype frequencies, as shown in Fig. 1.
[c] From a cross of the *bld* strain with strain J650. For *bld-101J* the data were obtained from crosses with strains J1501 and 1258.
[d] From a cross of the *bld* strain with strain 1514.
[e] NT, Not tested.

were used to transduce J668, which carries the *bldA39* allele (17), to Vio^r. The large majority of the transductants in each case were Bld$^-$, indicating that the new alleles are allelic with *bldA39*. Therefore they have been named *bldA209*, *bldA216*, *bldA301*, and *bldA309*.

(ii) Class II. Since the class II phenotypes were very similar to the phenotype reported for *bldB* mutants (17), mapping experiments were performed to determine whether the mutations were alleles of *bldB*, which maps at 5 o'clock (17) (see Fig. 5). In Fig. 2 is presented the mapping scheme for *bld-249*. The frequency at which the *bld-249* allele was recovered among recombinants indicated that it was very close to *strA1*. Segregation was independent with respect to *uraA1*, but not with respect to *mthB*. Therefore, the position counterclockwise to *strA* was chosen for the *bld-249* mutation. Very similar data were obtained for *bld-112* and *bld-186* (Table 3). Thus, the class II mutants were close to *bldB*. Complementation tests with the ϕC31 *bldB*$^+$ clone KC628 (Table 1) were performed as described earlier for the *bldA* mutants. Results of these tests (data not shown) indicate that *bld-112* and *bld-186* are allelic to *bldB43*; these were therefore named *bldB112* and *bldB186*.

The result with *bld-249* was less clear. Vio^r *bld-249* lysogens were essentially Bld$^-$, but sporulated very sparsely and weakly after 7 to 10 days at 30°C. They never produced the pigmented antibiotics. Therefore, it is unlikely that *bld-249* is an allele of *bldB*.

(iii) Class III. A position clockwise of *uraA* was chosen for *bld-101J* because it segregated with the *uraA*$^+$ allele but independently of the *argA*$^+$ allele in the cross shown in Fig. 3. Moreover, in a second cross (Fig. 3B) *bld-101J* was shown to be donated at a very high frequency in an NF × SCP1$^-$ cross, and it again showed linkage to *uraA*.

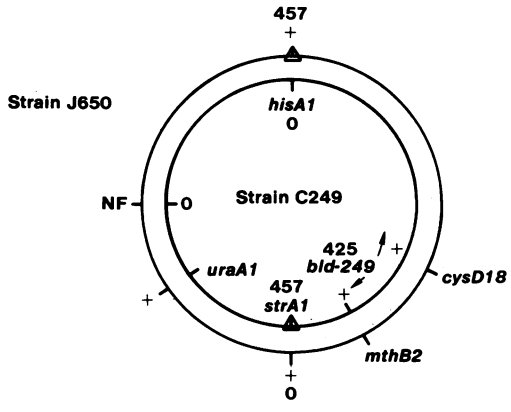
In crosses comparable to that shown in Fig. 3B, the *bld-103* and *bld-107* mutations were also mapped to a position near *uraA*. Linkage to other markers around the map was also tested (Table 3); *bld-103* and *bld-107* were linked only to *uraA1*. For each mutant, all of the phenotypes associated with the original isolates cosegregated in all *bld* recombinants, suggesting that the failure to produce antibiotics and the failure to produce aerial mycelia were due to the same mutation.

It remains to be established whether *bld-101J*, *bld-103*, and *bld-107* are allelic. Allelism tests are difficult to perform in *Streptomyces* species because the only system for establishing stable merodiploids requires that the wild-type allele first be cloned in ϕC31 (18). However, when NF strains of *bld-101J* and *bld-103* or *bld-101J* and *bld-107* were crossed against each other in nonselective crosses, no wild-type recombinants occurred, suggesting that they are closely linked. Therefore, these mutations are tentatively named *bldG101J*, *bldG103*, and *bldG107*. When a *bldG*$^+$ clone is obtained, allelism tests and fine-structure mapping of the *bldG* locus will be possible.

(iv) Class IV. For mapping the two representatives of class IV, the scheme illustrated for *bld-109* in Fig. 4 was used. Most of the *strA1 hisA*$^+$ recombinants were *bld-109*, and the *bld-109* allele segregated with the *mthB*$^+$ allele. To confirm that *bld-109* is counterclockwise of *mthB*, an NF *bld-109* recombinant was picked and crossed with strain J650 (Fig. 4B). Similar data were obtained for the *bld-181* mutation (Table 3). For both of these mutations, *bld* recombinants retained all of the phenotypes of the original mutant isolates.

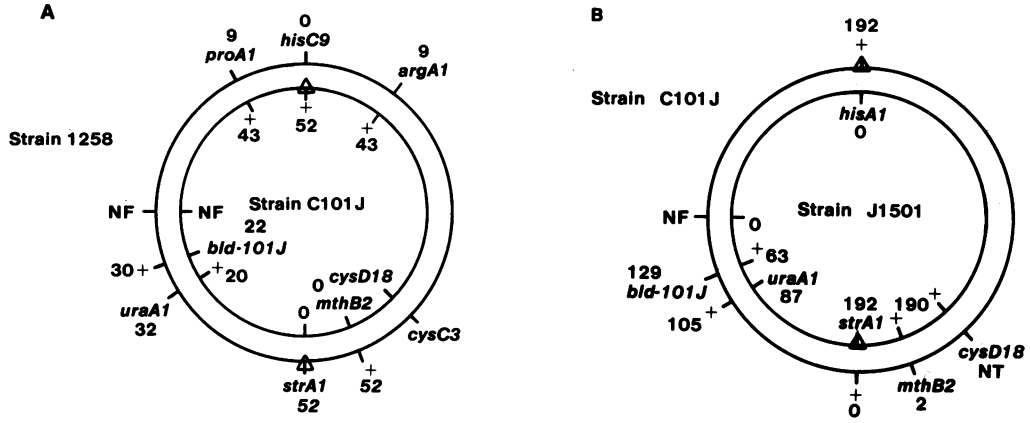
Because *bld-109* and *bld-181* mapped to roughly the same location as the *bldB* locus, complementation tests with the *bldB*$^+$ ϕC31 clone KC628 were performed. Not surprisingly, as the class IV phenotype is distinct from the *bldB* phenotype, Vio^r transductants of *bld-109* and *bld-181* remained Bld$^-$. Therefore, these two mutations are not alleles of *bldB*.

When NF strains of *bld-109* and *bld-181* were crossed against each other in nonselective crosses, no wild-type



	ura$^+$	*uraA1*		*mth*$^+$	*mthB2*
bld$^+$	28	4	*bld*$^+$	9	23
bld-249	62	29	*bld-249*	77	1
	p= 0.015			p< 0.001	

FIG. 2. Mapping of *bld-249*. Strain C249 was crossed with strain J650. Selection (triangles) excluded parental genomes but allowed analysis of recombination in the lower arc. A total of 93% of the *hisA*$^+$ *strA1* recombinants were *bld-249*. Segregation of *bld-249* with respect to the *uraA1* and *mthB*$^+$ alleles is tabulated.



	ura^+	uraA1	arg^+	argA1
bld^+	0	30	28	2
bld-101J	20	2	15	7

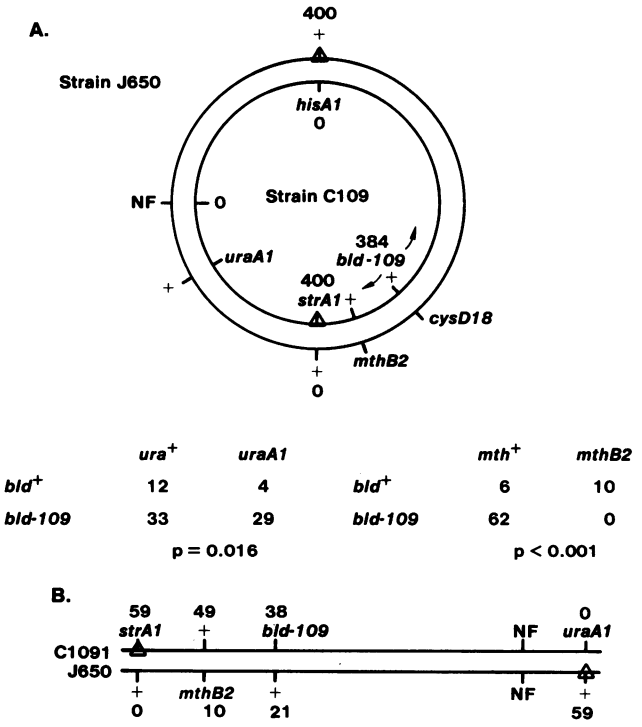
p < 0.001 p = 0.03

	ura^+	uraA1
bld^+	3	60
bld-101J	102	27

p < 0.001

FIG. 3. Mapping of *bld-101J*. (A) Strain C101J was crossed with strain 1258, with selection (triangles) for $hisC^+$ *strA1*. The numbers indicate allele frequencies for the 52 recombinants tested. Segregation of *bld-101J* with *uraA* and with *argA* is tabulated. (B) Strain C101J was crossed with strain J1501, with selection as indicated by triangles. Segregation of *bld-101J* with *uraA* is tabulated.

recombinants were obtained, suggesting that they are closely linked. Again, in the absence of definitive complementation tests, these mutations, which define a new locus, are tentatively named *bldH109* and *bldH181*.



	ura^+	uraA1		mth^+	mthB2
bld^+	12	4	bld^+	6	10
bld-109	33	29	bld-109	62	0

p = 0.016 p < 0.001

FIG. 4. Mapping of *bld-109*. (A) Strain 109 was crossed with strain J650, with selection as indicated by triangles. A total of 96% of the $hisA^+$ *strA1* recombinants were *bld-109*. Segregation of *bld-109* with *uraA* and *mthB* is tabulated. (B) A *bld-109* $hisA^+$ *strA1 uraA1* recombinant, C1091, from the cross described above for panel A, was crossed with strain J650 with selection for *strA1* $uraA^+$. The allele frequencies in 59 recombinants are indicated.

DISCUSSION

In this report I have presented an analysis of *S. coelicolor* mutants which are pleiotropically blocked in aerial mycelium formation and antibiotic synthesis. Of the 12 mutants reported on here, at least two new loci named *bldG* and *bldH* are defined in 5 of the mutants.

The *bldG* locus maps at approximately 9 o'clock, between *uraA* and the integration site of SCP1 (Fig. 5). This region is relatively silent, containing only two other loci, *hut* (histidine utilization) and *dagA* (agarose degradation) (10). Chater and Hopwood (6) previously reported mapping a *bld* mutation to the 9 o'clock position. It is possible that these new mutants may define the same locus. Determination of the number of genes defined by this collection of mutations will await fine-structure genetic analysis and cloning of the genes.

The *bldH* locus mapped at approximately 5 o'clock (Fig. 5). Several other genes which are important to development mapped in this region, including *bldB*. The *bldH* mutants were distinctive in phenotype from *bldB* mutants, whereas

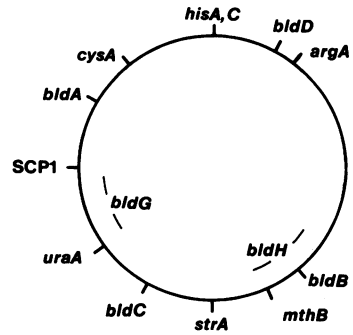


FIG. 5. Linkage map of *S. coelicolor* A3(2) showing, on the outside of the circle, previously known *bld* loci and mapping markers referred to in the text. Inside of the circle are the positions of the *bldG* and *bldH* loci.

bldH mutant differentiation was carbon source dependent, *bldB* mutant differentiation was blocked on all carbon sources. Furthermore, a cloned *bldB*$^+$ fragment did not complement the *bldH* mutants. Based on these criteria, the mutants have been assigned to a new locus, *bldH*. Previously described developmental mutations in other genes that map in this area (4, 7, 12) have phenotypes distinctly different from those of *bldH* mutations, so *bldH* is unlikely to be synonymous with any of these.

Of the remaining *bld* isolates reported on here, four mapped to the *bldA* locus and two, possibly three, mapped to the *bldB* locus. The complementation results for *bld-249* remain to be clarified. It is possible that the cloned *bldB*$^+$ allele was truncated, resulting in lysogens carrying a *bld-249* allele and a truncated *bldB* allele, a combination which partially restored sporulation. However, a 1.2-kilobase fragment internal to the 4-kilobase insert of KC628 displays *bldB*$^+$ activity (J. Piret, personal communication), which argues against the likelihood of a truncated *bldB* allele in KC628. Another possibility was that *bld-249* was a dominant allele of *bldB*. However, if *bld-249* were an allele of *bldB*, it should have been possible to recover Bld$^+$ Vio^r lysogens arising through homogenotization of the mutant and wild-type alleles. Alternatively, *bld-249* may be a mutation that is roughly in the same region as *bldB*, which is partially suppressed by two copies of *bldB*$^+$. Cloning of the *bld-249* allele should resolve this issue.

In an earlier study of *bld* mutants, Merrick (17) reported on 12 mutants. Of these, four mapped to the *bldA* locus and six mapped to the *bldB* locus. In this study, a preponderance of *bldA* and *bldB* mutants was also found. The especially high percentage of isolates which were *bldA* may result from the striking *bldA* colony morphology. Because *bldA* colonies are larger than the wild type and are yellowish, they stand out on a plate crowded with gray, fuzzy colonies. Thus, other mutants may be underrepresented because they are relatively overlooked in visual screens for mutants. No new *bldC* or *bldD* alleles were isolated in this study, but other, new loci were identified. Thus, it seems likely that genetic saturation of the *bld* genes has not yet been achieved and that additional *bld* genes may exist.

It is striking that many of the *S. coelicolor* mutants, including *bldA* and *bldD* as well as *bldG* and *bldH*, exhibited carbon source-dependent differentiation. Only the mutants with the *bldB* locus were blocked in differentiation, irrespective of carbon source. A clear explanation for the carbon source effect has not emerged. A suggested explanation is that *bldA* functions in one of two pathways which can lead to normal development; the second pathway may be glucose repressible and may be involved in morphological development but not in antibiotic synthesis (3). The phenotypes of *bldH* mutants suggest a somewhat different model. By this model, there are two alternative pathways which control differentiation, and the second pathway, a glucose-repressible pathway, is involved not only in morphological development but also in antibiotic synthesis. In this model, *bldADGH* would function in the pathway which is not glucose repressible. In the wild type, this pathway functions regardless of carbon source, but in a strain which has a mutation in one of these genes, only the glucose-repressible pathway is functioning, so that development occurs only on poor carbon sources. In this model, *bldA*, *bldD*, and *bldG*, but not *bldH*, would also be required for some function specific to antibiotic synthesis. Thus, *bldA*, *bldD*, and *bldG* mutants fail to develop on glucose but are able to develop morphologically on alternative carbon sources. In contrast, *bldH* mutants also failed to develop normally on glucose, but were completely unblocked on alternative carbon sources. The glucose repression system in *S. coelicolor* is not understood, but progress has been made in understanding the role of glucose repression in primary metabolism. Mutants which are unresponsive to glucose repression have all been found to be defective in glucose kinase (8, 21).

Some of the *S. coelicolor bld* genes may be functionally similar to *spoO* genes of *Bacillus subtilis*, which are also pleiotropically blocked in sporulation-associated characteristics. These genes are hypothesized to encode proteins which (i) assess the nutritional state of the cell; (ii) transduce such information to regulatory factors which initiate differentiation; or (iii) function as regulators, some of which are sigma factors (for a review, see reference 16). Cloning and sequencing of the *bld* genes may reveal whether these genes and the *spoO* genes are molecularly related.

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