A Morphological and Genetic Mapping Study of Bald Colony Mutants of *Streptomyces coelicolor*

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SUMMARY

Twelve *bld* mutations of *Streptomyces coelicolor* resulting in a lack of visible aerial mycelium were mapped genetically. The mutants were classified into three groups on the basis of colony morphology, production of antibiotics and morphology on different carbon sources. Four map locations were found for the *bld* genes and three of these were very near the loci of *whi* genes, which are also involved in differentiation. Closely linked *bld* mutations had similar phenotypes.

INTRODUCTION

Chater (1972) studied 50 *whi* mutants of *Streptomyces coelicolor* which were defective at various stages in aerial mycelium development. These mutants fell into six phenotypic classes and the mutations were mapped at eight loci. A further class of developmental mutants includes those in which a mutation prevents any aerial mycelium production. These mutants have been termed bald (*bld*) and one such mutation, *bldA1* (s48), has been mapped (Hopwood, 1967). Approximate map locations for some other *bld* mutations have also been reported (Chater & Hopwood, 1973; Hopwood *et al.*, 1973) but these mutations were not described in detail. This paper describes genetic mapping studies with a number of *bld* mutants and their classification using various criteria. A preliminary report of some of this work has been published (Chater & Merrick, 1976).

METHODS

Organisms. All strains were ultimately derived from the wild-type *Streptomyces coelicolor* strain A3(2) (Hopwood, 1959). All *bld* mutants were isolated by visual selection following treatment with either *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (Delić, Hopwood & Friend, 1970) or 8-methoxypsoralen plus near-ultraviolet irradiation (Townsend, Wright & Hopwood, 1971). With the exception of *bldA1* and *bldD53*, the mutations were isolated in strain J650. All mutations in J650 were isolated at 35 °C; after purification, their morphology was examined at 25 °C for temperature-sensitivity. The genetically marked strains used for mapping are listed in Table 1. The locations of all the markers on the circular linkage map are shown in Fig. 1 and the genetic symbols are as listed by Hopwood *et al.* (1973).

Media and general methods of culture and genetic analysis. Minimal (MM) and complete (CM) agar media, supplements and general methods of culture were as described by Hopwood (1967). All carbon sources were used at 1 % (w/v) final concentration. Unless otherwise stated, incubation was at 30 °C.

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Table 1. *Strains of Streptomyces coelicolor* A3(2)

Explanations of symbols are given by Hopwood *et al.* (1973). Fertility designations are those of Hopwood & Wright (1976*a*) and morphological phenotypes of *whi* mutations are given by Chater (1972).

Strain no.	Genotype	Fertility type
285	<i>adeA3 argA1 cysB6 strA1 uraA1</i>	n.t.
948	<i>ammA5 hisA1 serA1</i>	NF
949	<i>argA1 cysA15 nicA1 proA1 uraA1 tps-30</i>	NF
1190	<i>hisA1 uraA1 strA1</i>	SCP1-
1514	<i>agaA1 argA1 cysA15 nicA1 proA1 uraA1</i>	NF
A200	<i>adeCv10 pheA1 strA5</i>	SCP1-
J192	<i>cysD18 leuB5 strA1</i>	NF
J200	<i>nicA1 pheA1 strA1 whiB218</i>	NF
J650	<i>agaA1 cysD18 mthB2</i>	NF
<i>whi</i> mutants		
J12	<i>hisA1 strA1 whiA72</i>	NF
J49	<i>leuB5 strA1 whiH119</i>	NF
J86	<i>leuB5 strA1 whiI17</i>	NF
J93	<i>argA1 strA1 whiA72</i>	n.t.
J95	<i>cysD18 strA1 whiH119</i>	NF
J118	<i>cysD18 strA1 whiG71</i>	NF
J119	<i>leuB5 strA1 whiG71</i>	NF
J127	<i>cysD18 strA1 whiI17</i>	NF
<i>bld</i> mutants		
1169	<i>bldD53 hisA1 mthB2 pheA1 strA1</i>	NF
J660	<i>agaA1 bldC18 cysD18 mthB2</i>	NF
J668	<i>agaA1 bldA39 cysD18 mthB2</i>	NF
J669	<i>agaA1 bldB43 cysD18 mthB2</i>	NF
J702	<i>agaA1 bldA39 mthB2</i>	NF
J707	<i>agaA1 bldA35 mthB2</i>	NF
J708	<i>agaA1 bldA16 mthB2</i>	NF
J714	<i>agaA1 bldB43 leuB5 strA1</i>	NF
J733	<i>agaA1 bldA35 cysA15 hisA1 uraA1</i>	NF
J741	<i>agaA1 bldC18 mthB2</i>	NF
J777	<i>argA1 bldD53 strA1 uraA1</i>	NF

n.t., not tested.

Antibiotic production. Production of antibiotics was assessed by growing a confluent lawn of the strain to be tested on CM for 3 days. A 16 mm diameter plug was then cut from this lawn using a sterile cork-borer. For tests against eubacteria, the plug was surrounded by Difco nutrient agar containing the assay organism at a concentration of about 10^8 cells ml⁻¹ and the plate was incubated overnight at 37 °C. When testing for antibiotic activity against strains of *S. coelicolor*, the plug was placed on a freshly spread lawn of spores of the assay strain on CM and the plate was incubated for 3 days.

Microscopy. Impression preparations were made and examined by the method of Chater (1972).

RESULTS

Colony morphology of bald mutants

Colonies were grown for 5 days on MM with glucose as the carbon source. Under these conditions the colony morphology of each strain was found to be stable and reproducible and the mutants could be divided into three distinct morphological classes. None of the mutations produced a temperature-sensitive *bld* phenotype.

Class 1. Mutants in this class had non-pigmented colonies which were smooth after 2 or

Fig. 1. Linkage map of markers employed. The map and gene symbols are based on those of Hopwood *et al.* (1973). Morphological markers are given inside the circle, with the allele numbers of the mutations at each locus.

3 days' growth but had a characteristic sculptured pattern on the colony surface after 5 or 6 days (Fig. 2). The colonies were very soft and could easily be broken up with a fine wire. Such mutants replicated well by the normal velvet replica-plating technique. Colony diameter was similar to or sometimes slightly greater than that of wild-type colonies grown under the same conditions. In impression preparations, the hyphae frequently appeared to be mis-shapen, with numerous septa (Fig. 3).

Class 2. These mutants had non-pigmented colonies which were smooth and rather flat with a feathery outline (Fig. 2). Their morphology did not change with age. The colonies were relatively hard, difficult to break up with a wire and replicated poorly. Colony diameter was similar to that of the wild type.

Class 3. Only one mutant (*bld-18*) was identified in this class. The colonies were smooth with a well defined border (Fig. 2). Young colonies were non-pigmented but often turned pink or red on ageing. They were relatively soft and replicated quite easily. Colony diameter was smaller than that of the wild type.

None of the three classes of mutant produced the earthy odour characteristic of *Streptomyces* spp.

Phenotypes of bld mutants on different carbon sources

The parent strain (J650) grew well and had normal colony morphology on arabinose, galactose, glucose, mannose, cellobiose, maltose, glycerol and mannitol. All the *bld* mutants could also utilize these carbon sources.

The morphology of the *bld* mutants was examined, using a binocular microscope and by impression preparations, after 5 days' growth on MM supplemented with each of the above carbon sources.

Class 1 mutants were bald on glucose, mannose and cellobiose but on the other five

Fig. 2. Colony morphology of *bld* mutants and wild type: (a) wild type; (b) class I mutant (*bldA39*); (c) class 2 mutant (*bldB43*); (d) class 3 mutant (*bldCr8*). Colonies were grown on MM at 30 °C for 5 days. Bar marker represents 0.25 cm.

Fig. 3. Impression preparations from: (a) wild type; (b) *bldA* mutant (phase contrast). Bar markers represent 10 μ m. The wild type shows chains of mature spores and coiled aerial mycelium with immature spores. The mutant (*bldA39*) shows branched mis-shapen hyphae with frequent septa.

Fig. 4. Morphology of a *bldA* mutant grown on 1 % mannitol as carbon source. (a) Colony morphology (*bldA35*) after 5 days at 30 °C. Bar marker represents 0.25 cm. (b) Impression preparation (phase contrast) from colonies shown in (a). The mutant shows chains of mature spores indistinguishable from those of the wild type (see Fig. 3a). Bar marker represents 10 μ m.

carbon sources they produced aerial hyphae and spores (Fig. 4) which, in impression preparations, were indistinguishable from those of wild-type colonies (Fig. 4). Such 'corrected' strains did not, however, produce the pigments typical of wild-type *S. coelicolor* A3(2). Class 1 mutants remained bald when growing on CM regardless of the sugar or alcohol used to replace glucose.

Class 2 mutants were bald on all eight carbon sources. Growth on arabinose and mannitol was rather poor and sparse tufts of aerial hyphae were sometimes observed under these conditions. Impression preparations of such material indicated that spores were not produced and the aerial hyphae were similar in appearance to those seen in *whiG* mutants (Chater, 1972).

The class 3 mutant was bald on arabinose, glucose, mannose, cellobiose and glycerol, and produced aerial hyphae and spores on galactose, maltose and mannitol. Impression preparations of these aerial hyphae were indistinguishable from the wild type.

The wild type and the mutants grew well on MM with 1 or 0.1 % glucose but grew only sparsely with 0.01 % glucose. The phenotype of class 1 mutants was examined on MM with 1 % mannitol further supplemented with glucose at 1, 0.1 and 0.01 %. Colonies were completely bald with 1 or 0.1 % glucose but sporulated when only 0.01 % glucose was present.

Although neither the presence nor any effect of cyclic AMP has previously been demonstrated in Streptomyces, the effects of glucose on *bld* colony morphology suggested that catabolite repression and cyclic AMP might be involved. However, both cyclic AMP and its *N*⁶,*O*²-dibutyryl derivative (added to MM at 5 mM) had no effect on the colony morphology of all three classes of *bld* mutants.

Streptomyces coelicolor A3(2) can utilize agar as a carbon source and will therefore grow on MM without added glucose. However, the parent strain 1650, in which the *bld* mutants were isolated, carries the *agaA1* mutation (Fig. 1) and cannot utilize agar as a carbon source (Hopwood *et al.*, 1973). The metabolic lesion causing the *aga* phenotype is not known. The mutation does not apparently alter utilization of any sugars but does

Table 2. *Phenotypic characteristics of bld mutants*

Character	Morphological class			
	Wild type	1	2	3
Colony morphology*	Wild type	Soft, frag- menting Sculptured surface	Hard, non- fragmenting Smooth surface	Soft frag- menting Smooth surface
Pigment production†	+	—	—	+
Antibiotic production‡				
Methylenomycin	+	—	—	+§
Actinorhodin	+	—	—	+
Morphology on different carbon sources				
Arabinose	+	+	—p	—
Galactose	+	+	—	+
Glucose	+	—	—	—
Mannose	+	—	—	—
Cellobiose	+	—	—	—
Maltose	+	+	—	+
Glycerol	+	+	—	—
Mannitol	+	+	—p	+
Representative <i>bld</i> loci		<i>bldA, bldD</i>	<i>bldB</i>	<i>bldC</i>

* See Fig. 2.

† +, Produces pigment; —, no pigment produced.

‡ +, Produces antibiotic; —, no production.

§ Antibiotic produced by *bldC* SCP1⁺ strains has an antibacterial spectrum similar to but greater than that of methylenomycin (see text).

|| +, Produces aerial hyphae and spores; —, bald; p, poor growth.

prevent utilization of a wide range of amino acids as carbon sources, e.g. histidine, proline or arginine (Hopwood & Merrick, unpublished results).

It was subsequently shown that the effects of the carbon source on the *bld* phenotype are the same in both *agaA1* and *aga*⁺ strains and it is therefore probably not necessary to use an *aga* strain in order to classify a particular mutation with respect to its phenotype on different carbon sources.

Antibiotic production by bld mutants

Streptomyces coelicolor A3(2) produces two well-characterized antibiotics. Strains carrying the plasmid SCP1 produce the antibiotic methylenomycin (Kirby, Wright & Hopwood, 1975; Wright & Hopwood, 1976*a*). Genes on SCP1 code for a number of steps in the biosynthesis of this antibiotic and also for resistance to it (Kirby, 1976). SCP1[—] strains do not produce methylenomycin and are sensitive to it. The second antibiotic, actinorhodin (Brockman *et al.*, 1966), is produced by both SCP1⁺ and SCP1[—] strains (Wright & Hopwood, 1976*b*) and mutations causing loss of actinorhodin production have been mapped to the chromosome (Wright & Hopwood, 1976*b*; B. A. M. Rudd, personal communication). The parent strain J650, from which most of the *bld* mutants were derived, carries the SCP1 plasmid integrated into the chromosome. Such strains are referred to as NF and, like SCP1⁺ strains, produce both methylenomycin and actinorhodin.

Bld mutants were tested for methylenomycin production by looking for antibiotic

Table 3. Preliminary mapping of *bld* mutations

Examples are given of crosses with representatives from one of each of the phenotypic classes. Each *bld* mutant (inner circle) was crossed with strain 1514 and *cysA*⁺ and *cysD*⁺ (indicated by triangles) were selected. The number against each allele in the diagrams gives the frequency of that allele among the recombinant progeny. In crosses with J660 and J669 the segregation of the *bld* mutation with respect to two potentially closely linked markers is tabulated and the probability that segregation of each pair of markers is independent is given. In each case this probability is very low for one of the markers, indicating that the *bld* mutation is linked to this marker.

activity against *S. coelicolor* (strain 1190), *Bacillus cereus* and *Bacillus mycoides*. Actinorhodin is a red/blue indicator pigment and therefore the production of this antibiotic was assessed visually, as well as by the activity of SCP1⁻ *bld* strains against *Staphylococcus aureus*.

Class I mutants produced neither methylenomycin nor actinorhodin on CM. SCP1⁺ wild-type strains produced small amounts of methylenomycin and normal amounts of

Table 4. *More precise location of bld-16, bld-35 and bld-39*

The *bld* mutants were crossed with strain 949 and *proA*⁺ *methB*⁺ (indicated by triangles) were selected. The number against each allele in the diagrams gives the frequency of that allele among the recombinant progeny. In each case the location of the *bld* mutation between *cysA* and *nicA* is consistent with the gradient of allele frequencies.

actinorhodin when grown on MM with 1 % glucose or 1 % mannitol. However, class 1 mutants grown on MM with 1 % mannitol and showing a 'corrected' phenotype did not produce methylenomycin or actinorhodin (Chater, personal communication).

Class 2 mutants produced neither methylenomycin nor actinorhodin on CM.

The class 3 mutant produced actinorhodin and at least one other antibiotic which had a similar activity spectrum to methylenomycin. However, this mutant also showed antibiotic activity against *Escherichia coli* K12 and *Salmonella typhimurium* LT2 whereas the wild-type SCP1⁺ strains showed no antibiotic activity against these Gram-negative bacteria. When the class 3 mutation was transferred to an SCP1⁻ strain only actinorhodin was produced, indicating that the other antibiotic activity was dependent upon the presence of SCP1. The phenotypic characteristics of each class of *bld* mutant are summarized in Table 2.

Genetic analysis of the bld mutants

In preliminary mapping studies, each mutant was crossed with the multiply-marked strain 1514 and *cysA*⁺ *cysD*⁺ recombinants were selected by plating on MM supplemented with all the growth requirements of the parent strains except cysteine. The genotypes of

Table 5. Mapping of *bldA39* with respect to *adeC* and *agaA*

Strain J668 was crossed with strain A200 using the selection indicated by triangles. Crossing-over in the upper arc of the map was due to the zygote structure in $NF \times SCP1^-$ crosses. The allele frequencies obtained are shown in the diagram and the frequencies of the recombinant genotypes are tabulated.

a sample of recombinants were then scored and the frequencies of each allele were inserted in a diagrammatic representation of the cross (Table 3). The frequency of the *bld* allele in the cross usually allowed two alternative locations, one in each of the arcs separating the selected markers. To decide between these locations, the segregation of the *bld* allele with respect to that of a potentially closely linked marker from each arc was tabulated. In most cases the segregation of the *bld* allele was dependent upon the segregation of one of the two chosen markers to which it was therefore linked, e.g. lack of independent segregation was found for *bld-43* and *mthB2* (Table 3).

For the three class 1 mutations (*bld-16*, *bld-35*, *bld-39*) originally mapped by this method, all or virtually all the recombinants were *bld*. These crosses therefore only indicated that the mutations were located between *nicA* and *cysD* in the upper arc of the chromosome. An example of one such cross (with *bld-39*) is given in Table 3.

All the six class 2 mutations were located in the interval between *mthB* and *cysD*, e.g. *bld-43* (Table 3), and the single class 3 mutation, *bld-18*, was located in the *uraA-strA* interval (Table 3). With this information further crosses were devised to map each of the mutations more precisely.

Class 1 mutants

Bld recombinants carrying *mthB2* were selected from the preliminary crosses and crossed with strain 949. Selection was made for *mth⁺ pro⁺* recombinants and in each case the *bld* mutation mapped between *nicA* and *cysA* (Table 4). This location is the same as that found for *bldA1* (Hopwood, 1967), which also has a class 1 phenotype. In order to examine whether all these class 1 mutations could be alleles of *bldA*, pairwise crosses were made between all four mutants in alternative coupling arrangements.

When crossing morphological mutants, recombinants carrying both mutations (double

Table 6. Mapping of *bldA35* with respect to *whiB218*

Strain J733 was crossed with strain J200 using the selection indicated by triangles. The allele frequencies obtained are shown in the diagram, and the frequencies of the recombinant genotypes are tabulated.

	150	122	150	41	32	0	
J 733	+	<i>uraA1</i>	+	<i>bldA35</i>	+	<i>cysA15</i>	
	---	△	---	△	---	---	
J 200				a	b	c	
	---	---	---	---	---	---	△
	<i>pheA1</i>	+	<i>nicA1</i>	+	<i>whiB218</i>	+	
	0	28	0	109	118	150	
Genotype							Crossover in intervals
<i>whiB218</i>							a
<i>bldA35 whiB218</i>							b
<i>bldA35</i>							c
Wild type							a, b, c
							Frequency
							109
							9
							32
							0
							Total
							150

mutants) are usually indistinguishable from one or both of the single mutants without further genetic analysis (Chater, 1975). As a result, ordering of the mutations is not possible by analysis of a single cross but relies on the relative frequencies of recovery of morphologically wild-type recombinants from crosses in alternative coupling arrangements. If the two mutations are in the same gene such recombinants will be rare in either coupling.

Bld nicA1 and *bld cysA15* strains were constructed with each of the four class 1 mutations, *bldA1*, *bld-16*, *bld-35* and *bld-39*. Each *bld nicA1* strain was then crossed to all the *bld cysA15* strains and *nic⁺ cys⁺* recombinants were selected. In no case were *bld⁺* recombinants recovered. On the basis of these data, the three new *bld* mutations were assigned to the *bldA* locus (Fig. 1). Unambiguous assignment of a *bld* mutation to a particular locus by complementation analysis is not possible at present in *S. coelicolor* as SCP1' plasmids are not available for the relevant sections of the genome (Hopwood & Wright, 1976a).

In order to define the location of *bldA* more precisely, strain J668 was crossed to strain A200 and *phe⁺ strA5* recombinants were selected. This selection was made solely to exclude parental genotypes; crossing-over in the 9 o'clock to 12 o'clock region of the genome was due to the zygote structure in NF × SCP1⁻ (UF) crosses (Hopwood *et al.*, 1969). Data from this cross indicated that *bldA* was located between *adeC* and *agaA* (Table 5).

Another mutation affecting development, *whiB*, is also located between *nicA* and *cysA* (Chater, 1972) and it was therefore of interest to map *bldA* relative to *whiB*. Strain J200 was crossed with strain J733 and *cys⁺ nic⁺ phe⁺* recombinants were selected (Table 6). When scoring recombinants from this cross, use was made of the ability to 'correct' the *bldA* phenotype by growth on mannitol. By replicating master plates with glucose as carbon source to plates with mannitol as carbon source, it was possible to identify *bldA35 whi⁺* and *bldA35 whiB218* recombinants and hence all four recombinant classes could be distinguished. The *bldA35* mutation was located between *whiB* and *nicA* (Table 6). Recombinants of genotype *bldA35 whiB218* grown on mannitol had the normal *whiB218* phenotype described by Chater (1972) and when grown on glucose they showed a normal *bldA* phenotype.

A number of other *bld* mutants were isolated by Hopwood at the same time as *bldA1* but were not extensively analysed. Two of these mutations, *bld-33* and *bld-62*, have been mapped between *cysA* and *nicA* and gave no *bld⁺* recombinants when crossed in alternative coupling arrangements with *bldA* strains. They were found to have a class 1 phenotype

Table 7. The location of *bld-53*

Strain 1169 was crossed with strain 949 and *his*⁺ *strA1* recombinants were selected. The segregation of *bld-53* with respect to *argA1* and *proA1* is tabulated and the contingency χ^2 shows that the segregation of *bld-53* is dependent upon that of *argA1*, and independent of *proA1*.

	<i>argA</i> ⁺	<i>argA1</i>	Total
<i>bld</i> ⁺	6	59	65
<i>bld-53</i>	33	2	35
Total	39	61	100

$P < 0.001$

	<i>proA</i> ⁺	<i>proA1</i>	Total
<i>bld</i> ⁺	29	36	65
<i>bld-53</i>	11	24	35
Total	40	60	100

$P = 0.2-0.1$

Table 8. Mapping of *bldD53* with respect to *whiA72*

Strain 1169 was crossed with J93, and strain J777 was crossed with J12 using the selection indicated by triangles. Replication to mannitol was used to distinguish *bldD53* and *bldD53 whiA72* recombinants. The allele frequencies obtained are shown in the diagram and the recombinant genotypes are tabulated.

Genotype	Crossover in intervals	Frequency
<i>bldD53</i>	a	12
<i>bldD53 whiA72</i>	b	48
<i>whiA72</i>	c	42
Wild type	a, b, c	0
Total		102

Genotype	Crossover in intervals	Frequency
<i>whiA72</i>	a	18
Wild type	b	48
<i>bldD53</i>	c	35
<i>bldD53 whiA72</i>	a, b, c	3
Total		104

Table 9. Mapping of *bldD53* with respect to *serA1*

Strain 1777 was crossed with 948 using the selection indicated by triangles. The allele frequencies are shown in the diagram and the recombinant genotypes are tabulated.

	50	34	28	0			
	+	+	<i>bldD53</i>	<i>argA1</i>			
J 777	---	△	---	---	---		
		a	b	c			
948	---	---	---	△	---		
	<i>hisA1</i>	<i>serA1</i>	+	+			
	0	16	22	50			
					Genotype	Crossover in intervals	Frequency
					<i>serA1</i>	a	9
					Wild type	b	13
					<i>bldD53</i>	c	21
					<i>bldD53 serA1</i>	a, b, c	7
						Total	50

with the exception that they did not show the same pattern of morphological responses to different carbon sources as the other *bldA* mutations. A further mutation, *bld-53*, had an exact class 1 phenotype and was therefore mapped to determine whether it was also a *bldA* mutation. The original mutant strain, 1169, was crossed with strain 949 and *his⁺ strA1* recombinants were selected. Analysis of the cross indicated that *bld-53* was between *hisA* and *argA* (Table 7) and it was therefore assigned to a new locus *bldD* (Fig. 1). The unusual linkage relationship between *uraA* and *pheA* in this cross is not understood, and the segregation of *nicA* was not scored.

Since *whiA* also lies between *hisA* and *argA*, *bld-53* was mapped with respect to *whiA*. The cross was carried out in both coupling arrangements. Strain 1169 was crossed with 193 and strain 1777 was crossed with 112; *arg⁺ his⁺* recombinants were selected. Replication to mannitol distinguished *bldD53* and *bldD53 whiA72* recombinants. The results from both crosses were consistent with a map location for *bldD53* between *whiA* and *argA* (Table 8).

Finally, *bldD53* was mapped with respect to *serA* by crossing strain 1777 with strain 948 and selecting *his⁺ arg⁺* recombinants. This cross located *bldD* between *serA* and *argA* (Table 9).

Class 2 mutants

Preliminary mapping located all of the six class 2 mutations (*bld-15*, -17, -28, -43, -57 and -59) between *mthB* and *cysD*. To refine this map location, *mthB2* recombinant derivatives of each mutant were crossed with strain 1192 and *mth⁺ cys⁺* recombinants were selected. In each case the *bld* mutation was located between *leuB* and *cysD*. To determine whether all six mutations could be in the same gene, *leuB* and *cysD* recombinant derivatives of each mutant were obtained. One of the mutants, *bld-43*, was then chosen as a tester strain and each of the other mutants was crossed to *bld-43* in both coupling arrangements, with selection for *leu⁺ cys⁺* recombinants. In all crosses the frequency of wild-type recombinants was lower than 0.01 % and the six class 2 mutations were therefore all assigned to the *bldB* locus (Fig. 1). It should be emphasized that, although these mutations had similar phenotypes, they could be non-allelic.

Three *whi* loci (*whiG*, *whiH*, *whiI*) are located between *cysD* and *leuB* and crosses were designed to locate *bldB* with respect to these loci. A *bldB43* strain (1669) carrying *cysD18* was crossed with *leuB5* derivatives of *whiG* (1119), *whiH* (149) and *whiI* (186) respectively, and crosses in the alternative coupling arrangement were made using a *leuB5 bldB43*

Table 10. Mapping of *bldB43* with respect to *whiG*, *whiH* and *whiI*

Strain J669 was crossed with strains J119, J49 and J86 and strain J714 was crossed with strains J118, J95 and J127. In all crosses *leu*⁺ *cys*⁺ recombinants were selected and their phenotypes were scored. The recombinant genotypes *bldB* and *bldB whi* were indistinguishable and so only minimum estimates (*) of the frequency of *whi* could be obtained.

	103	≤52	36	0	Phenotype	Genotype	Crossover in intervals	Frequency
J 669	+	+	<i>bldB43</i>	<i>cysD18</i>	Whi	<i>whiG</i>	a	51
	---	a	b	c	Wild type	Wild type	b	16
J 119	---	+	+	+	Bld	<i>bldB</i>	c	36
	<i>leuB5 whiG71</i>	+	+	+	Bld	<i>bldB whiG</i>	a, b, c	—
	0	≥51*	67	103			Total	103
J 669	100	83	≤83	0	Whi	<i>whiH</i>	a	17
	---	a	b	c	Bld	<i>bldB whiH</i>	b	83
J 49	---	+	+	+	Bld	<i>bldB</i>	c	0
	<i>leuB5</i>	+	<i>whiH119</i>	+	Wild type	Wild type	a, b, c	—
	0	17	≥17*	100			Total	100
J 669	56	24	≤24	0	Whi	<i>whiI</i>	a	32
	---	a	b	c	Bld	<i>bldB whiI</i>	b	24
J 86	---	+	+	+	Bld	<i>bldB</i>	c	0
	<i>leuB5</i>	+	<i>whiI17</i>	+	Wild type	Wild type	a, b, c	—
	0	32	≥32*	56			Total	56
J 714	0	≤63	63	101	Bld	<i>bldB</i>	a	63
	---	a	b	c	Bld	<i>bldB whiG</i>	b	35
J 118	---	+	+	+	Whi	<i>whiG</i>	c	3
	<i>leuB5</i>	+	<i>bldB43</i>	+	Wild type	Wild type	a, b, c	—
	101	≥38*	38	0			Total	101
J 714	0	43	≤53	81	Bld	<i>bldB</i>	a	43
	---	a	b	c	Bld	<i>bldB whiH</i>	a, b, c	10
J 95	---	+	+	+	Wild type	Wild type	b	28
	<i>leuB5</i>	+	<i>whiH119</i>	<i>cysD18</i>	Whi	<i>whiH</i>	c	—
	81	38	≥28*	0			Total	81
J 714	0	49	≤95	99	Bld	<i>bldB</i>	a	49
	---	a	b	c	Bld	<i>bldB whiI</i>	a, b, c	46
J 127	---	+	+	+	Wild type	Wild type	b	4
	<i>leuB5</i>	+	<i>whiI17</i>	<i>cysD18</i>	Whi	<i>whiI</i>	c	—
	99	50	≥4*	0			Total	99

Table 11. *More precise location of bld-18*

Strain J741 was crossed with strain 285 using the selection indicated by triangles. The allele frequencies obtained are shown in the diagram and the recombinant genotypes are tabulated.

		42	30	15	0		
J 741	---	+	<i>bld-18</i>	+	+	---	
		a	b	c			
285	---	<i>adeA3</i>	+	<i>cysB6</i>	<i>strA1</i>	---	
		0	12	27	42		
						Genotype	Crossover in intervals Frequency
						<i>cysB6</i>	a 12
						<i>bld-18 cysB6</i>	b 15
						<i>bld-18</i>	c 15
						Wild type	a, b, c 0
						Total	42

strain (J714) and *cysD18* derivatives of *whiG* (J118), *whiH* (J95) and *whiI* (J127). In all six crosses *leu⁺ cys⁺* recombinants were selected (Table 10).

Since *bldB* could not be induced to sporulate by changing the carbon source it was not possible to distinguish *bldB43* and *bldB43 whi* recombinants. Interpretation of the crosses, except by genetic analysis of all the *bld* recombinants, therefore depended on the frequency of morphologically wild-type recombinants in each cross and on the assumption that *bldB whi* recombinants had a *bld* phenotype. The presence of *bldB whi* double mutants amongst the recombinants also meant that only minimum estimates of the frequency of *whi* could be obtained.

In crosses between *cysD bldB* and *leuB whi* strains (Table 10), wild-type recombinants could be obtained by a single crossover only if *bldB* were between *whi* and *cysD*. Such recombinants were obtained only when *bldB* was crossed with *whiG*, suggesting that *bldB* is between *whiG* and *whiH*. In crosses between *leuB bldB* and *cysD whi* strains (Table 10), wild-type recombinants could be produced by a single crossover only if *bldB* were between *leuB* and *whi*. Wild-type recombinants were obtained in all three crosses but the low frequency (3 %) of such recombinants in the cross between *bldB* and *whiG* could be accounted for by multiple crossovers. The higher frequency of wild types in the other two crosses probably arose by single crossovers and hence these crosses are also consistent with a location of *bldB* between *whiG* and *whiH*. Assuming that this map location for *bldB* is correct, then in three crosses (J669 × J119, J714 × J95 and J714 × J127) *bldB whi* recombinants could arise only by triple crossovers and hence minimum estimates of the frequency of *whi* (assuming no triple crossovers) can be obtained (Table 10).

Recombination frequencies for the *leuB-bldB* interval varied from 50 to 65 % in five crosses, with a value of 17 % in cross J669 × J49. The precision of such estimates is, however, likely to be low owing to the multinucleate nature of most of the plating units and the possibility of selection against particular genotypes.

Class 3 mutants

In preliminary crosses *bld-18* was located between *uraA* and *strA* and this map position was refined by crossing a *methB2 agaA1 bld-18* recombinant (J741) with strain 285. Selection was made for *ade⁺ strA1* recombinants and *bld-18* was found to map between *adeA* and *cysB* (Table 11). This location defined the fourth *bld* locus, *bldC* (Fig. 1).

The relatively close linkage of *bldC18* to *cysB* prompted investigation of whether the *bld⁺* allele of *bldC18* was carried by SCP1'-*cysB* (Hopwood & Wright, 1973). An SCP1'-*cysB*, *bldC18* strain was constructed and was found to have a *bldC* phenotype indicating

either that the *bldC*⁺ allele was not carried by the SCP1'-*cysB* plasmid or that the *bldC*⁺ allele was carried by SCP1'-*cysB* and *bldC* was dominant to *bldC*⁺. If the latter were the case then *bld*⁺ homogenotes would be expected to arise in the same way as *cys*⁺ or *cysB* homogenotes arise (Hopwood & Wright, 1976*b*). Such *bld*⁺ segregants were not observed and it was therefore concluded that the *bldC* gene is not carried by SCP1'-*cysB*.

DISCUSSION

Four distinct map locations designated *bldA*, *bldB*, *bldC* and *bldD* have been identified and in each case mutations at the same location gave rise to a similar phenotype. Methods of selection for SCP1' plasmids carrying genes from any chosen region of the genome are not yet available and hence complementation tests between the *bld* mutations are not possible at present. Some of the clustered *bld* mutations may therefore be non-allelic as was noted for the *whi* mutations by Chater (1972).

The close linkage of three of the *bld* loci to *whi* loci is of particular interest. Mutations in *bldA* and *bldD* appear to produce an identical phenotype, as do mutations in *whiA* and *whiB*, and this similarity is heightened by the fact that *whiA* is closely linked to *bldD*, and *whiB* is closely linked to *bldA*. Whether these phenotypic and linkage relationships could reflect biochemical similarities between the genes concerned is not known.

Studies on the cell-wall composition of *bldA* mutants have indicated that the wall composition differs from that found in *bld*⁺ strains although the precise alteration involved has not yet been identified (unpublished results).

The *bldB* mutations have the phenotype which might be predicted for a mutation which allows only the production of vegetative mycelium, i.e. a hard leathery type of colony which cannot be induced to form aerial hyphae by changing the carbon source. Hence it is particularly interesting that *bldB* maps between *whiG* and *whiH* which are apparently mutations in very early steps in aerial mycelium formation (Chater, 1975).

There have been a number of reports of the possible involvement of plasmid-borne genes in aerial mycelium development (Okanishi, Ohta & Umezawa, 1970; Kähler & Noack, 1974; Redshaw *et al.*, 1976). However, all the *bld* mutations mapped so far in *S. coelicolor* can be assigned unequivocally to map locations on the circular chromosome, although experiments using curing agents such as ethidium bromide have not been carried out.

Relatively few mutations have been investigated in this study and it is very likely that other *bld* loci await discovery. The mutants described were selected at random on the sole criterion that they should be completely bald and have a stable phenotype; other mutants were often found which produced sparse aerial mycelia and which sporulated poorly. Such phenotypes could frequently be due to trivial pleiotropic effects of mutations in genes not directly connected with colony development but, even if they represent mutations in important loci, their analysis would be extremely difficult owing to problems in classifying recombinants.

Asporogenous variants have been described in a number of *Streptomyces* species (Appleby, 1948; Williams & McCoy, 1953; Perlman, Greenfield & O'Brien, 1954; Nakamura, 1965; Nikitina & Kalakoutskii, 1973*a, b*). In all cases these variants arose as secondary colonies or sectors during serial transfer and therefore their genetic origins are obscure. The lack of a genetic system in any of the organisms prevented genetic analysis of the variants and hence one cannot exclude the possibility that they are due to multiple

mutations. By contrast all the *bld* mutants described here have been shown to be due to single mutations.

The variants of *S. griseus* described by Williams & McCoy (1953), Perlman *et al.* (1954) and Nakamura (1965) did not produce the antibiotic, streptomycin, produced by the parent. The asporogenous strains described by Nakamura (1965) also fragmented considerably after 5 or 6 days' growth and failed to produce both the pigments and the characteristic earthy odour produced by the parent strain. Similarly, some of the asporogenous variants described by Nikitina & Kalakoutskii (1973*a, b*) showed a tendency to fragment and were referred to as pasty in texture whilst others were described as having a leathery texture. In all cases the variants produced similar quantities of antibiotic to the parent strains. All of these variants appear to share some characteristics of the *bld* mutants described here and might possibly result from similar changes in metabolism.

The pleiotropic effects of all the *bld* mutations on the production of both antibiotics and pigments suggests that these characteristics may have regulatory links with certain developmental processes, although mutations in characters such as cell-membrane composition might cause these pleiotropic effects by disrupting the function of certain membrane-bound enzymes.

The morphological correction of *bldA*, *bldC* and *bldD* mutants grown on certain carbon sources is particularly useful when characterizing *bld whi* double mutants. It also suggests that the lesions causing these *bld* phenotypes could be in some aspect of the organism's carbon metabolism. The lack of correction when *bldA* mutants are grown on a mixture of glucose and mannitol could be due to carbon catabolite repression but seems more likely to be attributable to preferential utilization of glucose. Comparable effects of carbon source on morphology were observed by Nikitina & Kalakoutskii (1973*b*) in asporogenous variants of *S. albocyaneus* and *S. anthocyaneus*. In both cases the variants were bald on fructose-containing minimal medium but produced aerial mycelium on minimal media with glucose or glycerol as carbon source. Nikitina & Kalakoutskii (1973*a*) also showed that the emergence of asporogenous variants of *S. roseoflavus* var. *roseofungini* grown on fructose was suppressed by addition of very low levels of mannitol.

This study of *bld* mutants in *S. coelicolor* A3(2) has considerably extended our knowledge of developmental mutants in this organism but further investigation of the mutants described here and isolation of more *bld* mutants (possibly denoting new loci) is now required. Aspects such as the phenotypic suppression of some *bld* phenotypes by changes in the carbon sources, and the pleiotropic effects of *bld* mutations on antibiotic production may well be areas in which further study could be rewarding.

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