Construction and Phenotypes of Double Sporulation Deficient Mutants in *Streptomyces coelicolor* A3(2)

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**SUMMARY**

In *whiA, B, G* and *H* mutants of *Streptomyces coelicolor* A3(2), aerial hyphae develop but sporulation septa are not formed. Septa are produced by *whiD* mutants but are spaced abnormally far apart. Mutants in each locus have a distinctive aerial mycelium morphology, except for *whiA* and *B* mutants which are closely similar. Seven strains were made with pairwise combinations of *whiA* and *B* mutations with *whiG, H* and *I* mutations and with each other. The genotypes of these strains were confirmed by suitable crosses and their aerial mycelium morphology examined. An indirect procedure was used to determine the aerial mycelium morphology of *whiGH, GI* and *HI* double mutants. The double mutants always closely resembled one of the single mutant parent strains in morphology and a consistent scheme of epistasis was obtained – *whiG* being epistatic to *whiH, A, B* and *I*; *whiH* to *whiA, B* and *I*; and *whiA* or *B* to *whiI*. These results point to the absence of any complex interactions between gene products, which might have been revealed by the occurrence of novel phenotypes in double mutants or by inconsistencies in the epistasis scheme.

**INTRODUCTION**

Previous papers have described the isolation, phenotypes and genetic mapping of white colony (*whi*) mutants of *Streptomyces coelicolor* A3(2) defective in the conversion of aerial hyphae into mature spore chains (Hopwood, Wildermuth & Palmer, 1970; Chater, 1972; Chater & Merrick, 1974; McVittie, 1974). Eight *whi* genes (*A* to *E, G* to *I*) have been identified; the status of a ninth (*whiF*) is doubtful (Chater & Hopwood, 1973; Chater & Merrick, 1974). Of these, *whiA, B, G* and *H* mutants were apparently unable to form the special sporulation septa (Wildermuth & Hopwood, 1970) required to subdivide the long aerial hyphal cells into spore-sized compartments; *whiI* mutants produced aberrantly spaced sporulation septa (Hopwood *et al.* 1970); the single *whiC* mutant was oligosporogenous (Chater & Merrick, 1974); and *whiF* mutants were defective in the formation of mature spores from spore compartments (McVittie, 1974). The frequency with which *whiA, B, G, H* and *I* mutants occurred suggested that few if any other genes were specifically involved in the early stages of sporulation of aerial hyphae (Chater & Hopwood, 1973).

Most of these mutants had aerial hyphal morphology resembling intermediate stages in spore formation in the wild type. The sequence in which these stages take place should be determinable by studies of the morphology of strains carrying two different *whi* mutations. In this paper I describe the construction and phenotypes of a set of double mutant strains involving the five genes *whiA, B, G, H* and *I*. 
Double sporulation mutants of Streptomyces

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.

METHODS

Strains. All strains (Table 1) were originally derived from Streptomyces coelicolor A3(2) (Hopwood, 1959). The locations on the circular linkage map of all the markers employed are given in Fig. 1.

Media, culture methods, mating techniques and light microscopy. These were all as described by Chater (1972), microscopy being done on impression preparations.

Fertility testing. Fertility tests to distinguish IF and NF types were as described by Vivian & Hopwood (1970), with UF strains 1190 or A200 as testers.

RESULTS

Morphology of constructed double whi mutants containing whiA or whiB mutations

The sections immediately following describe the synthesis of seven strains containing pairwise combinations of the two mutations whiA170 and whiB218 with whiG71, whiH250 or whiI80, and with each other. The genetic confirmation of the presence of the pairs of whi mutations in each strain is also described. To facilitate understanding of some of the genetics, the phenotypes of the double whi mutants and the single mutant parent strains are described first.

The mapping and phenotypes of all five mutations were given by Chater (1972) and are summarized in Figs. 1 and 2 and in Table 1. Each is typical of mutations of its locus; whiA and B mutants usually have type II phenotype (non-fragmenting, often tightly coiled aerial hyphae); whiG mutants have type I phenotype (non-fragmenting aerial hyphae showing no coiling); whiH mutants have type III phenotype (non-fragmenting, loosely coiled hyphae); whiI mutants have type IV phenotype (coiled, much-fragmented aerial hyphae).

Each double whi strain was found to resemble closely one of the single whi parent strains (Fig. 2, Table 1). These data suggested that whiG and H were epistatic to whiA and B, and whiA and B to whiI, while the whiAB double mutant was indistinguishable in phenotype from either single mutant parent strain. These conclusions were supported by data, given
Fig. 2. Phase-contrast photomicrographs of impression preparations of the aerial hyphae of single and double whi mutants. All bar markers indicate 20 μm. (a) whiA170 (phenotype II); (b) whiB218 (II); (c) whiA170B218 (II); (d) whiA170G71 (I); (e) whiB218G71 (I); (f) whiG71 (I); (g) whiA170H250 (III); (h) whiB218H250 (III); (i) whiH250 (III); (j) whiA170I80 (II); (k) whiB218I80 (II); (l) whiI80 (IV). Phenotypic designations (Roman numerals) are those of Chater (1972).
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Table 1. List of strains

Explanations of symbols and fertility type designations are given in Hopwood, Chater, Dowding & Vivian (1973). Morphological phenotypes are those given by Chater (1972) (see also Fig. 2).

<table>
<thead>
<tr>
<th>Strain no.</th>
<th>Genotype</th>
<th>Fertility type†</th>
<th>Morphological phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>602</td>
<td>hisA1 pheA1 strA1 acrA9</td>
<td>NF</td>
<td>w.t.</td>
</tr>
<tr>
<td>1107</td>
<td>hisA1 cysD18 leuB5 strA1</td>
<td>NF</td>
<td>w.t.</td>
</tr>
<tr>
<td>1190</td>
<td>hisA1 uraA1 strA1</td>
<td>UF</td>
<td>w.t.</td>
</tr>
<tr>
<td>1258*</td>
<td>hisC9 proA1 argA1 uraA1 cysD18 strA1</td>
<td>NF</td>
<td>w.t.</td>
</tr>
<tr>
<td>A200</td>
<td>adeC10 pheA1 strA1</td>
<td>UF</td>
<td>w.t.</td>
</tr>
<tr>
<td>3354</td>
<td>cysD1</td>
<td>NF</td>
<td>w.t.</td>
</tr>
</tbody>
</table>

Single whi mutants

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
<th>Fertility type†</th>
<th>Morphological phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>3279</td>
<td>cysD18 strA1 whiA170</td>
<td>n.t.</td>
<td>II</td>
</tr>
<tr>
<td>3277</td>
<td>proA1 strA1 whiA170</td>
<td>n.t.</td>
<td>II</td>
</tr>
<tr>
<td>3271</td>
<td>cysD18 strA1 whiB218</td>
<td>n.t.</td>
<td>II</td>
</tr>
<tr>
<td>3961</td>
<td>hisC9 argA1 uraA1 cysD18 strA1 whiB218</td>
<td>IF</td>
<td>II</td>
</tr>
<tr>
<td>3118</td>
<td>cysD18 strA1 whiG71</td>
<td>n.t.</td>
<td>I</td>
</tr>
<tr>
<td>3119</td>
<td>leuB5 strA1 whiG71</td>
<td>n.t.</td>
<td>I</td>
</tr>
<tr>
<td>3116</td>
<td>hisA1 argA1 mthB2 strA1 whiH250</td>
<td>n.t.</td>
<td>III</td>
</tr>
<tr>
<td>3198</td>
<td>leuB5 strA1 whiH250</td>
<td>n.t.</td>
<td>III</td>
</tr>
<tr>
<td>4009</td>
<td>cysC3 strA1 whiH250</td>
<td>n.t.</td>
<td>III</td>
</tr>
<tr>
<td>3159</td>
<td>hisA1 argA1 mthB2 strA1 whi80</td>
<td>n.t.</td>
<td>IV</td>
</tr>
<tr>
<td>386</td>
<td>leuB5 strA1 whi17</td>
<td>n.t.</td>
<td>IV</td>
</tr>
<tr>
<td>3127</td>
<td>cysD18 strA1 whi17</td>
<td>n.t.</td>
<td>IV</td>
</tr>
</tbody>
</table>

Double whi mutants

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
<th>Fertility type†</th>
<th>Morphological phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>11003</td>
<td>uraA1 strA1 whiA170 whiB218</td>
<td>IF</td>
<td>II</td>
</tr>
<tr>
<td>1481</td>
<td>mthB2 strA1 whiA170 whiG71</td>
<td>n.t.</td>
<td>I</td>
</tr>
<tr>
<td>3158</td>
<td>mthB2 strA1 whiA170 whiH250</td>
<td>n.t.</td>
<td>III</td>
</tr>
<tr>
<td>3960</td>
<td>mthB2 strA1 whiA170 whiB80</td>
<td>n.t.</td>
<td>II</td>
</tr>
<tr>
<td>3159</td>
<td>mthB2 strA1 whiB218 whiG71</td>
<td>IF</td>
<td>I</td>
</tr>
<tr>
<td>3156</td>
<td>mthB2 strA1 whiB218 whiH250</td>
<td>NF</td>
<td>III</td>
</tr>
<tr>
<td>3153</td>
<td>mthB2 strA1 whiB218 whi80</td>
<td>NF</td>
<td>II</td>
</tr>
</tbody>
</table>

* Genotype given incorrectly by Chater (1972).
† n.t., Not tested.

Fig. 3. Synthesis of a whiAG double mutant. Strains 390 and 3279 were crossed and hisA+ argA+ cysD+ recombinants selected (triangles). These were screened for homoserine-requirement (the mthB2 phenotype). A sample of mthB2 recombinants was then tested for inheritance of the two whi mutations as shown in Tables 2 and 3. Similar crosses, in which strain 390 was replaced by strains 3116 or 3159, were used to construct whiAH and AI double mutants.
Table 2. Analysis of a strain (1481) carrying whiA and G mutations

Strain 1481 was crossed with 1258 and selection (triangles) made for proA\(^+\)mthB\(^+\) recombinants. These were analysed for non-selective markers, and samples of whi recombinants re-streaked and examined microscopically. The phenotypic classification is that of Chater (1972) (see also Fig. 2). The frequency of each allele among the progeny is given in the diagram, the frequency of whi recombinants being determined by the inheritance of whiA (see text).

<table>
<thead>
<tr>
<th>Frequency of recombinants</th>
<th>Phenotype of aerial mycelium in a sample of recombinants</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotype</td>
<td>Type I</td>
</tr>
<tr>
<td>uraA whi</td>
<td>12</td>
</tr>
<tr>
<td>whi</td>
<td>120</td>
</tr>
<tr>
<td>cysD uraA whi</td>
<td>4</td>
</tr>
<tr>
<td>cysD whi</td>
<td>8</td>
</tr>
<tr>
<td>argA cysD uraA whi</td>
<td>1</td>
</tr>
<tr>
<td>hisC argA cysD uraA</td>
<td>1</td>
</tr>
<tr>
<td>hisC cysD uraA</td>
<td>1</td>
</tr>
<tr>
<td>hisC uraA cysD*</td>
<td>1</td>
</tr>
<tr>
<td><strong>Total</strong> 148</td>
<td></td>
</tr>
</tbody>
</table>
* Multiple crossover class.

below, from crosses involving the double mutants, where the segregation of parental whi mutant phenotypes among whi recombinants was only explicable in terms of the epistatic relationships summarized above.

Synthesis and testing of whiAG, AH and AI double mutants

The diagrams and Tables relating to this section all describe in detail the situation for the whiAG double mutant. To save space and preserve clarity, the methods and results for the whiAH and AI mutants will merely be summarized in the text.

Fig. 3 illustrates the type of cross used to synthesize these strains. The selection of strains requiring only homoserine from these crosses ensured that, unless multiple crossing-over had occurred, the recombinants would inherit both the whiA and the whiG, H or I mutation.

In the crosses in which the presence of both whi mutations in each double mutant was confirmed (Tables 2, 3) the double whi strain was always counterselected at mthB2, the counterselection against the tester strains being such that the two whi mutations were not separated
Table 3. Further analysis of a strain (1481) carrying whiA and G mutations

Strain 1481 was crossed with 1107 and selection (triangles) made for mthB+ leuB+ recombinants. For further explanation, see Table 2 and text.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Frequency of recombinants</th>
<th>Phenotype of aerial mycelium in a sample of recombinants</th>
</tr>
</thead>
<tbody>
<tr>
<td>hisA cysD</td>
<td>1</td>
<td>—</td>
</tr>
<tr>
<td>hisA cysD whi</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>hisA whi</td>
<td>39</td>
<td>6</td>
</tr>
<tr>
<td>whi</td>
<td>261</td>
<td>6</td>
</tr>
<tr>
<td>cysD whi*</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>hisA*</td>
<td>2</td>
<td>—</td>
</tr>
<tr>
<td>Total</td>
<td>309</td>
<td></td>
</tr>
</tbody>
</table>

* Multiple crossover class.

by the selection points. As a result, when the segregation of white and grey colony colour was scored, a whi map location would be obtained corresponding to that of the whi mutation situated nearer to the counterselection point of the tester strain. In Table 2 this point was at proA, so that white colony colour segregated as if determined by whiA, while in Table 3 it was at leuB, so that the pattern of whiG segregation determined the frequency of whi recombinants. These data confirmed the genotype of the whiAG strain. Very similar data were obtained in crosses with the whiAH and AI strains, confirming their genotypes.

Assuming negligible multiple crossing-over, all whi recombinants should have the whiA mutation, and some should also have the whiG mutation. The whiG mutation, however, should only be found among cys+ recombinants. Since the phenotype of the parental double mutant was identical to that of a whiG mutant, it follows that some cys+ recombinants (i.e. those carrying whiA and whiG) should have the whiG (type I) phenotype, and the rest the whiA (type II) phenotype, while all cys− recombinants should have the type II phenotype. This was indeed observed (Table 2), confirming the epistasis of whiG over whiA. Similarly, with the equivalent whiAH double mutant test cross, segregation of aerial mycelium phenotype was observed only among cys+ recombinants (3 type II, 6 type III) while five cys−
recombinants tested were all type II. This confirmed the epistasis of whiH over whiA. The prediction was different for the equivalent whiAI double mutant test cross: since all whi segregants should carry the whiA mutation and whiA appears epistatic to whiI, segregation of aerial mycelium type was not expected. This was the case, all of nine cys+ and two cys- recombinants having the type II phenotype.

Similar consideration of the diagram in Table 3 leads to the prediction that, for the whiAG and AH crosses, all whi recombinants should have the whiG or whiH phenotype. However, in the whiAI cross all cysD hisA whi and most cys+ hisA whi recombinants should inherit only the whiI mutation and hence the type IV phenotype, while a few cys+ hisA whi recombinants, and all cys+ his+ recombinants, should also have the whiA mutation and hence the epistatic type II phenotype. These predictions were confirmed, no segregants of type II phenotype being observed with the whiAG or AH crosses, while the expected segregation of type II and IV phenotypes occurred in the whiAI cross. In this latter cross all 23 cys+ his+ whi recombinants tested were type II, while only one of 16 cys+ hisA whi recombinants tested was type II, the rest being type IV.

It is concluded from these results that the proposed genotypes of the prospective double whi mutants were correct, that whiG and H are epistatic to whiA, and that whiA is epistatic to whiI.

Synthesis and testing of whiBG, BH and BI double mutants

As in the previous section, data for only one double mutant are given in detail (Fig. 5), similar data for the other strains being mainly summarized in the text.

Fig. 4a illustrates the cross used to synthesize a whiBH double mutant. Ignoring multiple crossing-over, strains inheriting whiH250 could have inherited whiB218 as well. To identify those that did, mthB2 recombinants (arrayed in patches on master plates) were replica-plated to a lawn of spores of UF strain 1190. After a period of growth, these ‘plate crosses’
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Fig. 5. Analysis of a strain (526) carrying whiB and H mutations. Strains 526 and 1258 were crossed and selection (open triangles) made for hisA+mthB+ recombinants. These were analysed for non-selective markers, and samples of whi recombinants re-streaked and examined microscopically. (a) The allele frequencies obtained by considering only cysD recombinants (additional selection indicated by black triangle) which are all presumed to be whiB but are free to segregate for whiB218 or whiB+. All the cysD whi recombinants tested had the whiB (type II) phenotype. (b) The allele frequencies obtained by considering only proA uraA recombinants (black triangles) which are all presumed to be whiB+ but are free to segregate for whiH50 or whiH+. All the proA uraA whi recombinants tested had the whiH (type III) phenotype.

were replica-plated to selective medium lacking histidine and homoserine (Fig. 4b). This allowed the identification of any IF strains among the patches on the master plate, as these gave few if any recombinants with the tester strain. It can be seen (Fig. 4a, b) that such IF recombinants should have inherited the whiB218 mutation. The plate cross with strain 1190 also allowed the identification of NF strains carrying the whiB218 mutation. Selection for his+ in the plate cross ensured that the whiB region of any NF strain tested should be inherited by most of the recombinants, while the selection for mthB+, together with the asymmetrical nature of zygote structure in NF x UF crosses (Hopwood, Harold, Vivian & Ferguson, 1969) ensured that the whiH+ allele of strain 1190 would be inherited by almost all recombinants. Thus the predominant colour, white or grey, of the aerial mycelium of the recombinant patches after 4 days' incubation indicated the presence or absence of the whiB218 mutation in the appropriate patch on the master plate. The whiBH double mutant chosen for study, 526, was NF (Fig. 5), as was the whiBI double mutant used, 953 (Table 4). The whi BG double mutant studied, however, was IF. (The fertility type of these recombinants had significance only for genetic analysis, and did not affect morphology).

The presence of the expected whi mutations in the double mutants was confirmed by crossing with strain 1258 and, for the whiBI double mutant, also with strain 1107. In the crosses with strain 1258 (Fig. 5), the selection was different from that described earlier (Table 2) for whiAG, AH and AI double mutants, since the two whi mutations were separated by the points of selection and segregated independently to a large extent. To show that whiB218 was present, only recombinants requiring cysteine were considered (Fig. 5a), since the whiH+ allele of strain 1258 would be present in nearly all these progeny. Among these, a whi mutation giving the whiB218 phenotype was found to segregate in a way consistent
Table 4. Further analysis of a strain (1953) carrying whiB and I mutations

Strains 1953 and 1107 were crossed, and selection (triangles) made for mthB+ leuB+ recombinants. For further details, see Tables 2 and 3 and text.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Frequency of recombinants</th>
<th>Phenotype of aerial mycelium in a sample of recombinants</th>
</tr>
</thead>
<tbody>
<tr>
<td>hisA cysD</td>
<td>17</td>
<td>- Type I1 -</td>
</tr>
<tr>
<td>hisA cysD whi</td>
<td>1</td>
<td>- Type I11 -</td>
</tr>
<tr>
<td>hisA whi</td>
<td>16</td>
<td>0 Type I11 15</td>
</tr>
<tr>
<td>whi</td>
<td>45</td>
<td>15 5</td>
</tr>
<tr>
<td>Total</td>
<td>79</td>
<td></td>
</tr>
</tbody>
</table>

Only with a location between proA and uraA. Conversely, considering only proA uraA recombinants, nearly all of which should be whiB+ (Fig. 5b), a whi mutation having the whiH250 phenotype segregated in a way consistent only with a map location in the mthB-cysD interval. Thus the presence of the two whi mutations was confirmed in a single cross. In addition, the aerial mycelium phenotype of proA+ uraA+ (and hence whiB218) recombinants was examined. All those inheriting cysD should be whiH+ and therefore have the type II (whiB218) phenotype. However, a proportion of the cys+ recombinants should inherit the whiH250 mutation and therefore should have the type III phenotype shown by the parental double mutant (strain 1526; Table 1, Fig. 2). These predictions were confirmed; all three cysD recombinants scored were type II, as compared with only two out of 32 cysD+, the rest of which were type III. This substantiated the earlier conclusion that whiH was epistatic to whiB.

This kind of analysis gave a similar and equally unambiguous result with the whiBG mutant, confirming its genotype and the epistasis of whiG over whiB. With the whiBI double mutant, too few recombinants of the appropriate genotype were obtained in the cross with strain 1258 to confirm the presence of whiB80, though the presence of whiB218 was amply confirmed. A further cross, with strain 1107, was therefore used (Table 4). The pattern of white colony segregation was compatible only with the presence in strain 1953 of a whi mutation between the leuB and cysD genes, i.e. in the region of the whiI gene. The morphology of whi recombinants was also examined, in order to test the predicted pattern of
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Fig. 6. The synthesis of a *whiA*170 *whiB*218 double mutant. (a) Strains 327 and 961 were crossed and selection (triangles) made for *proA*+, *hisA*+, *argA*+, *cysD*+ recombinants, most of which should also have inherited *whiA*170 from strain 327. The recombinants were classified for fertility (F or NF) and uracil requirement, and seven *ura*1 F recombinants picked. Most of these should have inherited *whiB*218 from strain 961. The presence of *whiA*170 was confirmed by the absence of *whi*+ recombinants in a backcross with strain 327 (see text). (b) For confirmation of the presence of *whiB*218, strain 1003 was crossed with strain 602 and selection (triangles) made for *pheA*+ *acrA* (acriflavine-resistant) recombinants. After recombinant analysis, the nine *whi* recombinants were re-streaked and examined microscopically. All were of phenotype II, and they included one carrying *hisA*1. Allele frequencies are given on the diagram.

segregation. The *whiB*1 parent strain (1953) had type II (*whiB*) morphology (Table 1, Fig. 2), so that the presence or absence of *whiB*218 rather than *whi*80 should have been decisive in controlling the phenotypes of recombinants. This led to the prediction that a proportion of the *hisA*+ recombinants should have been type II, all other *whi* recombinants being type IV. This was confirmed (Table 4).

**Synthesis and testing of a *whiAB* double mutant**

The cross used to synthesize a strain carrying *whiA*170 and *B*218 is illustrated in Fig. 6a. The presence of *whiA*170, in seven presumptive double mutants, was confirmed by back-crossing to the parent *whiA*170 strain 1327. In the case of strain 1003, finally selected, all 100 re-streaked recombinants were white. Similarly, no grey recombinants were observed with any of the other six strains. The presence of *whiB*218 in strain 1003 was confirmed by a cross (Fig. 6b) in which the white/grey colony colour segregated with a frequency indicating the presence of a *whi* mutation located between the *hisA* and *uraA* genes.

**Epistasis among *whiG*, *H* and *I***

The *whiG*, *H* and *I* loci are all situated between *leuB* and *cysD* (Fig. 1) (Chater, 1972), and with the currently available methods of gene transfer in *S. coelicolor* it would be exceedingly arduous to make and confirm *whiGH*, *GI* and *HI* double mutants. A different method was therefore used to test epistasis. The frequency of different mutant phenotypes was scored among the progeny of crosses where the double *whi* genotype was expected to occur with a significant and predictable frequency. This situation was provided in a series of crosses made between *cysD*18 (or in one case *cysC*3) and *leuB*5 mutants carrying *whi*+, *whiG*71, *H*250 or *I*17, with selection for *leu*+ *cys*+ recombinants (Table 5).

In crosses homozygous for any *whi* allele, only *whi* (never *whi*+) recombinants arose, and
Table 5. Epistasis among whiG71, H250 and I17

Nine crosses involving whiG71, H250 and I17, both singly and in pairwise combinations, were carried out and leu⁺ cys⁺ recombinants selected in each case (triangles). Data from crosses 1 to 6 were summed, as indicated by lower case letters, to give recombination frequencies for each interval. Roman numerals refer to the microscopic morphology of whi recombinants. The summed data were used to make predictions of the frequency of each whi phenotype among prototrophic recombinants in crosses 7 to 9. Observed and expected frequencies were compared by chi-square tests.

CROSSES 1 to 6

<table>
<thead>
<tr>
<th>Cross</th>
<th>leuB</th>
<th>whiG</th>
<th>whiH</th>
<th>whiI</th>
<th>cysCD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>leuB</td>
<td>G71</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>leuB</td>
<td>H250</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>leuB</td>
<td>I17</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>leuB</td>
<td>G71</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>leuB</td>
<td>H250</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>leuB</td>
<td>I17</td>
<td>+</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Summed frequency of crossing-over in each interval

leuB-whiG = a + g + j = 28/155
leuB-whiH = e + g + h + m = 139/263
leuB-whiI = e + j + k + m + n = 173/152
whiG-cysCD = b + h + i + k + l = 127/155
whiG-whiH = h = 11/50
whiG-whiI = k = 36/55
whiH-cysCD = d + i + n + o = 124/263
whiH-whiI = n = 37/111
whiI-cysCD = f + l + o = 42/215
CROSSES 7 to 9

<table>
<thead>
<tr>
<th>Cross</th>
<th>Phenotype</th>
<th>Observed frequency</th>
<th>Expected frequency for epistasis of</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>whiG71</td>
</tr>
<tr>
<td>7. *</td>
<td>leuB5 + H250 +</td>
<td>I 73</td>
<td>80 (\chi^2 = 3.3)</td>
</tr>
<tr>
<td></td>
<td>+ G71 + - cysD18</td>
<td>III 25</td>
<td>18 (P = 0.07)</td>
</tr>
<tr>
<td>8.</td>
<td>leuB5 + I17 +</td>
<td>I 45</td>
<td>47 (\chi^2 = 0.49)</td>
</tr>
<tr>
<td></td>
<td>+ G71 + - cysD18</td>
<td>IV 12</td>
<td>10 (P = 0.5)</td>
</tr>
<tr>
<td>9.</td>
<td>leuB5 + I17 +</td>
<td>III 47</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>+ H250 + - cysC3</td>
<td>IV 53</td>
<td>53 (P = 1.0)</td>
</tr>
</tbody>
</table>

* One whi* recombinant was obtained in this cross.
these were all of the expected microscopic phenotype (data not given). This confirmed the validity of the scoring of the phenotypes. In crosses 1 to 3 of Table 5, only the leuB<sub>5</sub> parent was a <i>whi</i> mutant. From these crosses were derived estimates of the relative frequencies of crossing-over in the leu<sub>B</sub>-<i>whi</i> and <i>whi</i>-cysD intervals. The validity of the scoring of microscopic phenotypes was again upheld.

Crosses 4 to 9 of Table 5 were heterozygous for all three possible pairwise combinations of <i>whi</i> mutations, each in both coupling arrangements with the selective markers. For example, crosses 4 and 7 involved <i>whiG</i><sub>71</sub> and <i>H250</i> in alternative coupling arrangements. Ignoring rare multiple crossover classes, in cross 4 frequent <i>whi</i><sup>+</sup> recombinants were generated by crossing-over between <i>whiG</i> and <i>H</i>, while crossing-over in the same region in cross 7 generated <i>whiG</i> <i>whiH</i> recombinants. Assuming no unknown selective effects, these two classes should be equally frequent. Three possible phenotypes can be envisaged for a given double <i>whi</i> mutant: like one <i>whi</i> parent, like the other, or different from both. In the present crosses, only the parental phenotypes were observed; thus the double mutants always resembled one of the parent strains. From the relative frequencies of the two <i>whi</i> phenotypes segregating in the cross this parent could be determined, with the result that <i>whiG</i> was found to be epistatic to <i>whiH</i> and <i>I</i>, and <i>whiH</i> to <i>whiI</i>. In each case very good agreement of the observed segregation with that expected from epistasis of one of the <i>whi</i> genes was obtained (Table 5).

**DISCUSSION**

The main conclusions arising from this work are: (i) the aerial mycelium of double <i>whi</i> mutants for the <i>whiA</i>, <i>B</i>, <i>G</i>, <i>H</i> and <i>I</i> genes is always morphologically similar to one of the single mutant parent strains; (ii) a consistent scheme of epistasis emerges, with <i>whiG</i> epistatic to <i>whiA</i>, <i>B</i>, <i>H</i> and <i>I</i>, <i>whiH</i> to <i>whiA</i>, <i>B</i> and <i>I</i>, and <i>whiA</i> and <i>B</i> to <i>whiI</i>. Thus no complex interactions were detected.

It is difficult to make definite interpretations of these results, although we may reasonably state that, directly or indirectly, the <i>whiG</i> gene product causes limited coiling, and the <i>whiH</i> product further coiling, of aerial hyphal cells, and that the <i>whiA</i> and <i>B</i> gene products are perhaps more directly involved in sporulation septation. It is certainly not reasonable to assume that the epistatic scheme reflects the temporal sequence of either gene expression or gene product activity in any simple way.

Combining all the available information on these <i>whi</i> genes, a very speculative model can be put forward. On this model, the <i>whiG</i> and <i>H</i> gene products would be involved in cell wall changes required for the initiation and localization of sporulation septation. The coiling observed would be a secondary consequence of these changes. The <i>whiA</i> and <i>B</i> gene products might be directly involved as structural or catalytic elements in the development of sporulation septa. Since this scheme takes away from the <i>whiI</i> gene product the task of regulating the spacing of sporulation septa, it may be that the <i>whiI</i> effect upon spacing (Hopwood et al. 1970) is indirect and might result from, for example, an effect upon the distribution of chromosomal material to spore compartments that occurs during normal sporulation septation.

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Double sporulation mutants of *Streptomyces*

REFERENCES


