Mutants of *Streptomyces coelicolor* Defective in Sporulation

By D. A. HOPWOOD, H. WILDERMUTH AND HELEN M. PALMER

John Innes Institute, Norwich NOR 70 F

(Accepted for publication 3 March 1970)

SUMMARY

Sporulation-defective mutants were isolated on the basis of altered colonial coloration. The mutants were classified into a number of morphological classes by means of phase-contrast and electron microscopy. Some mutants lacking sporulation septa, the special septa that lead to spore delimitation and separation in the wild-type, or differing from the wild-type in the spacing of such septa, were subjected to genetic analysis. This has so far revealed two, and possibly three, genes concerned with the formation and spacing of sporulation septa.

INTRODUCTION

Mutants defective in any complex biological process are likely to aid its understanding by dissection of the total phenomenon into unit processes, and recognition of their interrelationships. A familiar example is provided by the use of auxotrophic mutants in the description of biosynthetic pathways. In the analysis of morphogenesis, this approach has proved most valuable in one of the simplest systems, T4 bacteriophage (reviewed by Levine, 1969), where many genes controlling the assembly of different components of the virus have been identified and part of their sequence of operation has been determined.

In a more complex system, the endospore of the protokaryotic *Bacillus subtilis*, some progress has been made in the isolation and characterization of mutants with a defective sporulation ability (reviews: Balassa, 1969; Schaeffer, 1969). The prerequisites for such a study, well-developed experimental genetics combined with suitably complex morphological processes, are currently found in only one other protokaryote, *Streptomyces coelicolor*; it therefore seemed worth while to attempt an analysis of a morphogenetic sequence in this organism to provide a comparison with studies on the *B. subtilis* system, as well as with those on the more numerous eukaryotic systems.

The organization of *Streptomyces coelicolor* colonies is complex (Hopwood, 1960; Wildermuth, 1970). On an agar medium a spore germinates to give rise to a 'substrate' mycelium consisting of much-branched, septate hyphae, and branches of these hyphae later initiate the 'aerial' mycelium in which the spores develop. Several phases of colonial development might be analysed by means of mutants: spore germination, branching and septation of the substrate mycelium, development of the aerial mycelium, spore delimitation and maturation. An advantage of working with mutants defective in some aspect of the aerial mycelium is that the whole of this phase of the colony is dispensable: colonies totally lacking aerial mycelium can be propagated by sub-culturing substrate mycelium. Hence any mutants with specific defects in the
aerial mycelium should be viable. We chose spore delimitation for a first study because this process is the most regular part of the life cycle of the wild-type colony; hence description of the lesions in particular mutants, by comparison of their phenotype with that of the wild-type, would be simplified. In this paper we describe the isolation of such mutants and some information on their phenotypes and genetics. A preliminary report on this work has appeared (Hopwood, Wildermuth & Palmer, 1969).

![Linkage map showing the locations of markers referred to in the paper.](image)

**Fig. 1.** Linkage map showing the locations of markers referred to in the paper.

**Table 1. Characteristics of strains**

<table>
<thead>
<tr>
<th>Strain no.</th>
<th>cysC3</th>
<th>cysD18</th>
<th>leuB5</th>
<th>strA1</th>
<th>pheA1</th>
<th>uraA1</th>
<th>proA1</th>
<th>hisA1</th>
<th>hisC9</th>
<th>argA1</th>
</tr>
</thead>
<tbody>
<tr>
<td>A3(2)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>S</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>876</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>R</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1077</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>R</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>1107</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>R</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

**METHODS**

**Organisms.** The wild-type strain was *Streptomyces coelicolor* (*S. violaceoruber* sensu Kutzner & Waksman, 1959) strain A3(2) (Hopwood, 1959). The mutational and recombinational derivatives of this strain referred to in this paper are listed in Table 1. The locations of markers on the circular linkage map of the organism are indicated in Fig. 1. Markers of *S. coelicolor* have for some time been designated by three-letter symbols according to the recommendations of Demerec, Adelberg, Clark & Hartman (1966); accordingly the mutations described in this paper, leading to a white aerial mycelium, have been called whi.

**Mutagenesis.** Ultraviolet irradiation was carried out as described by Hopwood & Sermonti (1962). Mutagenesis by *N*-methyl-*N'*-nitro-*N*'-nitrosoguanidine (NTG) was performed according to the schedule of Delić, Hopwood & Friend (1970).

**Media and general methods of culture and genetic analysis.** These were as described
**RESULTS**

**Isolation of morphological mutants.** The aerial phase of *Streptomyces coelicolor* wild-type colonies is weakly pigmented; young colonies appear white, but as sporulation occurs the colonies take on a pale fawn coloration. This coloration was found to be intense enough for the visual recognition of mutant colonies devoid of pigmentation, and therefore appearing white even after prolonged incubation. Some, at least, of such white colonies were expected to be morphological mutants defective in a process necessary for the production of mature spores, and this expectation was fulfilled.

In the isolation of white mutants, plates of minimal medium were sown with mutagen-treated spore suspensions of the wild-type strain, at a density yielding many hundreds of colonies per plate. After 4 days incubation at 30° the plates were scanned with the stereoscopic microscope for white colonies. These were picked off with a fine needle and purified once or twice by streaking, before being examined in the phase-contrast microscope.

**Preliminary classification of mutants.** The stages in the development of spores in the aerial mycelium of wild-type *Streptomyces coelicolor* A3(2) have been described by Wildermuth & Hopwood (1970). At first the aerial mycelium consisted of long aseptate hyphae, before cross-walls divided them into cells. At least the apical cells, and probably also the non-apical ones, were next subdivided by special 'sporulation septa' into spore-sized compartments, each of which was destined to give rise to a spore. When the process was complete, the mycelium fragmented into spores, typically some 1 to 1.5 μm long. The aerial mycelium occasionally fragmented into separate cells by splitting at the sites of cross-walls, even before the formation of sporulation septa. Such separated units were observed to be viable (Hopwood, 1960); this was presumably due to the cross-walls being double (Wildermuth & Hopwood, 1970), and splitting between the components of the double wall left the cell on either side with an intact end-wall. However, such fragmentation probably does not occur spontaneously, and even in the artificial conditions of impression preparations few short fragments were seen; most of the hyphae in such preparations were very long.

As explained in the Introduction, we sought mutants defective in the subdivision of the cells of the aerial mycelium into spore-sized units by means of the sporulation septa. Such mutants should have been recognizable by the fact that their aerial mycelium did not fragment at maturity into pieces (spores) some 1 to 1.5 μm long as in the wild-type, but either failed to fragment or fragmented into longer pieces. We therefore looked for strains with such phenotypes among our collection of white mutants by examining them in the phase-contrast microscope. Out of about 100 white mutants,
four showed regular fragmentation into pieces consistently longer than wild-type spores; two of these mutants, whi-6 and whi-13, were chosen for further study. The majority of the others showed little spontaneous fragmentation of the aerial mycelium, and one mutant, whi-46, was chosen to represent this group. The remaining mutants fragmenting into units indistinguishable from wild-type spores were not studied further; some might have been expected merely to lack the pigment of the wild-type spores, if indeed their coloration was due to the presence of a pigment rather than to a structural colour, while others might turn out to have structural defects in spore maturation. As such, they could provide material for a separate study.

Fig. 2. Length distributions of samples of wild-type spores (+ :top), non-sporulating wild-type cells (+ :bottom), aerial mycelial fragments of mutants whi-13 and whi-6, and aerial mycelial cells of mutant whi-46. Mean spore, fragment or cell lengths are indicated. Sample sizes were between 60 and 155. Measurements were made on electron micrographs of negatively stained impression preparations.

Phenotypes of mutants defective in sporulation septation.

Phase-contrast photomicrographs of impression preparations of the three whi mutants and the wild-type are in Pl. 1, fig. 1 to 4. It is characteristic of Streptomyces coelicolor, and many other streptomycetes, that the aerial hyphae become helically coiled before spore-delimitation; vestiges of such coiling are still seen in spore chains approaching maturity (Pl. 1, fig. 1). The aerial hyphae of the three mutants also coiled. Mutants whi-6 (Pl. 1, fig. 2) and whi-13 (Pl. 1, fig. 3) differed from the wild-type in failing
Sporulation mutants of *Streptomyces coelicolor* to delimit spore-sized units; instead, the spiral hyphae fell apart into sausage-shaped pieces, those of *whi-6* being on average longer than those of *whi-13*. Mutant *whi-46* (Pl. 1, fig. 4) presented unfragmented helical hyphae at maturity.

More precise information on the lengths of the fragments, and of the cells of unfragmented hyphae, were obtained from electron micrographs of impression preparations (Wildermuth, 1970) stained negatively (Brenner & Horne, 1959). Randomly chosen fields of such preparations were photographed, and measurements were made on the prints, in which cross-walls, as well as fragment- or spore-ends were clearly visible. The lengths of curved objects were measured along the median line. For the wild-type, separate measurements were made of spores and of cells in the non-sporulating parts of sporulating hyphae. Typical specimens of wild-type spores (Pl. 2, fig. 5), *whi-6* (Pl. 2, fig. 6) and *whi-13* (Pl. 2, fig. 7) fragments and *whi-46* cells (Pl. 2, fig. 8) are shown. The results (Fig. 2) show that wild-type spores had a rather constant length, with a mean of 1.27 µm. (top panel of Fig. 2); cells not subdivided by sporulation septa had a mean length of 7.26 µm. (bottom panel of Fig. 2), suggesting that each cell was normally subdivided into some six spores. This conclusion might be invalidated if apical cells were consistently longer than sub-apical ones, a possibility that has not been excluded (Wildermuth & Hopwood, 1970). The data of Fig. 2 confirm that *whi-13* and *whi-6* fragmented into pieces of average length greater than wild-type spores, the length of *whi-13* fragments being twice that of wild-type spores, while that of *whi-6* fragments was some four times that length. The data also show that *whi-46* cells had the same length as those of the wild-type. Thus the lesion of *whi-46*, which represented a large class of mutants, could have been a simple disability in the production of sporulation septa. The lesions of *whi-6* and *whi-13*, which represented much smaller classes of mutants, may have been more complex and may have involved disturbance of the control of the spacing of sporulation septa rather than their absence; although no conclusive evidence of the production of such septa could be obtained from electron micrographs of thin sections (see below), the fact that the aerial hyphae of these mutants fragmented as readily as those of sporulating wild-type hyphae strongly suggests the operation of the fragmentation mechanism normally associated with the sporulation septa.

The fine structure of the aerial mycelium of each mutant in thin sections was next compared with that of the sporulating wild-type, which has already been described in detail (Wildermuth & Hopwood, 1970). In the wild-type, cells of the aerial hyphae bounded by double cross-walls (Pl. 3, fig. 9, CW) were subdivided by regularly spaced sporulation septa (SS) into spore-sized compartments. The developing sporulation septum had a characteristic appearance in thin sections; it was formed of two layers. the inner margin of the ingrowing annulus being clearly double (Pl. 3, fig. 10). When the annulus was complete, a double lamella therefore resulted, representing the end walls of adjacent spore compartments. At this stage each spore compartment was surrounded by a wall of constant thickness; the thickness of the normal hyphal wall (10 to 12 nm.). Subsequently, further layers of wall material were deposited, so that the wall of the mature spore was about 30 to 50 nm. thick (Pl. 3, fig. 11). Simultaneously, separation of adjacent spore compartments began marginally, by rupture of the originally continuous parent hyphal wall (and its overlying superficial fibrous sheath: Hopwood & Glauert, 1961) opposite the sporulation septa. Adjacent spores were finally attached only over a narrow central region (Pl. 3, fig. 11) before separating in
response to slight external forces. At maturity the internal details of the spore in thin sections had become ill-defined (Pl. 3, fig. 11), in comparison with those of earlier stages in sporulation and of vegetative hyphae, in which nuclear material, mesosomes and 'vacuoles' were clearly visible.

The fine structure of the aerial hyphal cells of the three mutants was not found to differ in any reproducible way from that of wild-type cells. In particular, cross-walls (CW) were observed in whi-46 (Pl. 3, fig. 12), whi-13 (Pl. 4, fig. 13) and whi-6 (Pl. 5, fig. 17), and these closely resembled in appearance those of the wild-type (Pl. 3, fig. 9). The internal structure of the cells of the mutants was also similar to that of the wild-type.

Mutant whi-46, which showed no spontaneous fragmentation visible in the light microscope, revealed no evidence in thin sections of the formation of sporulation septa; it resembled the wild-type at a juvenile stage before the onset of subdivision of the aerial hyphal cells. Mutant whi-13, on the other hand, showed rounding-off of adjacent units at the sites of septation (Pl. 4, fig. 14), presumably leading to the fragmentation observed in this mutant. Whether such septation was initiated by the formation of typical sporulation septa is not clear; stages in the formation of sporulation septa were rarely seen even in the wild-type (Wildermuth & Hopwood, 1970), and it was difficult to obtain unequivocal evidence for their presence in the mutant although the almost complete septum in Pl. 4, fig. 15 may represent a sporulation septum. Most of the fragments produced by this mutant were surrounded by unthickened walls, but a small proportion had thickened walls (Pl. 4, fig. 16) and therefore resembled spores.

Thin sections provided little or no information on the process of fragmentation in whi-6; as for whi-13, a failure to observe stages in the formation of sporulation septa in sections could not be taken as evidence for their absence. Occasionally, structures that could be interpreted as abortive septa were observed as ingrowths of wall material on one side only of a hypha. Such structures were more easily recognized in negatively stained preparations, as finger-like ingrowths from the wall, usually associated with mesosomes (Pl. 5, fig. 19), but they were also seen in sections.

**Genetics of the sporulation-septum mutants**

Each of the three mutants was crossed with one or more genetically marked strains of wild-type morphology, and recombinants were selected to contain one marker from each parent (Hopwood, 1967). Samples of these recombinants were then classified in respect of the non-selected markers. For the whi versus whi+ phenotype, classification was done on the basis of colony colour, and the phenotype of a proportion of the segregants was confirmed by phase-contrast microscopy.

Each cross gave rise to a clear segregation of mutant versus wild-type morphology, providing presumptive evidence for a single gene mutation affecting morphology. This conclusion was strengthened by the finding of an approximate map-location for each mutation by analysis of the segregation of whi in relation to that of other non-selected markers.

Mutants whi-6 and whi-46. Preliminary crosses of these mutants were made with strain 1077, selecting argA1+ and strA1. The allele ratio at the whi locus in each cross (see Table 2) indicated its location either between cysD and strA or between uraA and proA. A choice between these two locations was easily made on the basis of the crossovers needed to explain the observed recombinant classes. Assuming a location
in the interval \(cysD-strA\) (indicated in the diagrams of Table 2), only five and three recombinants, respectively, in the two crosses required more than the minimum of two crossovers, whereas a position in interval \(uraA-proA\) would have necessitated 18 and 19 multiple crossovers.

**Table 2. Preliminary location of whi-6 and whi-46**

Each whi mutant (inner circle) was crossed with strain 1077 (outer circle) and \(arg\) \(A^+\) and \(strA^+\) (indicated by triangles) were selected. Numbers in the diagrams are allele frequencies.

<table>
<thead>
<tr>
<th>Genotype*</th>
<th>Crossovers in intervals</th>
<th>(whi-6)</th>
<th>(whi-46)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(cysD)</td>
<td>a, d</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>(cysD\ \ uraA)</td>
<td>a, e</td>
<td>32</td>
<td>75</td>
</tr>
<tr>
<td>(cysD\ uraA \ proA)</td>
<td>a, f</td>
<td>9</td>
<td>32</td>
</tr>
<tr>
<td>(uraA)</td>
<td>b, d</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>(uraA \ proA)</td>
<td>b, e</td>
<td>5</td>
<td>8</td>
</tr>
<tr>
<td>(whi)</td>
<td>c, d</td>
<td>10</td>
<td>2</td>
</tr>
<tr>
<td>(whi \ uraA)</td>
<td>c, e</td>
<td>83</td>
<td>29</td>
</tr>
<tr>
<td>(whi \ uraA \ proA)</td>
<td>c, f</td>
<td>12</td>
<td>11</td>
</tr>
<tr>
<td>(whi \ cysD \ uraA)</td>
<td>a, b, c; e</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>(whi \ cysD \ uraA \ proA)</td>
<td>a, b, c; f</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>(cysD \ proA)</td>
<td>a; d, e, f</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>(whi \ proA)</td>
<td>c; d, e, f</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

* Wild-type alleles omitted.

The interval \(cysD-strA\) contains several known loci (Hopwood, 1967). Two series of crosses were analysed in which \(whi-6\) or \(whi-46\) was crossed with strains bearing the markers \(cysD\) (or \(cysC\), which is closely linked to it) and \(strA\) and each bearing a different marker in the interval between them. Recombinants were selected to contain \(cysC^+\) (or \(cysD^+\)) and \(strA\); thus recombination in the interval \(cysC(cysD)-strA\) was obligate. The segregation of \(whi\) in relation to the other non-selected marker (\(leuB_5\),
thiA1, mthB2, hisD3 or uvsB6) indicated a location for both whi-6 and whi-46 anti-clockwise of each of the markers. Data for the crosses involving leuB5, the most anti-clockwise of the markers, are summarized in Table 3. Thus these two whi mutations were consigned to the comparatively short interval of the map between cysC(cysD) and leuB.

Table 3. Location of whi-6 and whi-46 between cysD and leuB in crosses with strain 1107

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Crossovers in intervals</th>
<th>Number in cross involving</th>
</tr>
</thead>
<tbody>
<tr>
<td>strA + whi +</td>
<td>a</td>
<td>175</td>
</tr>
<tr>
<td>strA leuB whi +</td>
<td>b</td>
<td>13</td>
</tr>
<tr>
<td>strA leuB + +</td>
<td>c</td>
<td>11</td>
</tr>
<tr>
<td>strA + + +</td>
<td>a, b, c,</td>
<td>1</td>
</tr>
<tr>
<td>Totals</td>
<td>200</td>
<td>198</td>
</tr>
</tbody>
</table>

* Triangles indicate selected alleles.

Mutant whi-13. The results of a cross between mutant whi-13 and strain 876 are tabulated and analysed in Table 4. The allele ratio at the whi-13 locus indicated a position close to hisC. At first sight it should have been possible to choose a location for whi-13 clockwise or anti-clockwise of hisC on the basis of the segregation of whi-13 in relation to that of argA and proA; a clockwise position would have been indicated by the finding that all, or nearly all, whi-13 recombinants carried arg, while an anti-clockwise position would have followed from their carrying pro. In fact, three out of five carried pro but none carried arg, tending to favour the anti-clockwise position. However, further considerations show that this conclusion is not justified. The cross involves two fertility types (A. Vivian & D. A. Hopwood, unpublished results); the whi-13 strain, being a mutant derivative of the wild-type strain y3(2), is believed to be of the IF type, whereas 876 is of the NF type. In IF x NF crosses the IF strain contributes the complete chromosome to the majority, at least, of the zygotes, and the NF strain contributes a chromosome fragment; this fragment appears normally to include the 9 o'clock region of the genome (A. Vivian and D. A. Hopwood, unpublished results). Thus the majority, at least, of the zygotes able to give rise to selected progeny in the cross under consideration would have had the constitution shown in Fig. 3. Most of the segregants carrying the arg marker would therefore have required multiple cross-overs in the right-hand half of the map: in intervals f and g together with either a or b. Nine of the 12 arg segregants were manifestly members of multiple crossover classes since they did not carry cys and therefore arose by crossing over in f, g and b. It is therefore very likely that, if whi-13 were located between his and arg, most of all or the segregants carrying whi-13 would also have arisen by multiple crossover patterns; therefore the finding that all five such segregants lacked arg by no means excludes such a location.
**Sporulation mutants of Streptomyces coelicolor**

We thus conclude from these data only that whi-13 is close to hisC. As such it clearly defined a different locus from whi-6 and whi-46 and the analysis was taken no further at this stage.

**Table 4. Preliminary location of whi-13**

Frequencies of different genotypes* of selected recombinants from a cross of mutant whi3 (inner circle) and strain 876 (outer circle). hisC9+ and strA1, indicated by triangles, were selected.

![Diagram](image)

(i) whi classes
- pheA whi-13
- pheA proA whi-13
- cysC pheA whi-13
- cysC pheA proA whi-13
- argA pheA whi-13

(ii) whi+ classes
- pheA proA
- cysC pheA proA

* Wild-type alleles omitted.

**DISCUSSION**

This study has shown that morphological mutants of *Streptomyces coelicolor* with defects in a particular stage of colonial development can be isolated by a comparatively efficient visual selection procedure.

In all probability the group of mutants discussed in this paper serves to identify
genes concerned with the synthesis or spacing of the sporulation septa that subdivide the aerial hyphae during sporulation. The three mutants examined here have revealed two such genes; \textit{whi-13} is certainly genetically distinct from the other two mutants, whereas \textit{whi-6} and \textit{whi-46}, falling as they do in the same segment of the linkage map, could be allelic, in spite of their different phenotypes.

![Figure 3](image)

\textbf{Fig. 3.} Constitution of the majority of the zygotes in a cross of a prototrophic IF strain (inner circle) and marked strain 876 NF (outer arc). Continuous lines indicate sections of genome obligately included in all zygotes capable of yielding selected progeny, while dashed regions are variably included.

Although the three mutants discussed are the only ones to have been studied morphologically in any detail, many of the remaining \textit{whi} mutants have been mapped approximately; a few of the mutants map in the region of \textit{whi-13}, and the remainder in the region between \textit{strA} and \textit{cysD}. Thus, unless there is extensive clustering of genes, it would appear that mutation to the \textit{whi} phenotype involves few genes. If so, the situation resembles that in the eukaryote fungus \textit{Aspergillus nidulans}, where Clutterbuck (1969) found mutation in only two genes led to interruption in conidiophore development, a morphogenetic sequence roughly analogous with sporulation in \textit{Streptomyces coelicolor}.

A contrasting result has emerged from studies in the other protokaryotic system, the endospore in \textit{Bacillus subtilis}, where mutation in many genes leads to reduced or no spore production. Mapping studies have identified a minimum of 15 genes (Schaeffer, 1969), and an indirect argument leads to an estimate of hundreds of genes being involved (Balassa, 1969). At first sight this conclusion appears to conflict with the findings in \textit{Streptomyces coelicolor}. However, the \textit{B. subtilis} system is probably very much more complex than that in \textit{S. coelicolor}. Very many attributes of the endospore, its metabolism as well as its morphological components, are probably controlled by sporulation-specific genes which are not involved in the life of the vegetative cell;
Sporulation mutants of Streptomyces coelicolor

the spore is in many respects a separate 'organism'. In contrast, sporulation in S. coelicolor consists merely of segmenting a pre-existing hypha into separate units; mutants of the type described in this paper should contribute to an understanding of this simple morphogenetic sequence.

The finding that septation of the substrate and aerial mycelium by cross-walls appears to be normal, even in a mutant (whi-46) that totally lacks sporulation septa, tends to confirm the conclusion from morphological studies of the wild-type that the two kinds of septum are distinct (Wildermuth & Hopwood, 1970).

This work was begun in the Institute of Genetics, University of Glasgow, and we gratefully acknowledge the advice of Dr P. T. P. Oliver of that Institute during electron microscope studies of the wild-type and whi-6. We should like to thank Helen M. Ferguson for the isolation of some of the mutants and for skilled assistance with some of the genetic studies.

REFERENCES


EXPLANATION OF PLATES

PLATE 1

Phase-contrast photomicrographs of impression preparations. Magnification approx. $\times 1400$.

Fig. 1. Wild-type. The preparation consists largely of isolated spores and chains of spores of varying maturity.

Fig. 2. Mutant *whi-6*. The preparation consists largely of separated fragments and short chains of fragments.

Fig. 3. Mutant *whi-13*. Separated fragments.

Fig. 4. Mutant *whi-46*. Helically coiled aerial hyphae.

PLATE 2

Electron micrographs of impression preparations negatively stained with potassium phosphotung-state. Magnification approx. $\times 16,500$.

Fig. 5. Wild-type. Two isolated spores and a chain of younger spores.

Fig. 6. Mutant *whi-6*. Two fragments and part of a third. Note mesosomes.

Fig. 7. Mutant *whi-13*. Separated fragments and short chains of fragments.

Fig. 8. Mutant *whi-46*. Note two cross-walls delimiting one cell and parts of two adjacent cells.

PLATES 3, 4 AND 5

Electron micrographs of thin sections (Figs. 9 to 18) or of negatively stained preparations (Fig. 19).

Fig. 9. Wild-type. Non-sporulating hypha. Note cross-wall ($CW$), mesosomes, nuclear material. $\times 50,000$.

Fig. 10. Wild-type. Sporulating aerial hypha at Stage 2 (Wildermuth & Hopwood, 1970). Note sporulation septa ($SS$). $\times 65,000$.

Fig. 11. Wild-type. Part of a chain of mature spores. Note thickened spore walls surrounded by vestiges of fibrous sheath, and poorly defined spore contents. $\times 50,000$.

Fig. 12. Mutant *whi-46*. Aerial hyphal cell with cross-wall ($CW$). $\times 50,000$.

Fig. 13. Mutant *whi-13*. Aerial hyphal cell with cross-wall ($CW$). $\times 50,000$.

Fig. 14. Mutant *whi-13*. Two adjacent aerial mycelial fragments separating. $\times 65,000$.

Fig. 15. Mutant *whi-13*. Possible sporulation septum dividing aerial mycelial cell. $\times 50,000$.

Fig. 16. Mutant *whi-13*. A fragment with thickened wall. $\times 50,000$.

Fig. 17. Mutant *whi-6*. Aerial hyphal cell in a cross section of a helically coiled hypha (compare longitudinal section in Fig. 18) with cross-wall ($CW$). $\times 50,000$.

Fig. 18. Mutant *whi-6*. Longitudinal section of a helically coiled aerial hypha (compare cross section in Fig. 17). $\times 50,000$.

Fig. 19. Mutant *whi-6*. Negatively stained aerial hypha with possible abortive sporulation septum as a finger-like ingrowth of the wall associated with a mesosome. $\times 65,000$. 
Plate I

D. A. HOPWOOD, H. WILDERMUTH AND H. M. PALMER

(Facing p. 408)
D. A. HOPWOOD, H. WILDERMUTH AND H. M. PALMER