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A new class of slow-growing non-perithecial mutants of *Aspergillus nidulans*

By J. A. HOUGHTON*
Department of Genetics, The University, Liverpool, England

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SUMMARY

Twenty-four mutants of *Aspergillus nidulans* were isolated which grew more slowly than normal on glucose as sole source of carbon and were unable to utilize intermediates of the tricarboxylic acid cycle. By complementation tests they were divided into five groups, each group representing a different cistron and the cistrons were assigned to linkage groups by mitotic analysis. The mutant loci have been designated *sgp-1* to *sgp-5* respectively. Three mutants have been mapped by meiotic analysis.

1. INTRODUCTION

The majority of extrachromosomal mutants isolated in the fungus *Aspergillus nidulans* have been found to be somatically unstable, and consequently unamenable to detailed biochemical and genetical investigation (reviewed by Jinks, 1963). An attempt was made to isolate mutants of *A. nidulans* with metabolic lesions similar to those found in the stable, extrachromosomally inherited, respiratory-deficient mutants of yeast (Ephrussi, 1953; Nagai, Yanagishima & Nagai, 1961) and *Neurospora crassa* (Mitchell & Mitchell, 1952; Mitchell, Mitchell & Tissieres, 1953) in the hope that they might also prove to be both stable and inherited extrachromosomally. Mutants were isolated which were unable to grow on non-fermentable carbon sources (Raut, 1954; Ogur, Lindegren & Lindegren, 1954) and these were investigated further. It became apparent that these mutants differed from the respiratory-deficient mutants of yeast and *N. crassa*; nevertheless because of their interesting physiological and genetical characteristics they were studied in greater detail. This paper describes the isolation, general characteristics and genetic mapping of these mutants, their biochemistry will be reported in a subsequent paper (Houghton, 1970).

2. MATERIALS AND METHODS

Routine methods for *Aspergillus nidulans* described by Pontecorvo *et al.* (1953) were used.

(i) *Strains.* The mutations were induced in wild-type strain 13 of *A. nidulans* obtained from the Department of Genetics, University of Glasgow. Master strain D was kindly supplied by Dr E. Forbes. Other strains were obtained from the

* Present address: Department of Microbiology, University College, Galway, Republic of Ireland.

Fungal Genetics Stock Centre, Department of Biological Sciences, Dartmouth College, Hanover, New Hampshire, U.S.A. (F.G.S.C.).

(ii) *Media.* All chemicals used were analytical grade and obtained from British Drug Houses Ltd. unless otherwise stated. Slightly modified forms of the minimal medium (MM) of Pontecorvo *et al.* (1953) were used. Basal minimal medium consisted of MM without the glucose. Acetate minimal medium (MMA) consisted of basal medium + 2 % sodium acetate. The media were solidified by adding 1·5 % (w/v) Oxoid agar no. 3 and were sterilized by autoclaving at 15 lbs/sq.in. for 15 min. All incubations were carried out at 37 °C.

(iii) *Induction of mutants.* The mutants were induced by ultra-violet (u.v.) irradiation. Suspensions of conidia of *A. nidulans* strain 13 were exposed to u.v. irradiation until the viable count was reduced to 5 % of the original value (Pontecorvo *et al.* 1953). Three different methods for mutant isolation were then used.

(iv) *Replica-plating.* A modification of the technique of Mackintosh & Pritchard (1963) was used. An irradiated conidial suspension was diluted and plated on MM + 0·08 % (w/v) sodium desoxycholate (SD) so as to yield about 100 colonies per plate. After about 96 hr incubation, small compact colonies had developed which were replicated, using damp rayon-velvet pads, on to MMA + SD and on to fresh MM + SD. The plates were incubated for 48 h and colonies which grew on MM + SD but not on MMA + SD were isolated.

(v) *Filtration enrichment.* The method used was essentially that described by Woodward, De Zeeuw & Srb (1954). An irradiated conidial suspension was diluted to give about 5×10^4 viable conidia/ml and 1 ml aliquots were shaken at 37 ° in 10 ml liquid MMA for 48 h and the suspension was filtered through nylon fabric at 6 h intervals. Surviving conidia were plated on MM and the colonies which grew were tested for their ability to grow on MMA or basal medium + an intermediate of the tricarboxylic acid (TCA) cycle as sole source of carbon.

(vi) *Visual selection.* An irradiated conidial suspension was diluted and plated on MM to produce about 25 colonies per plate. The plates were incubated for about 72 h, when the majority of colonies were large with copious conidial heads. On examination under a dissecting microscope it was sometimes possible to distinguish, in addition, small, poorly conidiating colonies with scanty mycelium. These were tested for their ability to utilise different carbon sources.

(vii) *Growth tests.* Mutant colonies isolated by the foregoing techniques were tested for their ability to grow on MM, MMA and basal medium + an intermediate of the TCA cycle as sole source of carbon. Conidial suspensions of mutant and wild-type strains were spread on these media and the colonies compared throughout their growth. Growth rates were determined by measuring the diameters of colonies grown from point inocula at regular time intervals. The mutants were identified by their slow growth on MM and their inability to grow on media containing acetate or an intermediate of the TCA cycle as carbon source.

(viii) *Cytochrome systems.* The cytochrome systems of wild type and mutant strains were compared using the technique of Boulter & Derbyshire (1957). The Nadi test was also used to compare cytochrome oxidase activity.

(ix) *Resistance to respiratory inhibitors.* The resistance of wild type and mutant strains to respiratory inhibitors was compared. Plates of MM were prepared containing different concentrations (0·0–2·0 g/l.) of a number of respiratory inhibitors (cyanide, azide, rotenone, 2,4-dinitrophenol). Conidial suspensions at a range of concentrations (10^2–10^3 conidia/plate) were spread on the plates and incubated. Growth was estimated visually and compared with controls of MM.

(x) *U.v. survival curves.* The lethal effect of u.v. irradiation on wild-type and mutant strains was compared. U.v. survival curves were determined by the standard technique of Pontecorvo *et al.* (1953).

(xi) *Genetic analysis of mutants.* The heterokaryon test (Jinks, 1963) was employed to determine whether the mutants were chromosomally or extrachromosomally inherited. The mutants were separated into functional groups by complementation tests using stable trans-heterokaryons and diploids (Pontecorvo *et al.* 1953; Roper, 1952) synthesized between pairs of mutants. An alternative method for carrying out complementation tests was developed which gave the same results. Mutants did not form perithecia even after prolonged incubation. It was found, however, that when two mutants belonging to different groups were cross-inoculated on the same plate, perithecia were produced in as great a number as by wild-type strains. All the perithecia examined were crossed. Mutants from the same group never produced perithecia when cross-inoculated. Newly isolated mutants could readily be assigned to a functional group by cross-inoculating with representative members of the known groups and establishing with which ones they produced perithecia.

Mutants were assigned to linkage groups by haploidization with parafluorophenylalanine of diploids synthesized between a mutant strain and master strain D (Forbes, 1959) marked in each of the eight linkage groups (Pontecorvo, Tarr Gloor & Forbes, 1954; Forbes, 1959; Lhoas, 1961; McCully & Forbes, 1965). Because the viability of the mutant haploid segregants was low, only non-mutant segregants were analysed.

Meiotic analysis was employed to locate the positions of a number of the mutant sites relative to other known markers within the linkage groups (Pontecorvo *et al.* 1953). Calculations of the frequency of recombination occurring between the mutant loci and other markers involved in the cross enabled the distance between them to be ascertained. The genotypes of the mutant progeny were not scored because of their low viability.

3. RESULTS

(i) *Isolation of mutants*

(*a*) *Replica plating.* Following the examination of 3087 viable conidia of strain 13 two mutants were isolated. Therefore 0·065 % of the survivors of the mutagenic treatment were of the desired type.

(*b*) *Filtration enrichment.* Two mutants were isolated, but the percentage of mutants amongst the survivors was only 4×10^{-4} %.

(*c*) *Visual selection.* This technique gave the best results for mutant isolation. Nineteen mutants were isolated (0·157 % of survivors).

In addition, a spontaneous mutant was isolated from amongst the sexual progeny of a cross between F.G.S.C. strains number 92 and 93 (Faulkner, unpublished data).

Each mutant was given an isolation number preceded by the letter R.

(ii) *Characteristics of wild type and mutants*

(*a*) *Morphology on MM.* Wild-type conidia spread on MM germinated within 12 h to give colonies which grew quickly and vigorously, producing a dense mycelium bearing copious conidial heads (over 150/mm^2). The growth rate of the colonies, measured as increase in colony diameter, was 15·6 mm/24 h after an initial lag. After 7–10 days mature perithecia containing viable ascopores appeared in abundance.

The mutants, however, grew more slowly than wild type (6·9 mm/24 h) and the mycelium was much scantier and fewer conidial heads were produced (about 50/mm^2). The conidial heads were a paler shade of green and they were smaller than those of wild type. The diameters of the conidia were the same as wild type but were less viable. Only 55 % of mutant conidia gave rise to colonies when plated on MM, compared to 80 % of wild-type conidia. It was also observed that less brown melanin-type pigment was produced by the mutants, although this was not measured quantitatively. The mutants did not produce any sexual fruiting bodies. Even after prolonged incubation under conditions of low oxygen tension, although small clusters of Hulle cells were produced, perithecia were completely absent. This abnormal growth of the mutants could not be restored to normal by supplementation of the MM, variation of the glucose concentration, or temperature changes. It was decided that the mutants, in conformity with current genetical convention, be termed *sgp* (slow-growing non-perithecial) mutants.

The fine structure of hyphae from wild type and *sgp* strains was examined by light microscopy but no differences were apparent. Similarly electron microscopy revealed no differences in hyphal sections, and mitochondria were present in both wild-type and mutant sections.

Investigation of the ability of wild-type and *sgp* strains to grow on a variety of carbon sources showed that the wild type could grow, with varying success, on all the sources tested (Table 1). The mutants differed in that, whilst able to grow to a limited extent on carbohydrates and intermediates of the Embden–Meyerhof pathway up to fructose 1,6-diphosphate, they could not grow on any of the intermediates following its cleavage by aldolase. The mutants were unable to grow on any of the intermediates of the TCA cycle as sole carbon source.

(*b*) *Cytochrome systems.* No differences between wild-type and mutant strains were observed in their cytochrome systems or response to the Nadi test.

(*c*) *Resistance to respiratory inhibitors.* The growth of the mutant strains was inhibited by the same concentration of inhibitors (cyanide, azide, rotenone, 2,4-dinitrophenol) as the wild-type strain and showed no resistance.

(*d*) *U.v. survival curves.* The conidia of *sgp* strains were found to be more sensitive than those of strain 13 to the killing effects of u.v. (Fig. 1.). Mutant conidial viability fell to 5% in only about 2 min, half the irradiation period required to effect the same killing of strain 13 conidia. There was hardly any

Table 1. *Growth of wild-type and* sgp *mutant strains on various carbon sources after 96 h incubation*

(Symbols denote qualitative estimation of growth, from no growth (−) to very good growth (+ + +). The order of symbols is, −, +, + +, + + +.)

Carbon source	Wild type	Mutants
D(+)glucose	+ + +	+ +
D(−)fructose	+ + +	+ +
Glucose 6-phosphate	+ +	+
Fructose 6-phosphate	+ +	+
Fructose 1,6-diphosphate	+ +	+
Dihydroxyacetone	+ +	−
DL-glyceraldehyde	+ +	−
D(−)-3-phosphoglyceric acid	+ +	−
Sodium pyruvate	+ +	−
Sodium acetate	+ +	−
Sodium hydrogen citrate	+	−
Sodium succinate	+ +	−
Sodium fumarate	+ +	−
Sodium hydrogen malate	+ +	−
Oxaloacetic acid	+ +	−

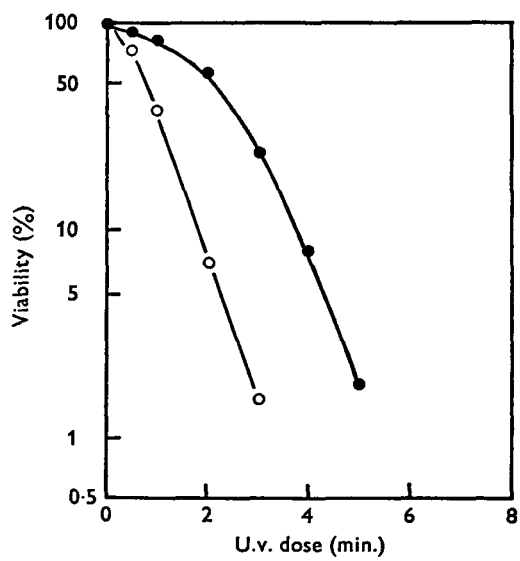

Fig. 1. The u.v. survival curves of wild-type strain 13 (●—●) and a *sgp* mutant (○—○). Viabilities are expressed as percentages of the colonies which grew from irradiated conidia, compared with colonies which grew on control plates spread with unirradiated conidia (this eliminates differences due to the lower viability of unirradiated mutant conidia compared to wild type).

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discernible lag phase before the killing effect on the mutants became apparent, whereas with strain 13 a lag of about period 1·5 min occurred (Lennox & Tuveson, 1967).

(iii) *Genetic analysis of the mutants*

(*a*) *Heterokaryon test.* The heterokaryon test (Jinks, 1963) is a useful means of establishing whether a characteristic is inherited chromosomally or extra-chromosomally and depends on the fact that haploid nuclei from different strains are present in a heterokaryotic cell. Since all divisions within that cell are mitotic a heterokaryon should produce conidia genotypically identical with those of the original strains. Five *sgp* mutants, one from each functional group (see section (*b*) below), were tested by forming heterokaryons between them and a white conidial mutant of strain 13. Four heterokaryotic heads, containing green and white conidial chains, from each heterokayron were examined by plating on MM and determining the genotypes of the colonies growing from the conidia. In all cases the genotypes of the original and extracted stains of the heterokaryons were the same. The test was repeated using a yellow conidial mutant of strain 13 and the same results were obtained. It was concluded that the mutants were inherited chromosomally.

Table 2. *Summary of the results of the mitotic haploidization analysis of the* sgp *mutants*

Cistron	Linkage group	No. of mutants isolated
sgp-1	VII	6
sgp-2	V	5
sgp-3	III	7
sgp-4	I	1
sgp-5	IV	5

(*b*) *Complementation tests.* The mutants were cross-inoculated together in all possible combinations and examined for perithecial production. They were found to fall into five groups, containing 6, 5, 7, 1 and 5 mutants respectively, members of the same group not forming perithecia when inoculated together. Standard complementation tests were performed between representative members of the functional groups. Members of the same group did not complement in stable trans-heterokaryotic or diploid association, members of different groups did complement. Each group was regarded as corresponding to a cistron (Benzer, 1957). The corresponding loci have been termed *sgp* loci and these are distinguished from each other by a number following the *sgp* description, namely *sgp-1* to *sgp-5* respectively. No cases of intra-cistronic complementation were found.

(*c*) *Mitotic haploidization.* By mitotic haploidization analysis of representative members of the functional groups the five cistrons were assigned to linkage groups VII, V, III, I and IV respectively. The results are summarized in Table 2.

(*d*) *Meiotic analysis.* R6;*paba1*;*w3* and R7;*paba1*;*w3*, both mutants of cistron *sgp-3*, were crossed with a strain carrying the markers *ad20, bi1;phen2; s3; cha.*

The mutant loci were found to recombine freely with all the markers involved in the cross except *phen2*. The recombination frequencies of the R6 and R7 mutant sites with *phen2* were 40·3 and 37·8 % respectively. No other suitable markers are present in this region of group III and so the mutants could not be mapped more precisely.

R10,*paba1;w3*, a mutant of cistron *sgp-4*, was crossed with strains carrying the markers *ad14,y,bi1; meth1*. The mutant locus was unlinked to *meth1* or *adh4* on the left arm of linkage group 1 but showed recombination frequencies of 40·7 and 40·0 % with *paba1* and *bi1* respectively. It was concluded that the mutant locus mapped to the right of *bi1*. Since it lay beyond the previously mapped extremity of linkage group I it could not be positioned with greater accuracy.

4. DISCUSSION

The *sgp* mutants can be seen to differ from the extrachromosomally inherited, respiratory-deficient mutants of yeast and *N. crassa* in several ways. The cytochrome system, mitochondrial structure, and resistance to respiratory inhibitors of the mutants were apparently normal, and they are inherited chromosomally (cf. Ephrussi, 1953; Nagai, Yanagishima & Nagai, 1961). Nevertheless, the *sgp* mutants are of considerable interest from both a genetical and biochemical viewpoint. They show a diversity of abnormal characteristics: they are unable to grow on a range of carbon sources and even on glucose their growth rate is slow and their morphology abnormal; their pigment production is reduced; and they show increased sensitivity to the lethal effects of u.v. irradiation. This is suggestive of some primary and fundamental defect which results in a wide range of secondary phenotypic effects (Houghton, 1970). Genetically the *sgp* mutants are unusual in that whilst mutants of the same cistron do not produce perithecia, crosses between mutants of different cistrons lead to the production of perithecia all of which are crossed. It is hoped that this characteristic may be of value in genetic complementation studies in *A. nidulans*.

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REFERENCES

Benzer, S. (1957). The elementary units of heredity. In *The Chemical Basis of Heredity*, pp. 70–93. (Ed. W. D. McElroy and B. Glass.) Baltimore: John Hopkins Press.

Boulter, D. & Derbyshire, E. (1957). Cytochromes of fungi. *Journal of Experimental Botany* **8**, 313–318.

Ephrussi, B. (1953). *Nucleo-cytoplasmic Relations in Micro-organisms: Their Bearing on Cell Heredity and Differentation*. Oxford: Clarendon Press.

Forbes, E. (1959). Use of mitotic segregation for assigning genes to linkage groups in *Aspergillus nidulans*. *Heredity* **13**, 67–80.

Houghton, J. A. (1970). Biochemical investigation of the slow-growing non-perithecial (*sgp*) mutants of *Aspergillus nidulans*. (In preparation.)

Jinks, J. L. (1963). Cytoplasmic inheritance in fungi. In *Methodology in Basic Genetics*, pp. 325–354. (Ed. W. J. Burdette.) San Francisco: Holden-Day, Inc.

LENNOX, J. E. & TUVESON, R. W. (1967). The isolation of ultraviolet sensitive mutants from *Aspergillus rugulosus*. *Radiation Research* **31**, 382–388.

LHOAS, P. (1961). Mitotic haploidisation by treatment of *Aspergillus niger* diploids with para-fluorophenylalanine. *Nature* **190**, 744.

MCCULLY, K. S. & FORBES, E. (1965). The use of para-fluorophenylalanine with 'master strains' of *Aspergillus nidulans* for assigning genes to linkage groups. *Genetical Research* **6**, 352–359.

MACKINTOSH, M. E. & PRITCHARD, R. H. (1963). The production and replica plating of micro-colonies of *Aspergillus nidulans*. *Genetical Research* **4**, 320–322.

MITCHELL, M. B. & MITCHELL, H. K. (1952). A case of 'maternal' inheritance in *Neurospora crassa*. *Proceedings of the National Academy of Sciences, U.S.A.* **38**, 442–449.

MITCHELL, M. B., MITCHELL, H. K. & TISSIERES, A. (1953). Mendelian and non-Mendelian factors affecting the cytochrome system in *Neurospora crassa*. *Proceedings of the National Academy of Sciences, U.S.A.* **39**, 606–613.

NAGAI, S., YANAGISHMA, N. & NAGAI, H. (1961). Advances in the study of respiration deficient (RD) mutation in yeast and other micro-organisms. *Bacteriological Reviews* **25**, 404–426.

OGUR, N., LINDEGREN, G. & LINDEGREN, C. C. (1954). A simple screening test for genetics studies of respiration deficiency in yeast. *Journal of Bacteriology* **68**, 391–392.

PONTECORVO, G., ROPER, J. A., HEMMONS, L. M., MACDONALD, K. D. & BUFTON, A. W. J. (1953). The genetics of *Aspergillus nidulans*. *Advances in Genetics* **5**, 141–238.

PONTECORVO, G., TARR GLOOR, E. & FORBES, E. (1954). Analysis of mitotic recombination in *Aspergillus nidulans*. *Journal of Genetics* **52**, 226–237.

RAUT, C. (1954). Heritable non-genic changes induced in yeast by ultra-violet light. *Journal of Cellular and Comparative Physiology* **44**, 463–475.

ROPER, J. A. (1952). Production of heterozygous diploids in filamentous fungi. *Experientia* **8**, 14–15.

WOODWARD, V. W., DEZEEUW, J. R. & SRB, A. M. (1954). The separation and isolation of particular biochemical mutants of *Neurospora* by differential germination of conidia, followed by filtration and selective plating. *Proceedings of the National Academy of Sciences, U.S.A.* **40**, 192–200.