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The two-way selection of mutants and revertants in respect of acetate utilization and resistance to fluoro-acetate in *Aspergillus nidulans*

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1. INTRODUCTION

Two-way selection, i.e. selection of auxotrophs from prototrophs and vice versa, can be carried out in the following way. Consider a metabolite A and its toxic analogue A′. A would be utilized to B and A′ to B′. Strains which fail to convert A to B will also fail to convert A′ to B′ and hence might become resistant to A′. These strains should be auxotrophic for B or for other metabolites coming after the block if the pathway involved is essential. Thus selection for resistance to an analogue might lead to the isolation of auxotrophic strains. Furthermore, selection for prototrophy from these auxotrophic strains should yield revertants sensitive to the analogue.

In *Aspergillus nidulans* this principle was applied successfully (Apirion, 1962) to the selection of mutants resistant to fluoro-acetate as a consequence of a block in the utilization of acetate. By plating these resistant mutants, unable to utilize acetate, in a medium in which the only source of carbon was acetate, it was then possible to select revertants able to utilize acetate and sensitive to fluoro-acetate.

The earlier work had shown that mutants selected because of their resistance to fluoro-acetate fell into two distinct classes. Those of one class grow extremely poorly on acetate as sole carbon source, and are designated *fac* (*f* in former publications, Apirion, 1962, 1963; Pontecorvo, 1963). Those of the other class are still able to grow almost normally on acetate and are designated *fan* (*fa* and *fe* in former publications).

The work reported here suggests that mutants of this second class also owe their fluoro-acetate resistance to blocks in the utilization of acetate.

In addition the present paper describes in detail the selective techniques, the properties of the mutants on which the selective techniques were based, the formal genetics of the mutants and revertants, and touches on some aspects of acetate metabolism in *Aspergillus nidulans*.

2. MATERIALS AND METHODS

For general techniques for *Aspergillus nidulans*, see Pontecorvo (1949); Roper (1952); Pontecorvo, Roper, Hemmons, McDonald & Bufton (1953); Pontecorvo

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& Käfer (1958); Käfer (1958); Forbes (1959, 1963) and *Aspergillus News Letter*, No. 4, 1963, pp. 12–14. 'Minimal' medium is an inorganic salt, glucose medium. 'Basal' minimal medium is the same but without glucose.

All chemicals used were of analytical grade unless otherwise stated and the water was singly distilled. Percentages are expressed as w/v.

(*a*) *Acetate Medium* (AM). Ammonium acetate, 12 g.; sodium chloride, 2 g.; magnesium sulphate ($7H_2O$), 0·5 g.; potassium dihydrogen phosphate, 3 g.; ferrous and zinc sulphate, traces; agar, 12 g.; pH adjusted to 6·1 by hydrochloric acid; final volume 1000 ml.

(*b*) *Fluoro-acetate Medium* (FAM). Glucose, 5 g.; sodium nitrate, 2 g.; potassium chloride 1 g.; magnesium sulphate ($7H_2O$), 0·5 g.; potassium dihydrogen phosphate, 3 g.; ferrous and zinc sulphate, traces; fluoro-acetic acid (technical), 40 g.; agar, 15 g.; pH adjusted to 6·1 by concentrated NH_4OH; final volume 1000 ml.

(*c*) *Succinate Medium* (SM). Basal minimal medium +1% succinic acid, pH 4–4·5 adjusted by NH_4OH.

(*d*) *Acetate Medium and Succinate* (AMS). Acetate medium plus succinate to give a final concentration of succinic acid 0·2% (succinate adjusted to pH 4 by NH_4OH).

(*e*) *Succinate Fluoro-acetate Medium* (SFAM). Succinate medium plus fluoro-acetate to give a final concentration of fluoro-acetic acid 0·5% (pH 4–4·5 adjusted by NH_4OH).

In all selections, at the plating densities employed, Grigg effects' (Grigg, 1952) were avoided. All but one of the strains used in this work were from the collection in the Department of Genetics, The University of Glasgow. Strain *bi1*; *orn9 cha* was kindly supplied by Dr E. Käfer, McGill University. Incubation temperature was 37°C. throughout all the work.

3. CHARACTERISTICS OF WILD-TYPE AND OF MUTANTS

(i) *Wild-type*

By auxanographic (Pontecorvo, 1949) and other growth tests it was found that: (*a*) acetate is a good sole carbon source for *Aspergillus nidulans* under suitable conditions (see Materials and Methods) while citrate, *cis*-aconitate, succinate, malate and fumarate are poor sources; (*b*) fluoro-acetate inhibits growth on either acetate, glucose, fructose, maltose or lactose; and (*c*) succinate or citrate inhibit growth on acetate.

(ii) fac *mutants*

fac mutants are isolated and identified because of their ability to grow on fluoro-acetate medium and their failure to grow as well as the wild-type on acetate medium. All of the forty-four *fac* mutants tested are able to grow to a very slight but varying extent on acetate medium, both in liquid culture and agar. The slight basal level of growth of *fac* mutants on acetate is taken to indicate the

existence of a minor pathway of acetate utilization alternative to a major one blocked in *fac* mutants (see Sections iii and iv). The slight growth of *fac* mutants on acetate medium is completely inhibited by the addition of 0·2% succinate.

All *fac* mutants tested can be made to grow better on acetate as sole carbon source by supplementing acetate medium with proline in amounts (0·01–0·02%) insufficient to provide the carbon for all the growth. Auxanographic tests show that growth of *fac* mutants on medium containing L-proline and acetate is superior to the combined growth of *fac* mutants on acetate and proline separately. This phenomenon will be referred to hereafter as the 'Proline effect'.

Citrate and *cis*-aconitate were found to be very poor carbon sources for both *fac* strains and wild-type, while succinate, fumarate and malate are better carbon sources for *fac* strains than for wild-type. This difference is sufficient to permit a clear distinction between *fac* and *fac*$^+$ strains (Plate I). *fac* mutants at one locus (*facA*) grow better on succinate medium than *fac* mutants at the other two loci (*facB* and *facC*). To demonstrate this difference plates should be incubated for 4–5 days.

(iii) fan *mutants*

fan mutants, like *fac* mutants, are resistant to fluoro-acetate, but unlike *fac* mutants they are able to grow on acetate medium, though somewhat more slowly than corresponding wild-type strains. Their growth on acetate medium can be completely inhibited by the addition of 0·2% succinate, while the corresponding wild-type, although somewhat inhibited, can still form colonies of appreciable size.

(iv) fac fan *strains*

Strains carrying the combination of a *fac* mutant and a *fan* mutant, regardless of the loci, are more resistant to fluoro-acetate and show less growth on acetate medium than either of the corresponding strains carrying one mutant singly. Those combinations which are completely unable to grow on acetate medium do not respond to proline.

On succinate medium *fac fan* strains resemble *fac* strains.

Supplementation of minimal medium (glucose 1%) with acetate (4%, pH adjusted to 6·5 with ammonia) inhibits the growth of wild-type strains as well as of *fac*, *fan*

Table 1. *Growth of* fac *and* fan *strains on various media*

	fac$^+$ *fan*$^+$	*fac*$^-$ *fan*$^+$	*fac*$^+$ *fan*$^-$	*fac*$^-$ *fan*$^-$
Glucose minimal medium	+ + +*	+ + +	+ + +	+ + +
Fluoro-acetate medium	−	+ −	+ −	+ +
Acetate medium	+	− +	+ −	−
Succinate medium	+	+ +	+	+ +
Acetate medium and succinate	+ −	−	−	−

* Symbols denote qualitative estimation of growth, from no growth − to very good growth + + +. The order of symbols is as follows: −, − +, + −, +, + +, + + +. Symbols on different media are not strictly comparable.

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and *fac fan* strains (see Vanderwinkel *et al.*, 1963). *fac*, *fan* and *fac fan* strains are indistinguishable from *fac*$^+$ *fan*$^+$ strains on minimal medium containing glucose as the sole carbon source. A summary of growth characteristics of the different mutants on various media is given in Table 1.

4. SELECTION OF FORWARD MUTANTS

(i) fac *mutants*

fac mutants were isolated by various techniques, only the most efficient two are described here.

(a) Sandwiching conidia between two layers of fluoro-acetate medium

Suspensions of up to about 10^8 conidia/ml. were spread in volumes of 0·1–0·2 ml. on the surface of fluoro-acetate medium (25 ml./dish). After a few hours' incubation a further thin layer of the same medium (3–5 ml./dish) was poured on top. Resistant mutants appeared after 3–4 days, and were isolated by the sixth or seventh day and, after purification, tested for their ability to grow on acetate medium. The frequency of the resistant mutants varied from $1–7 \times 10^{-7}$. About one-quarter of the resistant mutants failed to grow on acetate medium. A reconstruction experiment with one of those unable to grow on acetate showed that the selection technique is adequate for platings of a small number of resistant conidia together with up to $0·5 \times 10^6$ sensitive conidia per dish.

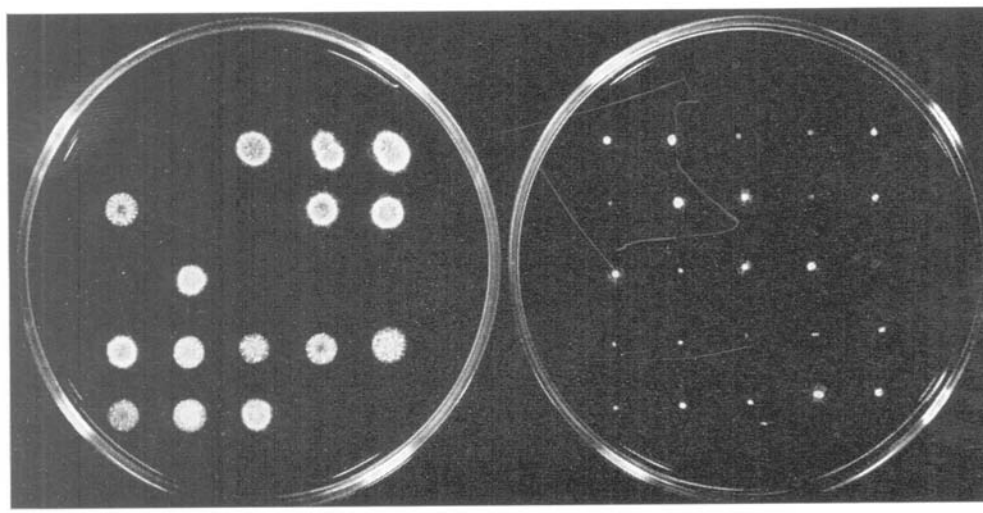
(b) Pre-germinating conidia on succinate medium before adding succinate fluoro-acetate medium

As *fac* strains grow slightly better than *fac*$^+$ strains on succinate medium (Plate I), selection of forward mutants is more efficient when both resistance to fluoro-acetate and better growth on succinate medium are used for selection. However, since fluoro-acetate prevents the germination of conidia on succinate medium, conidia of sensitive strains had to be pre-germinated before adding fluoro-acetate. The conidia were mixed with 3–5 ml. of succinate medium and poured on top of a previously poured layer of succinate medium (20–25 ml.). After overnight incubation, 3–5 ml. of succinate fluoro-acetate medium were poured as a top layer. Resistant colonies arose after 4–6 days' incubation, with a frequency of $5–20 \times 10^{-7}$.

The vast majority (eighty-six out of eighty-nine tested) of the mutants isolated by this method were both resistant to fluoro-acetate and unable to grow on acetate medium. This method was used extensively later in this work. In reconstruction experiments this technique was found to be adequate for platings of up to 10^7 sensitive conidia per dish.

(ii) fan *mutants*

fan mutants have been isolated either as first-step mutants like *fac* mutants using fluoro-acetate medium, or as additional mutants in *fac* strains. *fac* colonies



Growth of 26 strains (15 *facA*$^+$ and 11 *facA3*) from single ascospores of the cross *y*; *nic2 ribo5* × *w3*; *pyro4*; *facA3* segregating for *facA*$^+$/*facA3*. On acetate medium (left) *facA*$^+$ strains grow well, while *facA3* strains fail to grow. On succinate medium (right) *facA*$^+$ strains do not grow—they start to grow later—while *facA3* strains give appreciable spidery growth.

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growing on fluoro-acetate medium give rise to more vigorously growing sectors. These were found to result from further mutations, some of which were subsequently found to map at the same locus as *fan* mutants isolated as first-step mutants.

A reconstruction experiment showed that by sandwiching conidia between layers of fluoro-acetate medium recovery of *fan* mutants is satisfactory for platings of up to 3×10^6 wild-type conidia and a few *fan* conidia per dish.

5. SELECTION FOR REVERTANTS

(i) *Revertants of* fac *mutants*

Revertants were isolated by embedding conidia from *fac* strains in acetate medium plus the growth factors required by the various strains used.

Of forty-four *fac* strains tested for reversion, thirteen gave revertants able to grow on acetate. The revertants of eight strains were also tested for their resistance to fluoro-acetate. Revertants of seven *fac* mutants were like the wild-type, both able to grow on acetate and sensitive to fluoro-acetate, the exception—*facA3*—giving revertants able to grow on acetate but still resistant to fluoro-acetate.

Table 2 tabulates the reversion tests for the thirteen *fac* mutants which gave revertants. At least 2×10^8 conidia were tested from each of thirty-one further *fac* mutants without obtaining revertants: in one case (*facA4*) no revertants arose out of $2{\cdot}8 \times 10^9$ conidia. That the failure of these thirty-one *fac* mutants is genuine and not merely inability to detect revertants under the conditions of the test is indicated by the fact that nitrous acid induces reversion of the strain *facB101* which reverts spontaneously, but does not induce reversion of the strain *facA4* which does not revert spontaneously (Table 3).

Table 2. *Spontaneous reversion of* fac *mutants to the ability of growing on acetate as sole carbon source*

fac mutant tested	Total conidia plated ($\times 10^6$)	Back mutants per 10^6 plated conidia	Back mutants tested for resistance to fluoro-acetate	
			No. tested	% resistant
facA2	15	0·53	8	0
facA3	144	0·21	31	100
facC8	200	0·58	52	0
facC10	320	0·51	52	0
fac28	280	0·24	—	—
fac33	200	0·02	—	—
fac35	210	0·01	—	—
facB101	365	0·02	6	0
facC102	370	0·44	—	—
facC301	250	0·94	—	—
facA305	225	0·01	3	0
facC307	370	1·58	21	0
facB309	1040	0·07	24	0

Table 3. *Induction of reversion of* fac *mutants to the ability of utilizing acetate as sole carbon source by treatment with nitrous acid**

fac mutant tested	Treatment	Total conidia plated ($\times 10^6$)	Survival %	Revertants No.	Revertants Per 10^6 plated conidia
facB101	None	280	100	3	0·01
	Nitrous acid	32	58	24	0·75
facA4	None	300	100	0	0
	Nitrous acid	40	51	0	0

* Conidia treated with 0·0145 M nitrous acid for 10 min.; see: Siddiqi, 1962.

Satisfactory reconstruction experiments were achieved for platings of densities of up to 5×10^7 conidia per dish.

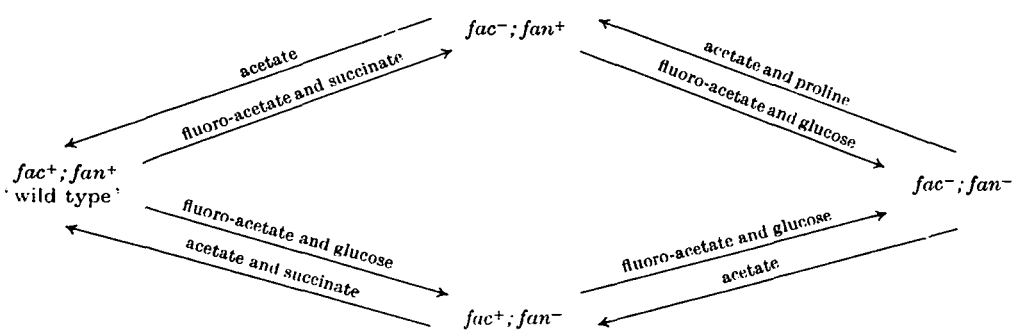
Revertants isolated are designated by the symbol 'b' (for backmutant) followed by the isolation number of the revertant and used as a prefix to the symbol of the *fac* mutant from which the revertant arose; e.g., *b2-facB101.*

(ii) *Revertants of* fan *mutants*

The growth of *fan* mutants on acetate medium is strongly inhibited by the addition of succinate or citrate. This property could be used for the selection of revertants. In reconstruction experiments good recovery was achieved of a few fac^+ fan^+ conidia from up to 6×10^6 fac^+ fan^- conidia. For this selection conidia have to be plated on the surface of the medium.

(iii) *Other selections*

Exploratory experiments showed that the other selections indicated in Fig. 1 are also effective. These selections are: (1) fac^- fan^- strains from either fac^- fan^+ or fac^+ fan^- strains, using fluoro-acetate medium (fluoro-acetate 3–4%, glucose



Text-fig. 1. A diagrammatic summary of the eight ways of 'forward' and 'back' selection based on the metabolism of acetate and fluoro-acetate. *For convenience genotypes arising as a result of suppression are ignored.*

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1%); (2) both *fac*$^+$ *fan*$^-$ and *fac*$^-$ *fan*$^+$ strains from *fac*$^-$ *fan*$^-$ strains, using acetate medium for the former and acetate medium and 0·01–0·02% L-proline for the latter. An idealized summary of the various selections is represented in Text-fig. 1.

6. FORMAL GENETICS OF MUTANTS

(i) fac *mutants*

Fourteen *fac* mutants, selected by means of one or the other of the techniques mentioned, were used for genetic studies. They include: eight mutants, *fac301*, *302*, *303*, *305*, *306*, *307*, *308*, and *309* isolated by the succinate–fluoro-acetate technique, and six mutants, *fac2*, *3*, *4*, *8*, *101*, and *102* isolated by the fluoro-acetate technique. Of these fourteen strains nine give revertants, while five do not.

(*a*) *Number of loci*

In crosses of the type *fac* × *fac*$^+$ a 1:1 segregation ratio of *fac* to *fac*$^+$ was always observed. In all cases tested (about 800 segregants from five different crosses), resistance to fluoro-acetate was inseparable by recombination from inability to grow on acetate as sole carbon source.

The fourteen *fac* mutants were crossed pairwise in a number of combinations. The analysis of these crosses shows clearly that these fourteen *fac* mutants map at three meiotically unlinked loci, designated *facA*, *B* and *C*; as crosses involving certain pairs of mutants gave no wild-type recombinants while crosses involving other pairs gave results not significantly different from a three to one ratio of mutant to wild-type in about 100 segregants tested. The mutants are given the symbol of the locus followed by the number given to each mutant when first isolated. The fourteen *fac* mutants analyzed are distributed as follows between the three loci:

locus *facA*: *fac2*, *fac3*, *fac4*, *fac303*, *fac305*, *fac306*
locus *facB*: *fac101*, fac *302*, *fac308*, *fac309*
locus *facC*: *fac8*, *fac102*, *fac301*, *fac307*

Mutants at locus *facA* are characterized by better growth on succinate medium than mutants at the other two loci.

In crosses between two *fac* mutants at different loci, only two types of colony could be distinguished, one having a *fac* phenotype and the other having a *fac*$^+$ phenotype, suggesting that the phenotype of a strain carrying two *fac* mutations at different loci is indistinguishable in these tests from that of a strain carrying a single *fac* mutation.

The cross *paba1 y*; *ad23*; *facA3* × *bi1*; *w3*; *facA4* was analyzed by plating a heavy suspension of ascospores on acetate medium plus *para*-aminobenzoic acid and an appropriately diluted suspension on minimal medium plus *para*-aminobenzoic acid. The proportion of *fac*$^+$ recombinants among the total *bi*$^+$ *ad*$^+$ recombinants of this cross was 0·1%, i.e. much higher than the reversion rates of the strains crossed. Thus the system might be suitable for fine genetic analysis.

(*b*) *Recessivity of* fac *mutants to their wild-type alleles*

The recessivity of *fac* mutants to their wild-type alleles was established by testing growth of diploids (Roper, 1952) heterozygous for *fac* mutants. Eleven *fac* mutants from the three loci were tested and all fell into the same pattern, i.e. they were able to grow on acetate medium and were sensitive to fluoro-acetate.

By synthesizing diploids between various pairs of *fac* mutants it was found that pairs of *fac* mutants at different loci complement, while pairs at the same locus do not. Fourteen combinations including all fourteen *fac* mutants which were located were tested.

(*c*) *Location*

By haploidization with *para*-fluorophenylalanine of diploids synthesized between a *fac* strain and a tester strain marked on all chromosomes (Pontecorvo, Tarr Gloor & Forbes, 1954; Pontecorvo & Käfer, 1956, 1958; Forbes, 1959, 1963; Morpurgo, 1961; Lhoas, 1961), locus *facA* was assigned to linkage group V, and loci *facB* and *facC* to linkage group VIII.

facA3 was crossed to a strain carrying the markers *nic2 ribo5*, but no linkage was detected. *facB101* was found to be located between *ribo2* (5% recombination) and *arg3* (18% recombination). To confirm this order, crosses of the type *facB101 ribo2* × *fac*$^+$ *ribo*$^+$ were made with *arg3* either in *cis* or in *trans* with *facB101*. Ascospores from these crosses were sandwiched between succinate and succinate fluoro-acetate media in order to select for *ribo*$^+$ *fac* recombinants. These recombinants were analyzed for the segregation of *arg3* (Table 4). The results confirmed the order *arg3 facB101 ribo2*.

Mutant *facC307* was used in crosses with most other known markers of the eighth linkage group (*orn7*, *arg3*, *ribo2*, *cha*, *palB7* and *gal7*), but no linkage between it and any of them was detected among about 200 colonies from each cross.

Table 4. *Order of the mutants* arg3 facB101 ribo2

Cross			No. of *ribo*$^+$ *fac* recombinants	
			Analyzed	Carrying *arg3*
arg3	*facB101*	*ribo2*		
			259	172
+	+	+		
+	*facB101*	*ribo2*		
			103	21
arg3	+	+		

ribo$^+$ *fac* recombinants were selected and the segregation of *arg3* measured among them. Selection was made by sandwiching between SM and SFAM ascospores from hybrid perithecia. The first selection resulted in 33·9% recombination between *facB* and *arg*, the second in 20·4%; this difference is probably due to poor viability of strains carrying the *arg3* marker.

(ii) *Revertants*

Crosses between revertant strains and wild-type strains showed that the tested revertants of mutants *facA3*, *facC8*, *facC102* and *facC307* were due to mutation at a supressor locus or loci unlinked to that of the mutant which had reverted, while the revertants of mutants *facB101* and *facB309* were due to mutation at a site very closely linked or even within the same cistron as that of the mutant which had reverted. From each cross at least 200 colonies were analyzed.

The synthesis of diploids homozygous for *facB101* and heterozygous for either of two of its suppressors threw some light on dominance relations. The diploids were as follows:

(1) *w3; pyro4; facB101 b1-facB101/y; s1; facB101*
(2) *w3; pyro4; facB101 b2-facB101/y; s1; facB101*

Both diploids, which in the relevant respects were of the type $\frac{facB101\ b\text{-}facB101}{facB101\quad +}$, were found to have a *fac*$^+$ phenotype on acetate and fluoro-acetate media. The combination of *facB101* and its suppressor is therefore dominant, as one would expect for an intra-cistron suppressor or for 'true' backmutation.

(iii) fan *mutants*

Genetic analysis was carried out by means of crosses of four types: (1) *fan* × *fac*, (2) *fan* × *fan*, (3) *fan fac* × *fan*, and (4) *fan fac* × *fan fac*. Analysis was carried out on acetate, fluoro-acetate and acetate-succinate media as required. On the first medium *fac* strains are distinguished from *fac*$^+$ strains irrespective of whether they are *fan* or *fan*$^+$. On the second medium *fac fan* strains are distinguished from either *fac* or *fan* or *fac*$^+$ *fan*$^+$ strains. On the third medium *fac*$^+$ *fan*$^+$ strains are distinguished from either *fac* or *fan* or *fac fan* strains (see Section 3 and Table 1).

Two *fan* mutants—*fanA1* and *fanE7*—isolated from a *fac*$^+$ *fan*$^+$ strain by sandwiching conidia in fluoro-acetate medium, were crossed with one another and were found to recombine freely. These two mutants were crossed with three *fac* mutants, each representing a different *fac* locus. The results of all six crosses showed that these two *fan* loci are unlinked meiotically to any of the three *fac* loci. This was repeated with a *fan* mutant, which had its origin in an additional mutation in a *fac* strain and was separated from it by outcrossing. Again in no case was meiotic linkage detected.

(*a*) *Number of loci*

Twelve *fan* mutants which had their origin in an additional mutation in *fac* strains (sectors from *fac* colonies on fluoro-acetate medium), and three *fan* mutants isolated from wild-type, were chosen for further genetic analysis. They were crossed in some of the possible pairs and the analysis of the crosses shows that

these mutants fall into five loci, *fanA*, *B*, *C*, *D*, and *E*, only two of which, *C* and *E*, are linked (20% recombination). Grouping of these mutants is as follows:

Locus *fanA fan1, 3, 4, 10, 11, 12, 14, 16*
,, *fanB fan52, 54, 60*
,, *fanC fan101*
,, *fanD fan 151*
,, *fanE fan5, 7*

From any *fac* colony on fluoro-acetate medium two morphologically distinct types of sectors grow out. One of these types results exclusively in *fac fanB* strains, while the vast majority of the other type of sector results in *fac fanA* strains.

In crosses between two *fan* mutants at different loci only two types of colony could be distinguished on the basis of growth on the various media, one having a *fan* phenotype and the other having a *fan*$^+$ phenotype. Thus, as in crosses between *fac* mutants, the double *fan* recombinant appears to be indistinguishable from either single *fan* parent.

(*b*) *Recessivity of* fan *mutants to their wild-type alleles*

Growth tests of diploids heterozygous or heteroallelic for *fan* mutants (Tables 5, 6) show that each mutant tested is recessive. Pairs of *fan* mutants mapping at different loci complement in the double heterozygote, but pairs of mutants mapping at the same locus do not complement.

(*c*) *Location*

By haploidization of diploids between mutants at each of four *fan* loci (*A*, *B*, *C*, and *D*) and tester strain MSD (Forbes, 1959, 1963), it was found that locus *fanA* is in linkage group V, *fanB* in VII, *fanC* in VI and *fanD* in VIII.

In an analysis of 200 colonies which arose from a cross involving the markers

Table 5. *Growth of diploids* heterozygous or heteroallelic for* fan *mutants on acetate medium plus succinate and on fluoro-acetate medium*

Combination	Media	
	Acetate and succinate	Fluoro-acetate
fanA1/fan$^+$	+	−
fanA3/fan$^+$	+	−
fanB52/fan$^+$	+	−
fanE5/fan$^+$	+	−
fanA1/fanA3	−	+
fanE5/fanE7	−	+
fanA1/fanB52	+	−
fanA1/fanE5	+	−
fanA1/fanE7	+	−
fanA3/fanE5	+	−

* Diploids are all homozygous *fac*$^+$

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Table 6. *Growth of diploids* heterozygous or heteroallelic for* fan *mutants on fluoro-acetate medium*

Combination	Growth**
facA303 fanA3/fac⁺ fan⁺	−
facA303 fanB52/fac⁺ fan⁺	−
facA3 fanC101/fac⁺ fan⁺	−
facB302 fanD151/fac⁺ fan⁺	−
facA303 fanB60/facA303 fan⁺	+
facA3 fanA1/facA303 fanA3	+ +
facA303 fanA3/facA303 fanA4	+ +
facA303 fanA3/facA303 fanA12	+ +
facA303 fanA3/facA303 fanB52	+

* Diploids are heterozygous or homozygous for *fac* mutants.

** − denotes growth like that of a wild-type strain on fluoro-acetate medium. + denotes growth like that of a *fac* strain on fluoro-acetate medium. + + denotes growth like that of a *fac fan* strain on fluoro-acetate medium.

nic2, fan*A3*, *facA303* and *ribo5* (all in the fifth linkage group) no obvious linkage was found between any pair of markers.

The mutant *fanB52* was crossed with strains carrying markers of the seventh linkage group (*nic8*, *palD8* and *mal1*) but it recombined freely with all of these markers.

No meiotic analysis of *fan* mutants of other loci was carried out.

7. DISCUSSION

The results reported here substantiate the principle outlined in the introduction for two-way selection and throw some light on acetate metabolism in *Aspergillus nidulans*.

The system might enable a thorough study of the mutational or recombinational aspects of one or more loci, for instance the study of intragenic suppressors.

The findings suggest that in wild-type *Aspergillus nidulans* two pathways operate in acetate utilization—a *major* and a *minor*. *fac* mutants are blocked in the major pathway and *fan* in the minor.

That *fac* and *fan* mutants belong to two pathways which operate in parallel in acetate utilization is indicated by the fact that their effects are cumulative in respect of acetate utilization and resistance to fluoro-acetate. Every strain carrying both a *fac* and a *fan* mutant, irrespective of its loci, utilizes acetate less well and is more resistant to fluoro-acetate than either of its parents. On the other hand, combinations of two *fac* mutants or *fan* mutants do not differ from the parental strains in these respects.

The fact that *fac* and *fan* strains are resistant to fluoro-acetate to roughly the same limited extent, as compared to *fac fan* strains might indicate that in both types of strain, in which two different pathways for acetate utilization are in operation, approximately the same amount of fluoro-acetate is metabolized.

These two pathways could possibly be two different permeases for acetate uptake, but this is unlikely in view of the fact that *fac* strains are able to utilize acetate ('proline effect'), and the fact that growth of *fac*, *fan* and *fac fan* strains on glucose is inhibited, as is that of the wild-type, in the presence of acetate.

The enhancing effect of proline on growth of *fac* mutants on acetate medium can be understood if endogenous proline is the limiting factor for protein synthesis under these conditions.

Inhibition of growth of wild-type and *fan* and *fac* strains on acetate medium by succinate might be due to inhibition and/or repression of the enzyme isocitratase, which is needed for growth on acetate as the sole carbon source (Kornberg & Elsden, 1961).

SUMMARY

An extension of two-way selection (i.e. selection of mutant from wild-type and vice versa within the same locus and with the same efficiency) to four different mutational or segregational situations was made possible by using acetate, fluoro-acetate and other substances related to their metabolism.

Two types of mutants resistant to fluoro-acetate were selected, the first of which (designated *fac*) cannot grow on acetate as the sole carbon source, while the second (designated *fan*) can.

Commencing with either a *fac* or a *fan* strain a double *fac fan* strain may be isolated, which is much more resistant to fluoro-acetate than either single mutant strain. Such double mutant strains may also be obtained by crossing a *fac* to a *fan* strain. Various characteristics of growth response of these strains on various media were observed.

fac mutants are recessive and map in three meiotically unlinked loci, one in linkage group V and two in linkage group VIII.

fan mutants are recessive and map in five loci, one in each of the linkage groups V, VII and VIII, and two linked in linkage group VI.

Most *fac* mutants isolated did not revert and this failure is considered genuine. Of the revertants tested, most resulted from extra-cistron suppressors, while revertants of two *fac* mutants resulted from very closely linked or intra-cistron suppressors.

It is argued that the findings indicate the existence of two pathways for acetate utilization in *Aspergillus nidulans*, a major and a minor; *fac* mutants are blocked in the major pathway, *fan* mutants in the minor pathway.

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REFERENCES

APIRION, D. (1962). A general system for the automatic selection of auxotrophs from prototrophs and *vice versa* in micro-organisms. *Nature, Lond.* **195**, 959–961.

APIRION, D. (1963). Ph.D. Thesis, Glasgow University.

Aspergillus News Letter (1963). Glasgow list of located, or partially located, mutants of *Aspergillus nidulans*. No. 4, pp. 12–14.

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

FORBES, E. (1963). A strain with all chromosomes marked for use in haploidization. *Aspergillus News Letter*, **4**, 15.

GRIGG, G. W. (1952). Back mutation assay method in micro-organisms. *Nature, Lond.* **169**, 98–100.

KÄFER, E. (1958). An 8-chromosome map of *Aspergillus nidulans*. *Adv. Genet.* **9**, 105–145.

KORNBERG, H. L. & ELSDEN, S. R. (1961). The metabolism of C_2 compounds by micro-organisms. *Adv. Enzymol.* **23**, 401–470.

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

MORPURGO, G. (1961). Somatic segregation induced by p-fluorophenylalanine (pfp). *Aspergillus News Letter*, **2**, 10.

PONTECORVO, G. (1949). Auxanographic techniques in biochemical genetics. *J. gen. Microbiol.* **3**, 122–126.

PONTECORVO, G. (1963). Microbial genetics: retrospect and prospect. *Proc. R. Soc.* B, **158**, 1–23.

PONTECORVO, G. & KÄFER, E. (1956). Mapping the chromosome by means of mitotic recombination. *Proc. R. phys. Soc. Edinb.* **25**, 16–20.

PONTECORVO, G. & KÄFER, E. (1958). Genetic analysis based on mitotic recombination. *Adv. Genet.* **9**, 71–104.

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

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

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

SIDDIQI, O. H. (1962). Mutagenic action of nitrous acid on *Aspergillus nidulans*. *Genet. Res.* **3**, 303–314.

VANDERWINKEL, E., LIARD, P., RAMOS, F. & WIAME, J. M. (1963). Genetic control of the regulation of isocitratase and malate synthase in *Escherichia coli K12*. *Biochem. biophys. Res. Commun.* **12**, 157–162.