Acriflavin resistance controlled by chromosomal genes in Neurospora

K. S. Hsu
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Abstract
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Acetate is metabolised in microorganisms via the glyoxylate cycle. The steps of the glyoxylate cycle which are not part of the citric acid cycle are those involving the conversion of isocitrate to glyoxylate and succinate and the synthesis of malate from glyoxylate and acetyl-CoA. These two steps require the enzymes isocitrate lyase and malate synthetase, respectively. The presence of isocitrate lyase in Neurospora grown on acetate has previously been reported by Turian (1962 NN1:6). Before acetate is metabolized it is first converted to its metabolically active form, acetyl-CoA, by the enzyme acetyl-CoA a 10-fold induction.

Mutants have been isolated (using N-methyl-N'-nitro-N-nitroso guanidine as a mutagen followed by filtration enrichment) which cannot grow on acetate but which can grow on sucrose. These mutants fall into 6 distinct complementation groups where all mutants of each group complement all members of other groups. No within-group complementation has been observed.

The mutants were grown up on sucrose medium, transferred to acetate medium for 7 hours as outlined above and assayed for the presence of the glyoxylate cycle enzymes.

Members of one complementation group appear to lack completely isocitrate lyase activity or have only low levels of it and are being considered as mutants of the structural gene for this enzyme. Two other groups can be tentatively regarded as consisting of structural gene mutants for malate synthetase and acetyl-CoA kinase, respectively.

Mutants in a fourth complementation group possess all the glyoxylate cycle enzymes, inducible by acetate as in the wild type. The metabolic deficiency which results in these mutants being unable to grow on acetate seems obscure at present.

In the mutants in a fifth complementation group the enzymes of the glyoxylate cycle are not induced by transfer to acetate; all mutants possess the low levels of enzyme activities typical of the repressed state on sucrose. These mutations are recessive in heterocaryons. Investigations to see if there is an acetate permease deficiency here are being carried out.

The sixth complementation group contains mutants in which isocitrate lyase is not induced by acetate but malate synthetase and acetyl-CoA kinase are. After transfer to acetate, isocitrate lyase specific activities remain at the low sucrose-grown levels. Presumably these mutants lack a gene-determined, cytoplasmic product necessary for the induction of isocitrate lyase. The mutant gene is recessive to its wild type allele in heterocaryons.

The presumed structural gene for isocitrate lyase has been mapped between pob-2 and asp on linkage group 'JR.

This work was supported by a Postgraduate Studentship of the Agricultural Research Council. Genetics Department, John Innes Institute, Bayfordbury, Hertford, England.

This note brings up to date results obtained with acriflavine-resistant mutants in N. crassa. Preliminary results have been reported in NN1:5 (1962) and Genetics 47:961 (1962).

Protophrotic strains of 74A and 73a background differed in sensitivity when 2 μg acriflavine (Nutritional Biochem. Co.) per ml was present in the minimal slants. A cross between resistant (STA 4) and sensitive (Pa) strains indicated a 2:2 segregation for sensitivity in the tetrad. The gene in strain KHI, responsible for this low level of resistance, was located in the left arm of linkage group I and designated acr-1. A second gene, in strain KHZ, was isolated when the sub-culture of a single strain continued to grow in the presence of 10 μg/ml of the dye while others did not. Again, the difference was due to a single gene, designated acr-2, which was located to the left of the group III centromere marker sc (scumbo) in a 3-point cross, acr-2 x sc tryp-1 (Table 2). Tetrad data (unordered) from a cross using the same markers is consistent with this order. Assuming the order acr-2 acr-1 sc tryp-1, 11 tetrads of the 141 analyzed were singles in I, 69 were singles in II, 3 were 4-strand doubles in II, 1 was single in I and double in II, and 5 were I, II doubles. (Map distance acr-2 - sc, 6.0 units, C = 0.6.) Since sc is

<table>
<thead>
<tr>
<th>Isolation No.</th>
<th>Locus</th>
<th>Strain of origin*</th>
</tr>
</thead>
<tbody>
<tr>
<td>KH1</td>
<td>acr-1</td>
<td>ST4</td>
</tr>
<tr>
<td>KH2</td>
<td>acr-2</td>
<td>acr-2; ntr-1(34547), aux(34508) S</td>
</tr>
<tr>
<td>KH4**</td>
<td>acr-2</td>
<td>cr1; cot(C102); ylo(Y30539y) A</td>
</tr>
<tr>
<td>KH5</td>
<td>acr-2</td>
<td>cr(B123); cot(C102) A</td>
</tr>
<tr>
<td>KH6</td>
<td>acr-2</td>
<td>cr(B123); bal(B56) A</td>
</tr>
<tr>
<td>KH8</td>
<td>acr-3</td>
<td>cr(B123); cot(C102) A</td>
</tr>
<tr>
<td>KH9**</td>
<td>acr-2</td>
<td>cr(B123); bal(B56) A</td>
</tr>
<tr>
<td>KH10**</td>
<td>acr-2</td>
<td>cr(B123); ylo(Y30539y) A</td>
</tr>
<tr>
<td>KH14</td>
<td>acr-3</td>
<td>pe(Y8743m), f1 A</td>
</tr>
<tr>
<td>KH15</td>
<td>acr-3</td>
<td>pe(Y8743m), f1 A</td>
</tr>
</tbody>
</table>

*All in the background of St. Lawrence stocks 74A and 73a except the pe f1 strains which are in Lindegren background.

**Isolated from the plates where conidia had been exposed to UV.
about 2 units right of the centromere (Hungate, F. P. 1945 Ph. D. Thesis. Stanford University), it seems possible that acr-2 is just left of the centromere.

For the isolation of mutants resistant to higher acriflavin concentration, conidia harvested from both macroconidial and microconidial strains were plated on the surface of minimal agar containing 50 μg acriflavin/ml. The plated conidia were exposed to ultraviolet irradiation giving about 75% killing for some of the macroconidial strains. Resistant colonies were isolated after 5 or 6 days at WC, not more than one for each culture. Eight strains so isolated were single-gene mutants. Five of them, KH4, KH5, KH6, KH9, and KH10, were assigned to the acr-2 locus because no wild-type progeny were observed from crosses with KH2. (40, 41, 54, 32, and 54 progeny were tested, respectively.) KH8, KH14, and KH15, were assigned to a third locus, ocr-3, in the left arm of group I, proximal to mating-type and probably proximal to ocr-1. That these three alleles presumably represent mutations at a single locus distinct from acr-1 was based on observations that recombinants were recovered in crosses involving KH1 and each of the three alleles, and that no wild type was obtained among 145 progeny tested from KH8 x KH14, nor among 130 from KH14 x KH15. Table 1 gives the loci and the strains of origin of these mutants. Linkage data of acr-1, acr-2, and ocr-3 based on random segregants from 3-point crosses are summarized in Table 2.

Table 2. Linkage data on random segregants from crosses involving acr-1, acr-2 and ocr-3. (The top number in each pair represents the class that has the + (or A, or g) allele of the leftmost marker.)

<table>
<thead>
<tr>
<th>Zygote genotype and recombination percent</th>
<th>Parental combinations</th>
<th>Recombination</th>
<th>Total and percent germination</th>
<th>Marker isolation numbers</th>
</tr>
</thead>
<tbody>
<tr>
<td>A acr-1^r cr</td>
<td>69</td>
<td>7</td>
<td>25</td>
<td>5</td>
</tr>
<tr>
<td>a acr-1^s +</td>
<td>80</td>
<td>2</td>
<td>24</td>
<td>3</td>
</tr>
<tr>
<td>a acr-1^s</td>
<td>36</td>
<td>7</td>
<td>10</td>
<td>3</td>
</tr>
<tr>
<td>A cr</td>
<td>36</td>
<td>1</td>
<td>11</td>
<td>2</td>
</tr>
<tr>
<td>+ SC tryp-1</td>
<td>76</td>
<td>1</td>
<td>31</td>
<td>216</td>
</tr>
<tr>
<td>acr-2^r +</td>
<td>74</td>
<td>6</td>
<td>28</td>
<td>95</td>
</tr>
<tr>
<td>+ SC tryp-1</td>
<td>36</td>
<td>3</td>
<td>13</td>
<td>0</td>
</tr>
<tr>
<td>A acr-3^r cr</td>
<td>95</td>
<td>1</td>
<td>8</td>
<td>207</td>
</tr>
<tr>
<td>a +</td>
<td>87</td>
<td>8</td>
<td>6</td>
<td>(49%)</td>
</tr>
<tr>
<td>+ ad-3b +</td>
<td>30</td>
<td>6</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>acr-3^r cr</td>
<td>35</td>
<td>4</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>+ ad-5 +</td>
<td>18</td>
<td>2</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>acr-3^r cr</td>
<td>12</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>+ leu-3 a +</td>
<td>35</td>
<td>4</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>9.3</td>
<td>31</td>
<td>2</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>A acr-3^r cr</td>
<td>34</td>
<td>4</td>
<td>11</td>
<td>0</td>
</tr>
<tr>
<td>a +</td>
<td>51</td>
<td>1</td>
<td>9</td>
<td>1</td>
</tr>
<tr>
<td>A acr-3^r cr</td>
<td>19</td>
<td>3</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>a +</td>
<td>22</td>
<td>1</td>
<td>5</td>
<td>0</td>
</tr>
</tbody>
</table>

The acriflavin concentrations mentioned above are not the maximum concentrations at which these alleles can survive, but rather the convenient concentrations for differentiating resistant from sensitive isolates. In fact, in acriflavin slants, KH1 still grew at 4 μg/ml, and other mutants continued giving slight growth at as high as 0.5 mg/ml. Correspondingly lower concentrations were required to differentiate resistance and sensitivity when the isolates were tested in liquid culture. The tolerance of
both resistant and sensitive strains was enhanced when hydrolyzed yeast nucleic acid was added to the acriflavin minimal medium. The double mutant acr-2\(^{-}\), acr-3\(^{-}\) was more resistant than either single mutant. In scoring acriflavin resistance, false negatives may result if inocula are too small.

acr-ir appeared to be recessive, while both acr-2\(^{-}\) and acr-3\(^{-}\) were dominant. This was inferred from the observation that at some concentrations, the amount of growth of acr-1\(^{+}\), acr-15 heterocaryons was close to that of the wild type, while growth of acr-2\(^{-}\), acr-2\(^{-}\), or acr-3\(^{-}\), acr-3\(^{-}\) heterocaryons was close to the mutant type. The dominance of acr-2\(^{-}\) and acr-3\(^{-}\} was in agreement with the fact that at least five of the seven mutants isolated from the macroconidial strains were heterocaryotic for the mutant alleles. Such heterocaryons would probably not survive the inhibitory effect of acriflavin if the mutant alleles were recessives.

All acr-2 mutants were cross-resistant to 3-amino-1,2,4-triazole, and all acr-3 mutants, to malachite green. The double mutant acr-2\(^{-}\), acr-3\(^{-}\) could grow in the presence of either chemical. The concentrations at which resistance and sensitivity could be differentiated were 0.25% in agar slants and 0.1% in liquid culture for 3-amino-1,2,4-triazole, and 2 \(\mu g\)/ml in slants and 0.5 \(\mu g\)/ml in liquid for malachite green. These mutants did not differ appreciably from wild type in their sensitivity to acridine orange, proflavine, thionine, and crystal violet.

Mutations from acriflavin sensitivity to resistance seem to be frequent. Mutants at either acr-2 or acr-3 have been recovered from most of the strains upon first testing when between \(10^6\) and \(10^7\) conidia from each strain were plated on the acriflavin plates. Mutants resistant to the dye have also been obtained by Howe and Terry (1962 Canad. J. Genet. Cytol. 4: 447) and by M. E. Case (personal communication). --- Department of Biological Sciences, Stanford University, Stanford, California.

Jho, K. K. Indole excretion by revertants derived from indole-accumulating try-3 (td) mutants.

Indole is known to be excreted by many try-3 mutants. Accumulation of indole in the medium is a consequence of the ability of the mutant protein to carry out the reaction: indole glycerol phosphates indole + triose phosphate (DeMoss and Bonner 1959 Proc. Natl. Acad. Sci. U. S. 45: 1405).

It seems that at least some of the prototrophic strains (assumed to be revertants because of their wild-type rate of growth) derived from indole-accumulating try-3 mutants also accumulate indole in the medium. A large number of phenotypic revertants from three try-3 mutants (td-2, td-71 and td-100) were isolated from UV-treated conidio by plating them on indole-supplemented minimal medium. The object was to isolate, if possible, some auxotrophic or non-accumulating strains which were still auxotrophic but could grow on either indole- or tryptophan-supplemented medium. Though such strains were not found, it was noticed that most of the prototrophic strains accumulated indole in the medium. The indole-accumulating revertants were four to seven times more frequent than the non-accumulating ones. Indole accumulation by a majority of isolates was expected because of the multi-nucleate, heterocaryotic nature of the conidio which gave rise to these strains. But the possibility was considered that the accumulation of indole was on inherent characteristic of the revertant nuclei.

Two of the indole-accumulating, fat-growing isolates (to avoid possible suppressed strains) were crossed to the closely-linked marker "fluffy" strain. Conidial, non-fluffy random ascospore isolates from each cross were found to contain both auxotrophs and prototrophs (23 prototrophs among 61 conidial isolates in one case and 92 isolates in the second cross). In both cores, with the exception of four isolates in the second cross (out of 75 isolates), all of the prototrophic, conidial isolates were found to accumulate indole in the culture medium; the four non-accumulating isolates could, presumably, represent cross-over products between the try-3 and td loci. Accumulation of indole by the isolates was quantitatively comparable to that of their parent auxotrophs.

These results indicate that the enzyme tryptophan synthetase in these revertants may be different from the wild-type enzyme. DeMoss (1962 Biochem. Biophys. Acta 62: 279) has shown that the wild-type enzyme does not permit accumulation of free indole in the synthesis of tryptophan from indolylglycerol phosphate. Esser et al. (1960 Z. Vererbungslw. 91: 219) have shown that about half the revertants from the indole-accumulating strain td-2 shared a tryptophan synthetase which could be distinguished from the wild-type enzyme by enzymatic and immunologic criteria. It was not reported whether or not some of the revertants accumulated indole.

This work was supported by grants to J. Weijer from the National Research Council of Canada. --- Department of Genetics, University of Alberta, Edmonton, Alberta, Canada.

Kolmark, H. G. Ureaseless mutants in Neurospora cressa.

The presence of the enzyme urease in Neurospora has been known for many years (Srb and Horowitz 1944 J. Biol. Chem. 154: 129). Mutation to an ureaseless condition is therefore to be expected.

In the present experiments it was first found that urease could very easily be demonstrated directly on a growing colony, simply by soaking a small piece of pH-indicator paper (range about \(pH\) 6-8) in an 8% solution of urea in water and placing it in contact with the mycelium or conidia. A color change towards the alkaline takes place in a few minutes due to liberation of ammonia when urea is enzymatically hydrolysed.

This change in \(pH\) was utilized in the screening methods finally worked out for isolation of ureaseless mutants.

Strains of Neurospora: Some earlier experiments with macroconidial strains of colonial morphology did not succeed in isolation of ureaseless mutants. A microconidial, small colonial strain, 398-28 A, was then tried in the expectation that mutants might more easily become phenotypically expressed when induced in mononucleate conidia. A morphological mutant, m-25, derived from this strain after treatment with ultraviolet light, was used in some of the later experiments. m-25 forms very small and extremely dense colonies.