Genetic nature of the slime variant of Neurospora crassa

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Abstract
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Davis, R.H. Lethality of Neurospora arginine mutants associated with a factor from wild type.

In the course of an analysis of Neurospora mutants lacking ornithine transcarbamylase (arg-12: Davis and Thwaites, Genetics, in press, 1963), crosses of such mutants to our stock of wild type 73a regularly yielded ratios of 2 wild: 1 arg-12: 1 "lethal." The last class was able to germinate, but grew very little after isolation to medium containing 200 μg arginine per ml. The progeny ratio suggested that another factor, derived from the wild type parent, was responsible for the "lethality" when associated with the arg-12 mutant.

We have been able to show that the 73a stock used is abnormal in having a low level of arginine, citrulline, and ornithine, and also in having a high ornithine transcarbamylase activity. The latter is probably a consequence of low arginine levels, by way of derepression. On the basis of high ornithine transcarbamylase, the progeny of a 73a x 74a cross was analyzed and segregation of 1 high to 1 low activity was observed. The factor responsible for all the effects described is denoted UM-300. The "lethal" category observed in crosses of a strain carrying UM-300 to an arg-12 mutant is the double mutant, UM-300, arg-12. Another absolute mutant in the arginine pathway (arg-11) also gives 1/4 "lethal" progeny when crossed to UM-300.

These results were consistent with the hypothesis that endogenously-synthesized arginine could not be maintained at a normal concentration (through loss or destruction) and exogenously-provided arginine could not be concentrated (because of an imperfect transport system or a rapid rate of destruction). We have been able to show that uptake is much slower in UM-300 than in our normal wild type; this was measured by disappearance from the medium and by the rate and extent of elevation of arginine in mycelia when added to the medium. These results suggest that an arginine concentration mechanism is deficient, and that rapid destruction is not the case. It should be noted that 1 mg arginine per ml medium will support the growth of UM-300, arg-12.

It is not clear whether UM-300 has a complete inability to concentrate arginine, since the internal concentrations of arginine have not been measured in the same units as external concentrations. Neither is it known yet how specific the transport system is for arginine; the only other amino acid studied in regard to uptake is ornithine, which UM-300 concentrates more poorly than does a normal wild type.

I should be interested to know whether other workers have had comparable experiences with arginine mutants or other mutants, and if possible, to exchange strains and test the genetic relationships between UM-300 and other mutants of this type. (A recent case reported by D. R. Stadler (Proc. XI Int. Cong. Genetics, vol. I, p. 52) appears to have similarities with the one described above.) Because more data will be available on UM-300 soon, I should appreciate that investigators wishing to cite this note write for more recent results, and that the note be cited only with permission.--Department of Botany, University of Michigan, Ann Arbor, Michigan.

Emerson, S. Genetic nature of the slime variant of Neurospora crassa. At the time cultures of slime were supplied to the Fungal Genetics Stock Center (see Neurospora Information Conference, NAS-NRC Pub. 950, 1962) little was known of the genetic basis of the slime phenotype except that it separated cleanly together with parental genes in isolations from heterocaryons with hyphal phenotypes. It is now known that three independently inherited characters are essential to, yet insufficient for, the persistent expression of the slime phenotype. The characters involved are: osmotic (os, linkage group IR, which was present in the irradiated parent of slime), fuzzy (fz, linkage group unknown, a morphological mutant), and spontaneous germination (sg, linkage group unknown, germinates without heat activation, has extremely poor surface growth habit). Ascospore isolates carrying os, fz, and sg usually germinate by slime flows instead of germ tubes but eventually change to a miserable hyphal growth. From some os fz sg isolates it has been possible by vegetative selection to recover strains with persistent slime phenotypes. No genetic difference has yet been established between the hyphal and plasmodioid forms of such os fz sg isolates. A fuller account with descriptions of the unit characters is scheduled for publication in No. 3, Vol. 34 (1963/64) of Genetica. (Supported in part by an
During the investigation of the effect of temperature on sexual reproduction in Neurospora, the phenomenon of the occurrence of asci with more than eight spores was observed. Such asci will be termed "multi-spored".

The strains used were cr (crisp), re-isolated from strain F945 kindly supplied by Dr. R. W. Barratt, and al-2 (albino), re-isolated from strain 15300 kindly supplied by Professor D. G. Catcheside. Batches of crosses were made by inoculating both strains together on the minimal reproductive medium of Westergaard and Mitchell. Part of each batch was incubated at 25°C, while the rest of the batch was incubated at 30°C. Some of the crosses from each temperature treatment were taken at intervals and the protoperithecia or perithecia fixed, stained and observed cytologically to determine the stage of development reached. Every 24 hours and during meiosis at every 12 hours, some crosses were transferred from incubation at 25°C to 30°C and some of these also were taken at intervals for cytological observation.

It was found that at 25°C protoperithecial growth begins after 24 hours and continues steadily for about a further 48 hours. Development did not take place beyond this stage in crosses incubated continuously at 30°C; the protoperithecia darkened but were ultimately devoid of any ascospores. At 25°C, however, the protoperithecia expand rapidly in the next 48 hours, Meiosis commenced late on the fifth day and the first wave of asci completed the three nuclear divisions late on the sixth day.

Provided protoperithecial growth had commenced at 25°C, crosses transferred from 25°C to 30°C did undergo meiosis and produce ascospores at 30°C. However, a high frequency of aberrant asci was present in all such crosses. As can be seen in Table I, many asci contained more than eight spores. Never more and usually fewer than eight black spores were found in all the multi-spored asci observed. The highest number of spores seen in any one ascus was 22 of which only 3 were black. Asci with fewer than eight spores were also frequent. In addition several asci contained grossly mis-shaped ascospores.

### Frequencies of normal and aberrant asci.

<table>
<thead>
<tr>
<th>Cross* and Treatment †</th>
<th>No. of spores per ascus:</th>
<th>Number of asci:</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Range Average</td>
<td>Normal 8-spored</td>
<td>Multi-spored</td>
<td>Less than 8-spored</td>
<td></td>
</tr>
<tr>
<td>1.</td>
<td>8 8.0</td>
<td>23 25</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>1-14 7.2</td>
<td>4 7</td>
<td>8</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>1-13 7.0</td>
<td>0 2</td>
<td>10</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>2-14 8.1</td>
<td>1 6</td>
<td>10</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td>4-15 9.1</td>
<td>5 8</td>
<td>13</td>
<td>4</td>
<td></td>
</tr>
</tbody>
</table>

* Samples 1-4. Cross: cr, + x +, al-2
Sample 5. Cross: +, + o x cr, al-2 o
† Estimated meioses occurred: (1) all at 25°C. (2) at 25°C, during change 25°C to 30°C and at 30°C. (3) during change 25°C to 30°C and at 30°C. (4) all at 30°C. (5) at 25°C, during change 25°C to 30°C and at 30°C.
Samples 1-5 each based on 25 asci selected at random.

The highest frequency of aberrant asci appeared to occur in those crosses in which meiosis was proceeding in some of the asci when the cultures were transferred from incubation at 25°C to 30°C. Cytolo-