Phenocopies of Neurospora mutants induced by biotin deficiency

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Abstract
Phenocopies of Neurospora mutants induced by biotin deficiency
The results show a somewhat lower frequency of oscospore abortion than the 50% often expected when single reciprocal translocations stocks are crossed with wild-type stocks. In addition, many of the abortion frequencies from these intercrosses were considerably lower than the average of the two parental translocation stocks crossed with normal. These low oscospore abortion frequencies observed in the intercrosses can be explained in terms of viable duplications and/or deficiencies.

Four-point recombination data were obtained with three marker genes and partial sterility to aid in the placement of the breakpoints for each of the translocation stocks. These translocations map with their breakpoints in the right arm (R) of both linkage groups I and IV, making intercrosses between them of the same-arms type. The only possible exception is T(I;IV)D304. This translocation has one breakpoint that maps very close to the centromere of linkage group I. If, in these some-arms intercrosses, each translocation involves one exchanged segment shorter and one exchanged segment longer relative to the other translocation, progeny can result that carry a duplication for both segments between the breakpoints without any deficiencies. Indications are that many of these combinations might be viable in Neurospora (Perkins 1971 Genetics 68: s50).

Duplications-producing intercrosses of the type described are often recognized by the frequencies of unordered octets, particularly by a deficiency of 6:2 ratios. A high number in this class is indicative of viable duplications since theoretically there is no other way to obtain such octets from reciprocal translocations. In intercrosses where one translocation has both exchanged segments shorter than the other translocation, or where the breakpoints are in opposite arms, all duplications would also be accompanied by deficiencies. Results show that several of these intercrosses are generating high frequencies of 6:2 octets. Compatible with these data are the low oscospore abortion results gained from random collections; certainly what one would expect with viable duplication situations. Breakpoints of T(I;IV)172 and T(I;IV)L19 map extremely close to each other and the oscospore abortion frequency from the intercross between them was only 3.6%. These two translocations have breakpoints that are either identical or at very nearly the same positions.

This work was accomplished at the University of Minnesota. Current address of the first author is the Department of Biology, Saint Mary's College, Winona, Minnesota 55987.


A number of oscospore shops mutants have been described in N. crassa and N. tetrasperma. Among these are indurated ascus (Dodge 1934 Mycologia 26: 360), round spore (Novak and Srb 1973 Con. J. Genet. Cytol. 15: 685), and triangle spore (Srb et al. 1973 J. Hered., 64: 242). Although several of these mutants have been studied cytologically and/or biochemically, we have, as yet, little information regarding alterations in spore shape. Using a backcrossed isolate of a wild strain of N. tetrasperma (Dunnellon 11-B-P542), we have observed that when a wild-type cross is made on purified agar (Difco) -- with no nutrients added -- indurated ascii, round spores and triangle spores, as well as wild-type spores, are produced. When such aberrant spores are isolated and allowed to cross, all progeny spores are wild-type in shape. This result, together with the fact that such deviant spore shops can be induced at will by altering the crossing medium, indicates that the aberrantly shaped spores are phenocopies rather than actual mutants.

In order to determine whether the presence of phenocopies could be attributed to the lack of a specific component of the standard crossing medium (Westergaard's, 1/2% sucrose), media were prepared with contained individual components or groups of components of Westergaard's medium. For this purpose, the components of the standard medium were divided into five groups: a) Westergaard's Salts -- 1 g KN03, 1 g KH2PO4, 0.5 g MgSO4·7H2O, 0.1 g CaCl2, 0.1 g NaCl, 1000 ml distilled water; b) trace elements -- as described by Beadle and Tatum, 1945, Am. J. Bot. 32: 678; c) biotin -- 5 micrograms/liter; d) sucrose -- 1/2% (w/v); e) agar -- 1% Difco purified agar. Wild-type crosses were made on the following media (all solidified with 1% purified agar): (1) standard Westergaard's (i.e. salts, trace elements, biotin, sucrose), (2) Westergaard's salts alone, (3) trace elements alone, (4) biotin alone, (5) sucrose alone, (6) purified agar, (7) Westergaard's salts + sucrose, (8) trace elements + sucrose, (9) biotin + sucrose, (10) Westergaard's minus biotin (i.e. salts, trace elements, sucrose).

All media were capable of supporting perithecial formation. Although the number of perithecia and the proportionate number of phenocopies present varied depending on the components of the medium, phenocopies were produced only on those media which lacked biotin. All crosses made on the same medium containing biotin resulted in the production of normal wild-type crosses. This was true even when biotin was the only nutrient added to the purified agar.

Similar, although less extensive, results have been obtained with N. tetrasperma strain T-220. Attempts to repeat the experiments described above, however, have been hampered by the fact that N. crassa crosses very poorly, if at all, on the various deficiency media listed above.
It should be noted that the above results do not imply that N. tetrasperma, as opposed to N. crassa, can grow in the absence of externally available biotin. Since biotin is required only in extremely small amounts and since no precautionary steps were taken to rid glassware, etc., of contaminating biotin, the growth observed in the absence of added biotin may well be due to the traces of biotin on glassware and/or in the components of the media.

In relation to the above results, Bennett and Lilly (1947 Am. J. Bot. 34: 196), while studying the effects of biotin deficiency on the crossing of S. limicola, also observed indurated ascospore formation in crosses on biotin-deficient media. Thus, the effects of biotin deficiency on ascospore formation do not seem to be limited to the genus Neurospora and the results may imply that one or more biotin-dependent steps are essential for the proper determination of spore shape. Section of Genetics, Development and Physiology, Cornell University, Ithaca, New York 14853.

PODOSPORO

Belcour, L. Loss of a cytoplasmic determinant through formation of protoplasts in Podospora. In Podospora anserina, protoplasts of an average diameter of 5 μm can be obtained, as described for Neurospora (Bochmann and Bonner 1959 J. Bacteriol. 78: 550) by treating young mycelia with small juice. If plated on Petri dishes, a variable number (see Table 1) can regenerate normal mycelia. The genetic analysis of very small quantities of cytoplasm made possible by protoplasts can reveal cytoplasmic heterogeneity which would not be easy to observe by other methods. Isolation from protoplasts of cytoplasmic (most probably mitochondrial) mutations displaying neither dominance nor suppressivity has already been reported (Belcours 1975 Genet. Res. Comb. 25: 155). We report here on the segregation of the two mutually exclusive cytoplasmic states (s) and (sS), obtained by the use of protoplasts.

Table 1. Isolation of (sS) strains by regeneration of protoplasts from (s) strains.

<table>
<thead>
<tr>
<th>experiment no</th>
<th>strain genotype</th>
<th>protoplasts regenerating (%)</th>
<th>no. of regenerated mycelia studied</th>
<th>no. of (sS) mycelia</th>
<th>% of (sS) mycelia</th>
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<td>s mt+</td>
<td>9</td>
<td>197</td>
<td>28</td>
<td>14</td>
</tr>
</tbody>
</table>

*Protoplasts were made by a method derived from that of Bochmann and Bonner (1959 J. Bacteriol. 78: 550) and described by Belcours (1975 Genet. Res. Comb. 25: 155).

The properties of the (s)/(sS) system can be summarized as follows: S and s are two alleles at one of the 9 well-known loci for protoplasmic incompatibility (Bernett 1965 Ann. Sci. Not. Bot. 6: 661). (s) and (sS) represent the two possible alternative cytoplasmic states of a strain containing the alleles. When s strains are cytoplasmically (sS) by (s) protoplasts, they become incompatible with S strains. In (s) x (sS) sexual crosses the (sS) and (s) properties follow a strict cytoplasmic (maternal) inheritance. Finally, the (s) state is highly infectious with respect to the (sS) state following anastomoses; the (sS) → (s) conversion never occurs spontaneously during vegetative growth, but has been observed after regeneration of conidiohhores isolated by micromanipulation. Briefly the (s) state depends upon the presence of the s gene plus that of a cytoplasmic factor, assumed to be necessary for maintaining the activity of this gene. (Pizet 1952 Rev. Trypt. Biol. Veg. 13: 51; Beisson-Schecroun 1962 Ann. Gen. 4: 1).

A significant proportion of the protoplasts obtained from an (s) strain yield (sS) mycelia after regeneration, as shown in Table 1. The (sS) mycelia thus obtained display all the properties of the (sS) strain previously investigated, in particular the ability to transform to the (s) state following cytoplasmic contact. The percentage of (sS) protoplasts varies from one experiment to another (1% to 16%) and does not seem to be correlated with the rate of protoplast regeneration.

The simplest interpretation of these results is that a passive and random distribution of cytoplasm occurs during protoplast formation. Those protoplasts receiving the s cytoplasmic factor would yield (s) mycelia, Those not receiving it would yield (sS) mycelia. A direct effect of the enzymatic treatment used for protoplast formation on the loss of the s factor may be excluded: experiments 3 and 4 have been carried out on two aliquots of the same culture, one treated with 5% enzyme (expt. no 3), the other with 20% enzyme (expt. no 4) bath for 4 hours. No significant difference in the ratio of (sS) mycelia was noted.

The hypothesis of a random distribution of the cytoplasm in protoplasts, and hence of the s cytoplasmic factor, allows a rough estimation of the concentration of s factors in the cytoplasm. Assuming that (sS) mycelia are those that received no s factor, the Poisson law allows the estimation of the mean-number of s factors per protoplast. The numbers thus obtained vary from 1.8 to 4.6 units per protoplast, depending on the experiment. The size of young protoplasts varies from 3 to 10 μm in diameter. Assuming a diameter of...