Ascospore arrangements in the aberrant asci of a Neurospora crassa mutant

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Ascospore arrangements in the aberrant asci of a Neurospora crassa mutant

Abstract
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Acetate stimulates macroconidiation in the wild type (Turian 1961 Compt. Rend. 252: 1374) and has also been found to favor the formation of microconidio in the mutant. A slightly modified Westergaard and Mitchell P synthetic medium (1947 Am. J. Bot. 34; 573) with either 1% Na acetate replacing half of the normal 2% sucrose (PSA) or just 2% Na acetate (PA) as single C source has therefore been used to produce an abundance of microconidio rather than the less well chemically defined enrichment of P formula with casein hydrolysate (Grigg 1965 NN7; 12) or Vogel's medium with complex extracts (Baylis and DeBusk 1965 NN7: 7).

After 12 days of stationary growth at 25°C on PSA or PA liquid medium (250 ml in 1 liter Fernbach flasks), the fluffy strain forms a dry surface mat which has an orange-brownish, powdery appearance due to a full cover of arbusculate microconidiophores. Each compartment (cell) of the microconidiophore has a repetitive capacity to produce uninucleate microconidio (Dodge 1935 Mycologia 27; 418). For the harvest, the mats were taken out of the flasks with a pair of forceps (fertile surface folded in) and vigorously shaken in a glass-stoppered cylinder in the presence of distilled water containing 1.2 drops per liter of a non-ionic detergent. The dense suspension obtained was filtered through a fine nylon net to remove hyphal debris and broken microconidio-phores. Centrifugation (3000 r.p.m. ) provided surprisingly dark, blackish-brown pellets which were then dispersed in acetone-water (1:1), united and diluted to a known volume in a glass-stoppered cylinder. Immediately after vigorous shaking, known aliquots were diluted for determining microconidial number in a hemocytometer. The net harvest of filtered microconidio averaged about 10^8-10^9 cells per flask of PSA medium.

After centrifugation, the carotenoids contained in the microconidial pellet were extracted 3 times with pure acetone and then transferred into petroleum ether for further extraction (a), while the remaining dark pellet was kept for melanin pigment extraction (b).

(a) Total carotenoids were estimated spectrophotometrically at 460 mg (Krzeminski and Quackenbush 1960 Arch. Biochem. Biophys. 88; 287) in the petroleum extracts after concentration under nitrogen; they averaged 2 pg per 103 microconidio on PSA and 6 pg per 103 conidia on PA. After overnight hydrolysis with an alcoholic KOH solution, the neutral first and subsequently, after HCl addition, the acidic carotenoid(s) could be separately transferred to the petroleum ether (epiphase and measured at 468 mg (neutral carotenoids) and 472 mg (acidic carotenoids); on both media a ratio of 2:1 was obtained in favor of the acidic carotenoid(s) which gave the typical absorption curve of neurosporaxanthin (Zalokar 1957 Arch. Biochem. Biophys. 70; 568). This ratio appears to be higher than that generally obtained from free carotenoid (Zalokar 1954 Arch. Biochem. Biophys. 50; 71).

(b) Melanin pigments were extracted by refluxing twice for 1 hour in 2 N NaOH. The total brownish-black extract gave a decreasing linear absorption slope from 400 to 600 mg. This is indicative of the melanin nature of this microconidial pigment(s), similar to that previously described in the mycelia of a black mutant (Schaeffer 1953 Arch. Biochem. Biophys. 47; 359).

Initial chemical fractionation of walls prepared from lyophilized microconidio first ground in dodecyl sulfate in the presence of glass beads and then cleaned according to Mahadevan and Tatum (1965 J. Bacteriol. 90; 1073) method has revealed that a major part of the melanin pigment remains associated with the chitin-containing fraction.

For the ultrastructural study of the microconidio, the cells have been fixed in 2% KmnO4 for 4 hours and subjected to a post-fixation with 1.5% uranyl acetate in 75% acetone. After dehydration, the specimens were embedded in an Araldite mixture (Ducupan ACM).

Microconidial ultrastructure is characterized by a high nucleo-cytoplasmic ratio expressed as maximal occupancy of the nucleus in the cell. The main features of the cytoplasm are: very few vacuoles, presence of a thin endoplasmic reticulum, small scattered mitochondria which have few cristae, lipid bodies, and dark, supposedly reserve, granules. On the microconidiophores harvested and fixed directly from the powdery surface, the cultures, interesting lamellated colerettes have been observed which surround the site of emission of the microconidio budded from their mother cell. - Laboratory of Microbiology, Institute of General Botany, University of Geneva, Switzerland.


G. Pincham, Cornell Univ.). The spindles of the second times overlapped such that the four resultant nuclei occur in various arrangements within the ascus instead of in a linear sequence. The purpose of this study was to ascertain the degree of consistency of spore arrangement by genotype in the peak ascus.

A new mutant strain was utilized which produced colorless ascospores when selfed. Crosses with wild type show a simple segregation of spore color. The mutation was found following treatment of the St. Lawrence standard wild type strain 74A with dimethyl
Camera lucida drawings were made of 128 aberrant ascii segregating for the colorless ascospore marker. The classes to which the asci were assigned and the frequencies obtained were: (1) distinct first-division segregation, 26; (2) distinct second-division segregation, 29; (3) most likely first-division segregation, 10; (4) most likely second-division segregation, 33; and (5) impossible to classify as first- or second-division segregation, 30.

These data indicate that the segregation pattern can be distinguished with absolute certainty in 43% and with a high degree of certainty in an additional 34% of the aberrant asci. The frequency of second-division segregation in the aberrant ascii was 53% (based only on classes 1 and 2). It was apparent, from studying the drawings, that a slight departure from the distinct first-division segregation pattern resulted in an ascospore arrangement that was difficult to classify. However, a slight departure from the distinct second-division segregation pattern usually remained recognizable as a second-division segregation pattern. This aspect biases the frequency of class 3 downwards and the frequency of class 5 upwards.

The cross of the colorless ascospore and peak-2 double mutant strain with the wild type strain yielded 149 first-division segregation patterns and 221 second-division segregation patterns. Therefore, the second-division segregation frequency in linear ascii was 60%. This value compared favorably with the frequency of second-division obtained in aberrant ascii (53%) although the following factors concerning the observed frequency of second-division segregation in the aberrant ascii may make such a comparison invalid: (1) the frequency would be increased by overlapping second-division spindles; and (2) the frequency would be decreased if a greater proportion of ascii with first-division segregation than with second-division segregation remained in a distinct pattern after the third division.

It is concluded that the ascospores in an aberrant ascus are highly ordered in approximately 77% of the ascii and that a reliable estimate of the gene-centromere distance was obtained for the colorless ascospore marker utilized.

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Cooke, F. Ascospore color mutants and low germination in Neurospora. Whereas ascospore color mutants have been much used in Sordaria and Ascoibolus to select for aberrant tetrad, such mutants have proved difficult to find in Neurospora. Those which are described, asc (Stadler 1956 Genetics 41: 528) and ts (Nakamura 1961 Bot. Mag., Tokyo 74: 110), have proved to have inviable ascospores and therefore to be of limited use for recombination studies. A pale-spored, pantothenic-acid-requiring mutant described by Threlkeld (1965 Can. J. Genet. Cytol. 7: 171) has proved to be more promising since the pale ascospores will germinate, but with a lower frequency than the wild type spores. This mutant has been used to investigate the relationship between paleness and germination frequency. It was found that the color of the spores could be altered by varying the amounts of pantothenic acid in the crossing medium.

Isotallelic crosses were set up on media with different concentrations of pantothenic acid (PA). Apart from a wild type control cross, all crosses were pan-2 (B3) x pan-2 (B3) a. With no PA added, no growth was possible. With 0.5 mg of PA per liter, vegetative growth was normal. The table shows the effect of the varying concentration of PA on perithecial and spore production and on spore viability. A wild type cross was carried out at each concentration of PA and without PA. In none of these cases were there noticeable differences in growth rates, perithecial production or spore color. Percentage germination of the wild type cross was 97%. In this cross, therefore, PA has no effect on growth rate, fertility or germination.

<table>
<thead>
<tr>
<th>Concentration of PA in mg/l</th>
<th>Growth and perithecial production</th>
<th>Spores</th>
<th>% Germination</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.05</td>
<td>weak growth = no perithecia</td>
<td>none</td>
<td></td>
</tr>
<tr>
<td>0.1</td>
<td>weak growth = few perithecia</td>
<td>very pale</td>
<td>33</td>
</tr>
<tr>
<td>0.2</td>
<td>moderate growth = few perithecia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.5</td>
<td>normal growth = many perithecia</td>
<td>pale</td>
<td>80</td>
</tr>
<tr>
<td>1.0</td>
<td>as above</td>
<td>pale</td>
<td>80</td>
</tr>
<tr>
<td>5.0</td>
<td>as above</td>
<td>dark</td>
<td>97</td>
</tr>
<tr>
<td>20.0</td>
<td>as above</td>
<td>dark</td>
<td>95</td>
</tr>
</tbody>
</table>

In the pan-2 x pan-2 cross the results show a progressive increase in spore darkness with increasing PA. The spore color at 5 mg/l and 20 mg/l of PA was indistinguishable from the wild type spore color. The results also show an increasing ability of the spores to germinate with increasing PA. The spore germination at 5 mg/l and 20 mg/l of PA was not significantly different from wild type spore germination. Both germination frequency and spore darkness decrease progressively with reduction in PA concentrations.

Since it was possible that some PA would break down during the autoclaving of the medium, the media were made up by...