Estimation of the frequency of multinucleate conidia in microconidiating strains

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Abstract
Frequency of multinucleate conidia in microconidiating strains

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Frequency of multinucleate microconidia.

<table>
<thead>
<tr>
<th>Experiment Number</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>ad-3 colonies x 10⁴ per ml conidial suspension</td>
<td>7.42</td>
<td>6.40</td>
<td>76.00</td>
</tr>
<tr>
<td>lry-3 colonies x 10⁴ per ml conidial suspension</td>
<td>37.00</td>
<td>358.00</td>
<td>3.8</td>
</tr>
<tr>
<td>Ratio ad-3 colonies/lry-3 colonies</td>
<td>1/5</td>
<td>1/56</td>
<td>20/1</td>
</tr>
<tr>
<td>Total viable conidia x 10⁴ per ml conidial suspension</td>
<td>44.42</td>
<td>364.40</td>
<td>79.0</td>
</tr>
<tr>
<td>Wild type colonies per ml conidial suspension</td>
<td>83</td>
<td>53</td>
<td>19</td>
</tr>
<tr>
<td>Frequency @colonies</td>
<td>0.836</td>
<td>0.982</td>
<td>0.048</td>
</tr>
<tr>
<td>Frequency ad-3 colonies</td>
<td>0.166</td>
<td>0.017</td>
<td>0.952</td>
</tr>
<tr>
<td>Per cent wild type colonies</td>
<td>0.0187</td>
<td>0.0014</td>
<td>0.0024</td>
</tr>
<tr>
<td>Estimated per cent multinucleate microconidia</td>
<td>0.067</td>
<td>0.041</td>
<td>0.026</td>
</tr>
</tbody>
</table>

* This value is obtained from the following equation:

\[
\text{\% wild type colonies} = \frac{2(\text{lry-3 colony frequency})}{(\text{ad-3 colony frequency})}
\]

The frequency of multinucleate conidia in microconidiating strains of N. crassa was determined by means of a technique employing forced heterocaryons. The strains used were of the following genotypes: ad-3A; pe, f1 (38701; Y8743m, L) and lys-3; pe, f1 (4545; Y8743m, L).

Heterocaryons were formed by placing drops of a mixed microconidial suspension on plates of minimal medium. The heterocaryons formed on the plates were transferred to minimal agar slants and incubated. Microconidia suspensions from three independent heterocaryons were analyzed. Each was filtered through Nitex 53 mesh and glass wool to remove conidial clumps and mycelial fragments. Aliquots of the filtered suspension were plated on minimal, adenosine-supplemented, and lysine-supplemented medium. From the plate counts and by application of the binomial theorem the frequency of multinucleate conidio was determined (Table). To simplify the calculations, all multinucleate microconidio are considered binucleate. The frequency of multinucleate microconidia varied little over a wide range of nuclear ratios. These percentages probably represent the upper limits of multinucleate microconidial frequencies since an undetermined fraction of the wild type colonies formed may have had their origin as multinucleate mycelial fragments or as newly-formed heterocaryons. The percentages of multinucleate microconidia obtained were less than 0.1% in all cases, and therefore somewhat lower than those reported by Barratt and Garnjobst (1949 Genetics 34: 351).

This work was supported in part by Atomic Energy Commission Contract AT-(40-1)-2788. Genetics Laboratories, Department of Biological Sciences, Florida State University, Tallahassee, Florida.

Lowry, R. J., T. L. Durkee and A. S. Sussman. Ultrastructural study of microconidium formation.

and show a much more extensive system of rough endoplasmic reticulum than do young vegetative hyphoe. A bulge in the hyphoe presages the start of microconidium formation, followed by the rupture of the outermost wall layers. A thick collar forms wound the protruding microconiophore due to extensive thickening of the inner wall layer of the parent hyphoe. At this stage the cytoplas of the developing microcoiniophore is still continuous with that of the hypho cell from which it arises and is contained by a wall which is derived from the thickened collar. The microconidium is finally isolated from the cytoplasm of the microconiophore by a centripetal extension of its wall, the material of which seems to be

The present data suggest that microconidia differ from macroconidia in their smaller size, denser array of ribosomes, more extensive endoplasmic reticulum, more conspicuously layered wall, fewer mitochondria, and single nucleus. These results confirm and extend those of Dodge (1932 Bull. Torrey Botan. Club 59: 347) and Moreau and Moreau (1939 Bull. Soc. Botan. France 86: 12) whose observations with the light microscope were the principal sources of information on the subject. Department of Botany, University of Michigan, Ann Arbor, Michigan 48104.


Microconidiating cultures of N. crassa strain peach-fluffy (Y8743m, L) (FGSC#659) were fixed at various times after the initiation of growth and examined with the electron microscope. Hyphoe from which microconidio form are markedly vacuolated and show a much more extensive system of rough endoplasmic reticulum than do young vegetative hyphoe. A bulge in the hyphoe presages the start of microconidium formation, followed by the rupture of the outermost wall layers. A thick collar forms wound the protruding microconiophore due to extensive thickening of the inner wall layer of the parent hyphoe. At this stage the cytoplas of the developing microcoiniophore is still continuous with that of the hypho cell from which it arises and is contained by a wall which is derived from the thickened collar. The microconidium is finally isolated from the cytoplasm of the microconiophore by a centripetal extension of its wall, the material of which seems to be

When N. crassa, strain 69-I 113A, is grown in standing culture on liquid medium at 25°C, the accumulation of trehalose in the vegetative mycelium begins during the second day. Rapid accumulation of this sugar follows, attaining a maximum on the third day. Conidiation begins during the third day, following which a rapid decrease in the trehalose of vegetative mycelium occurs. This decrease is closely related to the conditions to which the strain is exposed. If conidial production is delayed, mycelial trehalose continues to accumulate until spores are formed. Likewise, the trehalose concentration in acnoidal strains steadily increases beyond the time when conidiation normally occurs in other strains. Moreover, trehalose levels in the conidia are much higher than those found in the vegetative mycelium as considered separately from the mycelium.

The trehalose activity per unit dry weight of the vegetative mycelium of strain 69-I 113A when grown under standard conditions remains low for three days. Beginning with the fourth day, it increases rapidly until the tenth day of growth. Concomitant with