The relationships of trehalose and its metabolism to conidiation in Neurospora

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Abstract
Relationship of trehalose to conidiation

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Baylis, J. R., Jr. and A. G. DeBusk. Estimation of the frequency of multinucleate conidia in microconidializing strains. of Neurospora crassa. The frequency of multinucleate conidia in microconidializing 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, fl (38701; YB743m, L) and lyr-3; pe, fl (4545; YB743m, 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. Microconidial 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, adenine-supplemented, and lysine-supplemented medium. From the plate counts and by application of the binomial theorem the frequency of multinucleate conidia was determined (Table). To simplify the calculations, all multinucleate microconidia were 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, University of Florida, Tallahassee, Florida.

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

and show a much more extensive system of rough endoplasmic reticulum than do young vegetative hyphes. A bulge in the hyphal wall presages the start of microconidium formation, followed by the rupture of the outermost wall layers. A thick collar forms around the protruding microconidium due to extensive thickening of the inner wall layer of the parent hypha. At this stage the cytoplasm of the developing microconidium is still continuous with that of the hyphol 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 microconidiophore by a centripetal extension of its wall, the material of which seems to be derived from the collar.

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. There results confirm and extend those of Dodge (1932 Bull. Torrey Botan. Club 59: 347) and Moreau and Moreau (1939 Bull. Soc. Botan. France 86: 1) whose observations with the light microscope were the principal sources of information on the subject. - Genetics Laboratories, Department of Botany, University of Michigan, Ann Arbor, Michigan 48104.

Honks,* D. L. and A. S. Sussman. The relationships of trehalose and its metabolism to conidiation in Neurospora. When N. crassa, strain 69-1 113A, is grown in standing culture on liquid medium at 24°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 occurs. This is closely related to the decrease in conidial production. Mycelial trehalose continues to accumulate until spores are formed. Likewise, the trehalose concentration in aconidial 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 estimated separately from the mycelium.

The trehalose activity per unit dry weight of the vegetative mycelium of strain 69-1 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

<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 10^4 per ml conidial suspension</td>
<td>7.42</td>
<td>6.40</td>
<td>76.00</td>
</tr>
<tr>
<td>lyr-3 colonies x 10^4 per ml conidial suspension</td>
<td>37.00</td>
<td>358.00</td>
<td>3.00</td>
</tr>
<tr>
<td>Ratio ad-3 colonies/ lyr-3 colonies</td>
<td>1/5</td>
<td>1/56</td>
<td>20/1</td>
</tr>
<tr>
<td>Total viable conidia x 10^4 per ml conidial suspension</td>
<td>44.42</td>
<td>364.40</td>
<td>79.00</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: % wild type colonies = (lyr-3 colony frequency) / (ad-3 colony frequency).
the increase in mycelial trehalose activity on the fourth day is the cessation of mycelial growth and the rapid production of aerial hyphae and conidio. These events occur 24 hours prior to the depletion of the carbohydrate supply from the growth medium. In contrast, if the same strain is grown under conditions of suppressed conidiation, trehalose activity does not increase until the exogenous carbon supply has become depleted. Total trehalose activity produced by heavily conidioting strains is six-to ten-fold greater than that produced by aconidial or slowly conidioting strains or strains in which conidiation is suppressed. A comparison was made of the activities of 6 different enzymes from the mycelial fraction of strains 60-l 13A, B106a and STL6A during the ten-day growth period, including trehalase, β-galactosidase, alkaline phosphatase, ornithine transcarbamylase, tryptophan synthetase and invertase. The results indicate that trehalase is the only enzyme of those studied that appears to be correlated with conidiation.

The regulation of mycelial trehalose activity under the conditions of this study appears to be by catabolite repression. Evidence for this is as follows:

(1) The derepression of mycelial trehalose which is associated with conidiation occurs when the carbon supply is only partially depleted. However, this increase in activity coincides with a period of extremely rapid growth and, presumably, results from the decreased concentration of the repressor at this time.

(2) The derepression of trehalase in the absence of conidiation does not occur until the complete exhaustion of the carbon source in the growth medium.

(3) An aconidial mutant, strain STL6A, grown in media containing various sugars or L-amino acids as the sole carbon source, exhibits varying levels of trehalose activity. In each instance, a reciprocal relationship is found between the amount of derepression and growth rate upon the substrate used.

(4) The retardation of growth alone does not derepress trehalose. When the growth rate of various strains is severely retarded by any of several methods which do not involve the depletion or limitation of the exogenous carbon supply, trehalose remains repressed.

(5) The complete removal of exogenous carbon supply from rapidly growing mycelium of strain STL6A results in the rapid derepression of trehalose.

(6) When sucrose is added to the growth medium of strain STL6A, in which the trehalose activity per unit weight is high as a result of previous growth in mannitol, rapid repression follows.

An indication that trehalose may play a major role in the development of conidia in Neurospora is its presence in higher quantity in young aerial hyphae before the appearance of conidia. Trehalase activity per unit weight in these structures is three-to four-fold greater than is found in the vegetative mycelium. The derepression of mycelial trehalose during conidiation does not appear to be a primary factor in the developmental process. Rather, it seems to arise as a consequence of the effects of conidiation upon the vegetative mycelium.

Labeling and inhibitor experiments indicate that trehalose derepression represents de novo synthesis of the enzyme rather than activation of existing protein. • • • Department of Botany, University of Michigan, Ann Arbor, Michigan 48104. • Present address: Department of Botany, Brigham Young University, Provo, Utah.