Lowered Respiratory Response to Adenosine Diphosphate of Mitochondria Isolated from a Mutant B Strain of *Schizophyllum commune*

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Mitochondria were isolated from the mycelium of a B-factor mutant in *Schizophyllum commune* that was previously shown to have its energy metabolism partially uncoupled. These mitochondria were compared to mitochondria isolated from wild-type mycelia and were found to increase their respiration rate upon addition of adenosine diphosphate to only one-half the extent of the mitochondria of wild-type mycelia.

It has been recently shown that a genetically determined malfunction of energy metabolism that differs from any previously known occurs as a normal feature in the tetrapolar basidiomycete *Schizophyllum commune* (5).

The malfunction is associated with a specific phenotype that results from either of two distinct genotypes. Sexual morphogenesis in this organism converts sterile, vegetative, homokaryotic hyphae containing one nucleus per cell to potentially fertile dikaryotic hyphae containing two nuclei per cell. This process follows from the mating of two homokaryons that are heteroallelic for both of two incompatibility genes, the A factor and the B factor. A mating between two homokaryons that differs in only one factor results in the operation of only part of the morphogenetic sequence. Matings in which the A factor is homoallelic and the B factor heteroallelic (A = B*) yield heterokaryons with greatly reduced growth and altered hyphal morphology. This heterokaryon is closely mimicked for every known character by a homokaryon carrying a primary mutation in the B factor. Identical developmental controls are elicited by two compatible interacting B factors in the heterokaryon and by the single mutant B factor in the homokaryon. Studies to clarify the physiological basis for the poor growth of this phenotype have been made primarily with the mutant B homokaryon, a homogeneous system, in contrast to the A = B* heterokaryon, which contains homokaryotic as well as heterokaryotic elements (8).

Previous studies on intact mycelia of the mutant B homokaryon and the A = B* heterokaryon have shown this phenotype to have a partial uncoupling between energy-yielding and energy-conserving processes. The degree of uncoupling in isolated mitochondria should be indicated by their respiratory response to added adenosine diphosphate (ADP) (2). The present report notes the isolation of mitochondria from mutant B and wild-type homokaryons and the lowered respiratory response of the mitochondria of the mutant to ADP.

Mycelia were grown in a synthetic medium with glucose as the sole carbon source (5). Started in petri dishes, the mycelia were successively transferred to liquid media of increasing amounts with the final growth taking place in 2,800-ml Fernbach flasks containing 500 ml of the synthetic medium. The mycelia were blended in a Waring Blender at each transfer, and the final cultures were incubated at 30°C on a rotary shaker for 12 to 16 hr. This method of growth was necessary to prevent production of viscous polysaccharide by the wild-type mycelium.

The mycelia were harvested and washed by centrifugation and suspended in a breaking buffer containing 0.3 m mannitol, 10^{-3} m ethylenediaminetetraacetic acid (EDTA), 0.5% cysteine, and 0.1% bovine serum albumin, pH 6.8 (6). The cells were broken in a French pressure cell at 2,000 lb/inch². After breakage, the pH was adjusted to 6.8, and the suspension was centrifuged at 3,600 x g for 10 min. The supernatant fraction was then centrifuged at 8,700 x g for 30 min. The pellet was suspended in a wash medium containing 0.3 m mannitol, 10^{-4} m EDTA, and 0.1% bovine serum albumin, pH 6.8 (6), and the suspension was centrifuged at 3600 x g for 10 min. The supernatant fluid
Table 1. Respiratory response to added adenosine diphosphate (ADP) by isolated mitochondria of wild-type and mutant mycelia

<table>
<thead>
<tr>
<th>Strain of Schizopyllum commune</th>
<th>Expt no.</th>
<th>Protein in reaction medium* (mg)</th>
<th>Ratio of respiration with ADP/without ADP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Protein/ADP with ADP (5 x 10^{-4} M final conc)</td>
<td></td>
</tr>
<tr>
<td>Wild type</td>
<td>8</td>
<td>5.55</td>
<td>0.7</td>
</tr>
<tr>
<td>Wild type</td>
<td>9</td>
<td>4.6</td>
<td>0.7</td>
</tr>
<tr>
<td>Mutant B</td>
<td>10</td>
<td>4.5</td>
<td>1.3</td>
</tr>
<tr>
<td>Mutant B</td>
<td>11</td>
<td>4.6</td>
<td>1.3</td>
</tr>
</tbody>
</table>

*Reaction medium: 3 ml containing 0.3 M mannitol, 10^{-3} M potassium phosphate buffer, 10^{-2} M KCl, 5 x 10^{-3} M MgCl2, and 10^{-2} M succinate, pH 6.5 (6).

Isolated mitochondria were spun at 8,700 x g for 15 min. The resulting pellet was suspended in a Clark-type oxygen electrode. After a constant rate of respiration was attained, ADP was added to give a final concentration of 5 x 10^{-4} M. Protein was measured by the method of Lowry et al. (7).

Mitochondria of wild-type mycelia increased their rate of respiration by a factor of at least two upon the addition of ADP (Table 1). Mitochondria of mutant mycelia, on the other hand, increased their rate of respiration only about 50% upon the addition of ADP. To our knowledge, these mitochondria are the first isolated from this organism, or from any basidiomyete, that demonstrate energy-conserving properties. The rate of increase of respiration due to ADP in the mitochondria of the wild type compares favorably with mitochondria isolated from other fungi (1, 3, 4, 10, 11).

The reduced stimulation of respiration by ADP may indicate that the isolated mitochondria of the mutant are partially uncoupled. It is also possible, however, that the mitochondria are loosely coupled in that nearly normal phosphorylation may occur, but respiratory response to ADP is lessened (2).

The subnormal behavior of the mitochondria of the mutant parallels a corresponding impairment of energy metabolism of the intact mycelium of the mutant. Further experiments are in progress to elucidate the molecular basis of this phenomenon.

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LITERATURE CITED