Fatty acid composition of Neurospora plasma membrane

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Abstract
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TABLE I

<table>
<thead>
<tr>
<th>Fatty Acid Composition (%)</th>
<th></th>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>fatty acid *</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>whole cells</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>plasma membranes</td>
<td>14.0</td>
<td>15.0</td>
<td>16.0</td>
</tr>
<tr>
<td></td>
<td>0.925±0.33</td>
<td>0.925±0.15</td>
<td>30.5±3.8</td>
</tr>
<tr>
<td>plasma membranes</td>
<td>0.615±0.36</td>
<td>0.775±0.035</td>
<td>32.8±2.4</td>
</tr>
<tr>
<td>fatty acid *</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>whole cells</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>plasma membranes</td>
<td>16.1</td>
<td>16.2</td>
<td>18.0</td>
</tr>
<tr>
<td></td>
<td>5.25±2.1</td>
<td>1.2±0.25</td>
<td>3.8±0.75</td>
</tr>
<tr>
<td>plasma membranes</td>
<td>3.4±0.85</td>
<td>6.17±0.2</td>
<td>2.3±0.88</td>
</tr>
<tr>
<td>fatty acid *</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>whole cells</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>plasma membranes</td>
<td>18.1</td>
<td>18.2</td>
<td>18.3</td>
</tr>
<tr>
<td></td>
<td>7.6±0.25</td>
<td>4.6±4.6</td>
<td>5.3±4.7</td>
</tr>
<tr>
<td>plasma membranes</td>
<td>7.37±3.2</td>
<td>4.9±3.1</td>
<td>3.7±2.4</td>
</tr>
</tbody>
</table>

*Chain length: unsaturation.

The availability of Neurospora mutants with altered lipid metabolism and the feasibility of impaling Neurospora with microelectrodes is permitting us to investigate the relationship between membrane lipid composition and membrane electrophysiology (Friedman 1975, J. Membr. Biol. 32: 33-47; 36: 175-190). Such studies assume that the data obtained for total lipid extracts is an accurate reflection of the lipid composition of the plasma membrane. However, we are not aware of any data concerning the distribution of cellular lipids between cytoplasm and cell membrane in Neurospora or other fungi which would support this assumption.

By using the methodology of Scarborough (1975, J. Biol. Chem. 250: 1106-1111) for the isolation of the plasma membrane fraction of the Neurospora cell wall-less mutant slime, we have been able to obtain data which demonstrates that the composition of the plasma membrane fraction is in good agreement with the fatty acid composition of total hyphal extracts.

The slime strain was obtained from Dr. Eugene Scarborough and grown on 800 ml of Vogel's minimal media supplemented with 2% (w/v) mannitol, 0.75% (w/v) yeast extract, and 0.75% (w/v) nutrient broth on a rotary shaker (150 rpm) at 31°C. Cells were harvested and washed with buffer four times by centrifugation. Plasma membranes were isolated according to the procedures of Scarborough (1975). Harvested cells and isolated plasma membranes were freeze-dried for 24 hours. Phospholipids were extracted using procedures similar to those detailed by Friedman (1977). Fatty acid methyl esters were obtained using BF₃-Methanol Reagent and identified and quantified by gas chromatography (see Friedman 1977 for details).

Analyses of three experiments in which fatty acids were extracted from intact slime cells and three experiments in which fatty acids were extracted from isolated plasma membranes are summarized in Table I and Figure I. The means ± standard deviation are shown. To test the hypothesis that there is no difference in fatty acid composition between whole cells and plasma membranes (null hypothesis), we calculated the t-statistic for paired observations. The obtained (t=0.298) has a probability > 80% (for n=10). Statistically, therefore, there is no difference between fatty acid composition between whole cells and plasma membranes.

We believe our results indicate that the fatty acid analyses of "whole cell" Neurospora extracts is an accurate reflection of the fatty acid composition of Neurospora plasma membranes.