Decryption of Acid Phosphatase in Arthrospores of *Geotrichum* Species Treated with Dimethyl Sulfoxide and Acetone

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Decryptification of acid phosphatase in *Geotrichum* sp. arthrospores was accomplished using acetone or dimethyl sulfoxide treatment. Both dimethyl sulfoxide and acetone irreversibly destroyed the integrity of the spore membranes without solubilizing acid phosphatase.

Changes in the specific activity of acid phosphatase have been shown to occur during the life cycle of some fungi (9, 11). We wish to determine if acid phosphatase activity is correlated with arabinol and mannitol formation in the life cycle of *Geotrichum* sp. The present experiments were performed to develop a technique for acid phosphatase assay during differentiation of *Geotrichum* sp. Our initial attempts to isolate the known acid phosphatase activity from lysosome-like particles (10) in the arthrospores were unsatisfactory. Mechanical grinding followed by sonication with glass beads (1 μm in diameter) did not produce greater than 30% disruption of arthrospores.

Dimethyl sulfoxide Me₂SO has been used to alter the permeability of lysosomal membranes (7, 8) and to decryptify α-glucosidase of *Saccharomyces cerevisiae* (1). Treatment of 48-h-old washed arthrospores with this solvent did result in decryption of acid phosphatase. The procedure consisted of growing *Geotrichum* sp. in E-1 medium (glucose salts) for 48 h at 23.5 C (2). The resulting arthrospores were washed twice in distilled water by centrifugation and then placed in various concentrations of Me₂SO (Matheson, Coleman and Bell, Norwood, Ohio, reagent grade) in distilled water or buffer solutions for 30 min at 30 C. The total volume was 5 ml, with arthrospore concentrations ranging between 1.5 to 3.0 mg/ml. The treated spores were then washed twice with distilled water and suspended in 0.15 M sodium acetate buffer at pH 4.7. Portions of washed spores were also filtered onto Whatman glass fiber filters (GF/A, 4.25 cm) which were dried at 80 C for 24 h; such disks were used for dry weight determinations.

Acid phosphatase activity was measured using a modification of Wilson's method (11). Each sample of arthrospores was prewarmed at 30 C in 0.15 M sodium acetate buffer (pH 4.7) before being added to a prewarmed solution of *p*-nitrophenyl phosphate in acetate buffer. The final reaction mixture contained approximately 1 mg of arthrospores and 2 mg of *p*-nitrophenyl phosphate per ml in 2.5 ml of sodium acetate buffer (pH 4.7). This substrate concentration is 10 times the *Kₘ* value (5.6 × 10⁻⁴ M) for acid phosphatase of *Geotrichum* sp. (unpublished data). Reaction mixtures were incubated at 30 C for 30 min; 0.5-ml portions were taken at 0 time and 30 min and mixed with 5 ml of 10% Na₂CO₃ to stop the reaction. Arthrospores were pelleted by centrifugation, and the optical density of the Na₂CO₃ solutions was measured at 400 nm in a Bausch and Lomb Spectronic 20 spectrophotometer using the 0-time Na₂CO₃ solutions as reference.

Enzyme activity was optimal after treatment with 40 to 45% Me₂SO (Fig. 1). It should be noted that the supernatant was devoid of acid phosphatase activity; the enzyme(s) was apparently still bound to spores which were now fully permeable to neutral red. Untreated viable spores contained neutral red only in discrete vesicles presumed to be lysosomes (5, 6, 10). Germination studies demonstrated that the spores which stained uniformly with neutral red at a concentration of 1:10,000 had lost their viability and were not capable of producing germ tubes; a total of 400 spores was counted in each viability determination. This result is in contrast to studies showing that animal cells remain viable after Me₂SO treatment (4).

Treatment of spores with 50% Me₂SO quantitatively permeabilized the spore population (Table 1) but yielded less activity than treatment with 45% Me₂SO (Fig. 1). The data may indicate that a fraction of the enzyme was denatured with 50% Me₂SO treatment. At-
tempts to increase enzyme activity using modified Me₂SO treatments were successful (Table 2); however, Me₂SO concentrations greater than 45% again resulted in reduced enzyme activity (data not shown). It appeared that Me₂SO treatments could be used to decryptify acid phosphatase in Geotrichum sp. provided the concentrations were kept at 45%. Whereas the Me₂SO treatment appeared adequate for

![Graph](image)

**FIG. 1. Effects of various concentrations of Me₂SO in 10 mM phosphate buffer (pH 6.5) on decryptification of acid phosphatase.** The peak enzyme activity in each experiment was designated the 100% activity level to compensate for differences in spore concentrations in the four experiments. Enzyme activities at other Me₂SO concentrations were expressed as percentages of the peak enzyme activity. Symbols: ○, experiment 1; □, experiment 2; △, experiment 3; and ●, experiment 4.

**Table 1. Effect of increasing concentrations of Me₂SO in 10 mM phosphate buffer (pH 6.5) on the viability of arthrospores of Geotrichum sp.**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Germination (%)</th>
<th>Nonviable spores (%)</th>
<th>% of spores uniformly stained by neutral red</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (no Me₂SO)</td>
<td>99.3</td>
<td>0.7</td>
<td>0.5</td>
</tr>
<tr>
<td>30% Me₂SO</td>
<td>79.5</td>
<td>20.5</td>
<td>18.2</td>
</tr>
<tr>
<td>35% Me₂SO</td>
<td>76.1</td>
<td>23.9</td>
<td>26.5</td>
</tr>
<tr>
<td>40% Me₂SO</td>
<td>33.6</td>
<td>66.4</td>
<td>67.0</td>
</tr>
<tr>
<td>45% Me₂SO</td>
<td>5.5</td>
<td>94.5</td>
<td>93.6</td>
</tr>
<tr>
<td>50% Me₂SO</td>
<td>2.6</td>
<td>97.4</td>
<td>100.0</td>
</tr>
</tbody>
</table>

*Arthrospores were incubated on E-1 (0.4% glucose) medium at 23.5°C for 7 h.

*Calculated from percent germination data.

**Table 2. Effect of water and buffers on decryptification of acid phosphatase with 45% Me₂SO**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>% of spores permeable to neutral red</th>
<th>Sp act of acid phosphatase*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (no Me₂SO)</td>
<td>0.5</td>
<td>0.01</td>
</tr>
<tr>
<td>45% Me₂SO in deionized water Me₂SO</td>
<td>94.8</td>
<td>1.70</td>
</tr>
<tr>
<td>45% Me₂SO in 0.15 M sodium acetate buffer (pH 4.7)</td>
<td>97.2</td>
<td>1.50</td>
</tr>
<tr>
<td>45% Me₂SO in 10 mM phosphate buffer (pH 6.5)</td>
<td>91.3</td>
<td>1.10</td>
</tr>
</tbody>
</table>

*Specific activity of acid phosphatase was defined as optical density at 400 nm/mg (dry weight) of spores per 30 min.

our purposes, we sought a more reliable technique.

A modified acetone treatment (3) was found to be effective in decryptifying dormant arthrospores. Spores were suspended in 100% acetone at a concentration of 1 mg of spores/10 ml of solvent and stirred at -5°C for 5 min; the spores were then filtered onto glass fiber disks and washed with an additional 10 ml of acetone. The disks were allowed to dry at room temperature in a vacuum desiccator. The resulting acetone powders could be conveniently handled, since exact quantities of dry arthrospores could be resuspended in acetate buffer. Such suspensions possessed an acid phosphatase specific activity of 1.7, a result which compared well with the best Me₂SO decryptification. All enzyme activity remained associated with the arthrospores even though they were 100% uniformly stained with neutral red. The acid phosphatase activity was not affected by storing the acetone powders at -20°C for at least 6 months. We conclude that the acetone procedure is superior to the Me₂SO procedure because of better reproducibility and ease of handling.

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**LITERATURE CITED**