Macroconidial Germination in *Microsporum gypseum*

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Biochemical events which occur during macroconidial germination have been studied in the dermatophyte *Microsporum gypseum*. The specific activity levels of various metabolic enzymes have been assayed during germination time periods. The accumulated levels of several of these enzymes, as a function of exogenous carbohydrate source, have been investigated. *M. gypseum* was found to possess a constitutive glyoxalate shunt, a constitutive glucokinase, a fructose phosphoenolpyruvate transferase, and a mannitol phosphoenolpyruvate transferase. The integration of endogenous reserve utilization during germination is discussed. The purification and properties of an alkaline phosphatase and its possible relationship to sporulation and spore germination are also described.

There have been many studies of the biochemistry of fungal conidial germination. The data accumulated from much of this work are available in several excellent reviews (5, 8, 26). Most of the organisms examined thus far preserve the vegetative nuclear-cytoplasmic ratio in the conidium. In the dermatophyte *Microsporum gypseum*, the vegetative nuclear-cytoplasmic ratio of two nuclei per hyphal septated unit is not preserved during macroconidial development (Leighton and Stock, in preparation). The macroconidium of *M. gypseum* may contain from one to eight nuclei per septated spore compartment. Nuclear distribution analyses indicate that greater than 70% of the total nuclear population is distributed above the two nuclei per spore compartment level. The structural complexity of the macroconidium and its unusual nuclear distribution properties suggest that the analysis of macroconidial germination in *M. gypseum* could yield further information concerning fungal cell differentiation processes. This preliminary investigation describes several biochemical aspects of spore germination in *M. gypseum*.

**MATERIALS AND METHODS**

The organism, sporulation, and germination conditions were as previously described (14, 15).

Sporulation and spore germination in the presence of mannitol, fructose, and acetate. Mannitol (1%, w/v), fructose (1%, w/v), or sodium acetate (3%, w/v) was substituted for glucose (1%, w/v) where indicated.

Sporulation and spore germination were always carried out by using the same carbon source. That is, if fructose was the carbon source used for spore germination studies, these spores were harvested from a sporulation medium containing fructose as the single added carbohydrate.

**Preparation of cell-free extracts.** Sonically disrupted spore and mycelial extracts were prepared as previously described (15). Extracts were also obtained by the liquid nitrogen method of Bleyman and Woese (2). Debris was removed from the liquid nitrogen grindate by centrifugation at 10,000 × g for 10 min at 4°C. In all cases, cell-free extracts were buffered in 0.2 M tris(hydroxymethyl)aminomethane (Tris)-hydrochloride, pH 7.4.

**Analytical determinations.** Protein was estimated by the method of Lowry et al. (18). Crystalline bovine serum albumin was used as the standard. Total carbohydrate was determined by the anthrone reaction (19), with glucose used as the standard. Inorganic phosphate was quantitated by the method of Chen et al. (4). Ribonucleic acid (RNA) was measured by the orcinol reaction (22), with yeast RNA used as a standard.

**Carbohydrate extraction and identification.** The 80% alcohol and sulfuric acid-soluble extracts were prepared as described by Lingappa and Sussman (16). Alcohol-soluble extracts were desalted by passage through a mixed bed of Dowex-1-x-8 (Cl-)- and Dowex-50W-x-4 (H+). Immediately after extraction and desalting, carbohydrates in the alcohol-soluble pool were separated by descending paper chromatography by use of a pyridine-ethyl acetate-water (50: 120:40, v/v) solvent system. Sugars were detected by developing the chromatograms with silver nitrate (27) or benzidine (10) reagents. Unknown spots were identified by co-chromatography with authentic standards.

**Enzyme assays.** Enzyme activities were determined by following the changes in optical density of the
reaction mixtures at 25°C with an automatic recording spectrophotometer (model 2400; Gilford Instrument Laboratories, Oberlin, Ohio). Absorbance changes were measured with reference to reaction mixtures minus substrates. Measurements of reaction rates were made under conditions in which the initial measured rate was linear with respect to time. Specific activities are reported as the change in optical density units per milligram of cell-free extract protein per minute.

The following enzyme activities were measured: alkaline phosphatase, glucose-6-P-O4 dehydrogenase, aldolase, glyceraldehyde-3-P-O4 dehydrogenase, enolase, fumarase, and nicotinamide adenine dinucleotide phosphate (NADP)-linked isocitric dehydrogenase (as described in Biochimica Catalogue, Bohringer Mannheim Corp., New York, N.Y.); mannitol-1-P-O4 phosphatase (25); NADP-linked mannitol dehydrogenase (11); nicotinamide adenine dinucleotide (NAD)-linked mannitol-1-P-O4 dehydrogenase (25); fructokinase (24); mannitol kinase (24); gluco-kinase (24); transaldolase (6); transketolase (6); isocitrate lyase (23); glucose phosphoenolpyruvate (PEP) transerase (9); fructose PEP transerase (9), and mannitol PEP transerase (9).

Alkaline phosphatase purification. All steps of the procedure were carried out at 4°C unless otherwise stated. Alkaline phosphatase assays were carried out on all samples at each step of the procedure. Finely ground, solid ammonium sulfate was used to produce the various saturation levels.

Step 1. The cell-free extract of M. gypseum mycelia was centrifuged at 110,000 × g in a Spinco model L ultracentrifuge for 2 hr. The supernatant fluid was removed and stored in an ice bath. The pellets were suspended to original volume in 1.0 M Tris-hydrochloride buffer (pH 8.0) containing 10−4 M MgCl₂ prior to determination of alkaline phosphatase activity.

Step 2. With 100 ml of the cell-free extract supernatant fraction, 50% saturation was achieved by the addition of 25 g of ammonium sulfate with constant stirring on an NaCl ice bath. The mixture was allowed to stand for 10 min and then centrifuged at 13,000 × g for 15 min. The supernatant fluid was brought to 60% saturation by the addition of 7 g of ammonium sulfate in the previously described manner. After standing for 10 min, the suspension was centrifuged as before, and the pellet was discarded. The supernatant fraction was brought to 65% saturation through the further addition of 3.5 g of ammonium sulfate and allowed to stand for 10 min. The suspension then was centrifuged as before. The pellet was retained and held at 0°C. The supernatant liquid was then brought to 75% saturation by the further addition of 7 g of ammonium sulfate and again allowed to stand for 10 min. The suspension was centrifuged as before, and the pellet was retained and stored at 0°C. The supernatant fluid was brought to 80% saturation by the addition of 3.5 g of ammonium sulfate. The suspension was allowed to stand for 10 min and then was centrifuged as before. The pellet was retained, and the supernatant fraction was discarded. The pellets from the 65 to 80% saturations were dissolved and pooled in 1 M Tris-hydrochloride buffer (pH 8.0) containing 10−4 M MgCl₂ to a final volume of 15 ml. The solution was dialyzed for 24 hr against two successive 2-liter volumes of 0.02 M Tris-hydrochloride buffer (pH 8.0) containing 10−4 M MgCl₂.

Step 3. Diethylaminoethyl ether (DEAE) cellulose was equilibrated with 0.02 M Tris-hydrochloride buffer (pH 8.0) containing 10−4 M MgCl₂, and then packed to a height of 10 cm above a piece of finely meshed nylon in an 11-mm diameter glass column. A 7.5-ml volume of the dialyzed enzyme preparation from purification step 2 was applied to the top of the column. After the enzyme solution had entered the column, the phosphatase was eluted from the column by using 300 ml of Tris-hydrochloride buffer (pH 8.0), containing 10−4 M MgCl₂, in a linear gradient from 0.02 to 0.25 M. Five-milliliter fractions were obtained by using a Warner-Chilcott fraction collector (Warner-Chilcott Inc., Richmond, Calif.). The absorbance of the fractions was read at 280 nm with a Gilford model 2400 spectrophotometer. Those fractions containing the major portion of enzymatic activity were pooled and concentrated to a 10-ml final volume with a Dialvo apparatus, by using a membrane of 1,000 molecular weight retention. The pooled, concentrated enzyme preparation was then dialyzed for 24 hr against two successive 2-liter volumes of 0.02 M Tris-hydrochloride buffer (pH 8.0) containing 10−4 M MgCl₂.

Step 4. Column chromatography was carried out on this enzyme preparation in the same manner as described in Step 3, except that the total volume of buffer used in the gradient was reduced to 200 ml. The absorbance of fractions was read at 280 nm and those containing the major portion of enzymatic activity were pooled and concentrated to a final volume of 10 ml as before. The final enzyme preparation was frozen and stored at −70°C as 1-ml volumes until required.

Disc gel electrophoresis. Disc gel electrophoresis (pH 9.3) was carried out by the procedure for the Canalco model 6 system (Canalco, Rockville, Md.).

Activity of alkaline phosphatase on various phosphorylated compounds. The concentration of all phosphorylated compounds employed was 8.5 × 10−4 M. Reaction mixtures contained 50 μl of purified enzyme preparation, 6.0 ml of 1 M Tris-hydrochloride buffer (pH 8.0), 5.0 ml of distilled water, and 1.0 ml of substrate. Control reaction mixtures contained all constituents except the enzyme preparation. All assays were carried out at 37°C, allowing 10 min of incubation for each of the various substrate reaction mixtures and control mixtures to equilibrate to temperature. Samples of 2.0 ml were taken from both control and reaction mixtures at set time intervals and added to 1.0 ml of cold 1 N H₂SO₄. The amount of inorganic phosphate in the reaction and control samples was determined by the procedure of Chen et al. (4).

The background level of inorganic phosphate in the control mixtures was subtracted from that of the reaction mixtures for each substrate. Rates of hydrolysis by the alkaline phosphatase were calculated and compared for each substrate.

Chemicals. All substrates for enzyme assays were
purchased from Boehringer Mannheim Corp., with the exception of fumaric acid, isocitric acid, and D-mannitol, which were purchased from Calbiochem (Los Angeles, Calif.). Semi-carbazide was purchased from Fisher Scientific Co., Fairlawn, N.J. All analytical standards and carbohydrates were obtained from Calbiochem. Mannitol-1-PO₄ was the generous gift of L. Klungsøyr (Physiological Institute, University of Bergen, Bergen, Norway). Reagents for disc gel electrophoresis were obtained from Canalco, Rockville, Md. Tris, N-tris(hydroxymethyl)methyl-2-aminoethane sulfonic acid (TES), and N-2-hydroxyethylpiperazine-N'-2-ethane sulfonic acid (HEPES) were purchased from Calbiochem.

RESULTS

Macroconidial utilization of alcohol- and acid-soluble carbohydrates (saline-germination system). Utilization of endogenous carbohydrate reserves is essential for the germination of conidia in many fungi. Since M. gypseum macroconidia are capable of germinating in the absence of exogenous nutrients (14), we thought it was possible that endogenous carbohydrate reserves could provide part of the energy necessary for this germination process.

Chromatographic analysis of the zero-time 80% alcohol-soluble pool indicated the presence of glucose, fructose, mannitol, trehalose, and inositol. The mannitol spot was no longer detectable at 3 hr after initiation of spore germination. Trehalose and inositol spots were no longer detectable after 8 hr. Figure 1 illustrates the time course utilization of alcohol- and acid-soluble carbohydrate fractions during germination. By 8 hr, nearly all the available alcohol-soluble pool had been utilized. No further germ-tube elongation was observed after this time.

Enzyme specific activity changes during periods of spore germination (glucose-germination system). It was our experience that a 0.2 M Tris-hydrochloride buffer, pH 7.4, was superior to 0.2 M phosphate buffer, pH 7.4, for the preparation of cell-free extracts. Phosphate at 0.2 M appeared to be an inhibitor to several of the enzymes assayed. Neither HEPES nor TES at 0.2 M, pH 7.4, gave appreciably higher specific activities when compared with Tris-hydrochloride buffer. Experimental variance in single enzyme specific activities between subsequent experiments was never greater than 10%. The enzyme assays cited were found to produce optimal reaction rates in this system (unpublished data).

Table 1 depicts the time course of the 12 metabolic enzymes assayed. Each enzyme also has been assigned an activity ratio which is the zero-time specific activity divided by the 24-hr specific activity. The observed pattern does not appear to be an artefact of cell disruption. Spores disrupted by sonic oscillations or liquid nitrogen gave identical activity ratio level profiles.

Mannitol metabolism in germinating macroconidia (mannitol, fructose, or glucose-germinating system). Sporulation on the various carbohydrates was completed by 7 days, and the harvested spores from each carbohydrate source exhibited similar germination time periods. That is, spores harvested from the homologous carbohydrate source germinated to the 100% level by 8 hr in all cases (unpublished data). Macroconidia of M. gypseum contained an NAD-linked mannitol-1-PO₄ dehydrogenase, a mannitol-1-PO₄ phosphatase, and an NADP-linked mannitol dehydrogenase.

Table 2 lists the specific activities of glucose PEP transferase, fructose PEP transferase, and mannitol PEP transferase after 24 hr of germination in the presence of glucose, fructose, or mannitol.

Table 3 lists the specific activities of glucokinase, fructokinase, and mannitol kinase after 24 hr of germination in the presence of glucose, fructose, or mannitol.

The above data suggested the presence of a "constitutive" glucokinase and that mannitol PEP transferase and fructose PEP transferase accumulated to higher specific activities when their respective substrates were added exogenously. It is of interest that both the fructose and
manitol-phosphate
Transaldolase
Transketolase
Glucose-6-phosphate phosphatase
Alkaline dehydrogenase
......................................
Enolase...Glyceraldehyde-3-phosphate isocitrate lyase...
Fumarase ....................................

2.4 hr after in the presence phospho-

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PEP enzymes phosphatase, cumulation cose, mannitol, ute
tivities of phospho-

Fructose during enzymes ods, tive glyoxylate apparent that glyoxylate shunt (acetate or pyruvate transfer-

trans-enolpyruvate trans-

trans-enolpyruvate ferase.

Activity 4

Evidence for the occurrence of a constitutive glyoxylate shunt (acetate or glucose-germination system). Table 5 lists the changes in specific activities of glyoxylate and tricarboxylic acid cycle enzymes during macroconidial germination. It is apparent that M. gypseum possesses a constitutive glyoxylate shunt. It is also evident that ger-

mation in acetate resulted in increased levels of isocitrate lyase and fumarase and a decrease in the level of isocitrate dehydrogenase.

Purification and properties of an alkaline phosphatase. Alkaline phosphatase-type activities are known to be associated with the terminal steps in sporulation of fungi and related organisms (7, 17, 20). Since an alkaline phosphatase appeared to accumulate maximally during terminal sporulation of M. gypseum (unpublished data), we were interested in investigating the function of this enzyme and its behavior during spore germination.

Table 6 lists the recovery and purification of alkaline phosphatase throughout the isolation procedure. The purified enzyme was found to be homogeneous with respect to alkaline phosphatase activity as judged by disc gel electrophoresis. The enzyme has an apparent affinity constant of $6.3 \times 10^{-4}$ (moles of $p$-nitrophenyl phosphate/liter) at 37°C, pH 8.0 (Fig. 2). It appears that the enzyme is inhibited by orthophosphate (Fig. 3).
Table 4. Activities of mannitol metabolism enzymes after 24-hr germination

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Carbohydrate source for sporulation and spore germination</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Glucose specific activity</td>
</tr>
<tr>
<td>Mannitol dehydrogenase</td>
<td>0.540</td>
</tr>
<tr>
<td>Mannitol-1-phosphate dehydrogenase</td>
<td>0.300</td>
</tr>
<tr>
<td>Mannitol-1-phosphate phosphatase</td>
<td>9.5</td>
</tr>
</tbody>
</table>

*a Specific activity = Δ optical density per minute per milligram of cell-free extract protein.

*b Specific activity = micromoles of inorganic phosphate released per 0.18 mg of cell-free extract protein per 75 min at 37 C.

Table 5. Glyoxalate and tricarboxylic acid cycle enzyme activities during macroconidial germination

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Carbohydrate source for sporulation and spore germination</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Acetate specific activity</td>
</tr>
<tr>
<td>Isocitrate lyase</td>
<td>0.022</td>
</tr>
<tr>
<td>Isocitrate dehydrogenase</td>
<td>0.061</td>
</tr>
<tr>
<td>Fumarase</td>
<td>0.048</td>
</tr>
</tbody>
</table>

*a Specific activity = Δ optical density per minute per milligram of cell-free extract protein.

The rate of phosphorlytic activity of the enzyme against several products of intermediary metabolism, as measured by release of orthophosphate, is summarized in Table 7. The enzyme has high activity against fructose-6-PO₄ and glucose-6-PO₄ (5). Since both of these compounds are important

Table 6. Purification of Microsporum gypseum alkaline phosphatase

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Total enzyme units*</th>
<th>Protein mg/ml</th>
<th>Enzyme units/mg of protein</th>
<th>Yield (%)</th>
<th>Purification (fold)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell-free extract</td>
<td>92</td>
<td>5.1</td>
<td>18</td>
<td>100</td>
<td>1</td>
</tr>
<tr>
<td>Supernatant from 110,000 × g centrifugation</td>
<td>92</td>
<td>1.0</td>
<td>92</td>
<td>100</td>
<td>5</td>
</tr>
<tr>
<td>65 to 80% saturation with (NH₄)₂SO₄</td>
<td>80</td>
<td>0.24</td>
<td>320</td>
<td>87</td>
<td>18</td>
</tr>
<tr>
<td>Pooled fractions from the final DEAE-cellulose column</td>
<td>22</td>
<td>0.0052</td>
<td>4,230</td>
<td>24</td>
<td>240</td>
</tr>
</tbody>
</table>

*a One unit of enzyme activity was defined as the quantity of enzyme that catalyzes the release of 1 μmole of p-nitrophenol in 1 min under the conditions specified.

*b Values corrected for the fact that only one half of the total volume from the 65 to 80% ammonium sulfate saturation fraction was applied to the first diethylaminoethyl (DEAE)-cellulose column.
TABLE 7. Substrate specificity of alkaline phosphatase

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Relative reaction velocity</th>
</tr>
</thead>
<tbody>
<tr>
<td>p-Nitrophenyl phosphate</td>
<td>1.0</td>
</tr>
<tr>
<td>Fructose-6-phosphate</td>
<td>1.7</td>
</tr>
<tr>
<td>Glucose-6-phosphate</td>
<td>1.5</td>
</tr>
<tr>
<td>Ribose-5-phosphate</td>
<td>1.4</td>
</tr>
<tr>
<td>Mannitol-1-phosphate</td>
<td>1.2</td>
</tr>
<tr>
<td>a-Glycerophosphate</td>
<td>1.2</td>
</tr>
<tr>
<td>b-Glycerophosphate</td>
<td>1.2</td>
</tr>
<tr>
<td>3-Phosphoglyceric acid</td>
<td>0.8</td>
</tr>
<tr>
<td>Fructose-1-6-diphosphate</td>
<td>1.0</td>
</tr>
<tr>
<td>Bis-p-nitrophenyl phosphate</td>
<td>0</td>
</tr>
</tbody>
</table>

TABLE 8. Relative concentrations of spore ribonucleic acid and protein in zero-time and 24-hr cell-free extracts

<table>
<thead>
<tr>
<th>Time</th>
<th>RNA</th>
<th>Protein</th>
<th>Ratio RNA:protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>hr</td>
<td>µg/ml</td>
<td>µg/ml</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>396</td>
<td>7,200</td>
<td>0.055</td>
</tr>
<tr>
<td>24</td>
<td>910</td>
<td>6,500</td>
<td>0.140</td>
</tr>
</tbody>
</table>

substrates for intermediary metabolism and reserve synthesis, these data suggested that the alkaline phosphatase may be active in the shutting off of intermediary metabolism and the synthesis of carbohydrate reserves during the terminal periods of sporulation.

RNA to cell-free extract protein ratios during spore germination (glucose-germination system). Table 8 lists the RNA to cell-free extract protein ratios in zero time and 24-hr spores. It is apparent that RNA is not concentrated in the spore and evidently is synthesized to a greater extent than cell-free extract protein during spore germination.

DISCUSSION

Since there has been only one previous study of macroconidial germination in M. gypseum (1), this preliminary investigation was undertaken to ascertain what basic processes occurred during spore germination, and hopefully to suggest possible germination-specific proteins which might be examined subsequently in further detail.

The composition of the alcohol-soluble pool was very similar to that observed in Aspergillus (11, 12) and Neurospora (12, 16). The ordered disappearance of mannitol and subsequently trehalose and inositol was very similar to the Aspergillus system. Horikoshi et al. (11) have shown that mannitol is a repressor of trehalase and must be utilized prior to any trehalase degradation.

The utilization pattern of the alcohol-soluble pool was found to be very similar to that reported for Neurospora (16). The biphasic nature of the utilization curve is probably the result of mannitol utilization prior to the degradation of trehalose and inositol. It appears that M. gypseum is unique in its ability to utilize a considerable portion of the acid-soluble pool during spore germination.

The pattern of enzyme specific activity changes observed during spore germination was somewhat unusual. All enzymatic activities either remained constant or decreased to some extent during the germination and hyphal outgrowth time period. This may be explained most easily by the fact that the vegetative nuclear-cytoplasmic ratio was not preserved in the macroconidium. Upon germination, the concentrated spore nuclei migrate into the growing germ tube and re-establish the lower mycelial nuclear cytoplasmic ratio (Leighton and Stock, in preparation). Since germination enzymes of necessity would have to be synthesized prior to and during germ-tube elongation, i.e., "concentrated" prior to rapid germ-tube outgrowth, there could be a disproportionate amount of non-enzymatic proteins synthesized during the outgrowth time period. This would give rise to an apparent decrease in enzyme specific activities during spore germination. In this type of a system, enzymes which maintain a constant specific activity during germination may in fact represent a considerable amount of enzyme accumulation.

It has been suggested by other workers that mannitol dehydrogenase and isocitrate dehydrogenase are involved in fungal spore germination (3, 8, 11; J. C. Galbraith and J. E. Smith, Proc. Soc. Gen. Microbiol., p. 12, 1969). It is interesting that these two enzymes have a low activity ratio in the M. gypseum germination system. It is possible that these enzymes are involved in germination processes. Further de novo synthesis studies are presently in progress to clarify this observation.

Investigations involving other fungi and related organisms have suggested that alkaline phosphatase (7, 17, 20) and isocitrate lyase (3, 8; Galbraith and Smith, Proc. Soc. Gen. Microbiol. 1969:xii) are present at high levels during fungal sporulation. In addition, it has been shown that M. gypseum alkaline phosphatase is produced maximally during periods of terminal differentiation (Leighton and Stock, in preparation).
The specificity data obtained for this enzyme have suggested that it may function in the shut-off of intermediary metabolism and carbohydrate reserve synthesis. We also knew that the release of inorganic phosphatase is an essential feature of macroconidial germination in *M. gypseum* (Leighton, Page, and Stock, in preparation). This phosphatase could be derived easily from the action of alkaline phosphatase on fructose-6-P or glucose-6-P. Since the enzyme is inhibited by high concentrations of orthophosphate, it may regulate its own shut-off by a type of end-product inhibition. This enzyme is also being studied for its potential synthesis during germination time periods since its high activity ratio suggested it may not accumulate during spore germination.

Many fungi possess a constitutive glyoxylate shunt (5), and *M. gypseum* appears to be no exception. When spores of this organism were germinated in acetate, isocitrate lyase accumulated to a higher level than in glucose-germinated spores. In the presence of acetate, the isocitric dehydrogenase level decreases. This may be explained most readily by the observation of Osaki and Shiio (21), which indicates that isocitrate dehydrogenase is repressed by glyoxylate cycle products. Hence, an increase in glyoxylate cycle activity would be expected to result in a decreased level of isocitrate dehydrogenase activity. The fact that fumarase levels were high during acetate germination growth may be a result of increased succinate levels and apparently a lack of inhibition of fumarase by glyoxylate cycle products.

It is not surprising that *M. gypseum* macroconidia do not contain a high ratio of RNA to cell-free extract protein. The terminal steps of sporulation occur under what are essentially starvation conditions (William Page, unpublished data). This type of environment does not favor a high ratio of RNA to cell-free extract protein.

Fungal conidia cannot survive temperature extremes (26). In the case of *M. gypseum*, the macroconidia are capable of surviving temperatures of 50 to 55°C for only short periods of time (14). However, macroconidia are capable of surviving for several months in the range 4 to 25°C. The ability of the macroconidium to survive does not seem to reside in its possessing a protective physical environment, as is the case with fungal ascospores (26). We suggest that the increased nuclear-cytoplasmic ratio in the macroconidium results in a large number of copies of essential information in the spore. This selective concentration of nuclei increases the number of available targets and concomitantly the number of potentially lethal events that the spore is capable of surviving.

**ACKNOWLEDGMENT**

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**LITERATURE CITED**


