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# Simple Procedure for Disruption of Fungal Spores†

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A simple, inexpensive method for disrupting dormant fungal spores is described.

The lack of suitable procedures for disrupting fungal spores has impeded many physiological and biochemical studies of spores. In this report we describe a simple, quick, and inexpensive method for breaking dormant fungal spores. The procedure works for a variety of fungal spores and has been used succesfully in several laboratories in addition to our own.

The method, which is slightly modified from that described by Lipinsky et al. (2) for disrupting yeast cells, consists of placing 5 to 10 g of glass beads (Bronwill Scientific Co.) in a 15- or 30-ml Sorvall Corex tube together with 3.5 to 5 ml of buffer and 25 to 1,000 mg of spores. The sample is homogenized for 5 to 120 s with a Vortex mixer set at maximum speed. The choice of bead size for a particular spore is a critical variable in the procedure. Table 1 lists the conditions that gave 90 to 100% breakage of spores of seven species. These examples include a range of spore types, i.e., ascospores of *Neurospora crassa*, uredospores of a rust fungus (*Uromyces phaseoli*), teliospores of the corn smut fungus (*Ustilago maydis*), sporangiospores of *Rhizopus stolonifer*, pycnidiospores of *Botryodiplodia theobromae*, conidia of *Aspergillus nidulans*, and spores of the slime mold *Dictyostelium discoideum*.

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The effect of homogenization time and spore concentration on breakage and recovery of soluble protein from spores of both *R. stolonifer* and *B. theobromae* is reported in Table 2. Polysomal profiles obtained from *R. stolonifer* spores germinated for 4 h are depicted in Fig. 1; these data illustrate two things. First, extensive breakage occurs very rapidly, some within 5 s; second, the procedure disrupts spores at all stages of germination (germ tubes begin to appear at 4 h in *R. stolonifer* spores). It should be noted that excessive homogenization (e.g., 60 s) shears polysomes. However, excellent polysomal profiles can be obtained from 50 mg of spores homogenized for 5 to 30 s.

We have also used the procedure to extract intact ribonucleic acid from *R. stolonifer* and *B. theobromae* spores. At our suggestion, others have employed the procedure for the extraction of steroid methyltransferases from dormant *U. phaseoli* spores (H. Knoche, personal communication), adenosine 5'-triphosphate from dormant *D. discoideum* spores (D. Cotter, personal communication), and deoxyribonucleic acid with a molecular weight greater than $30 \times 10^6$ from dormant *A. nidulans* spores (R. Morris, personal communication). Although we have not attempted to isolate organelles from dormant

TABLE 1. *Optimal conditions for 90 to 100% breakage of a variety of fungal spores*

| Organism | Spore type and approx size (μm) | Optimal conditions | | | | | |
|---|---|---|---|---|---|---|---|
| | | Spores (mg) | Glass beads | | Buffer (ml) | Corex tube size (ml) | Homogenization time (s) |
| | | | Size (mm) | Amt (g) | | | |
| *B. theobromae* | Pycnidiospore (16 by 22) | 200 | 1.0 | 10 | 5 | 30 | 60 |
| *N. crassa* | Ascospore (15 by 25) | 50 | 1.0 | 10 | 5 | 30 | 30 |
| *U. maydis* | Teliospore (7 by 10) | 50 | 1.0 | 10 | 5 | 30 | 75 |
| *U. phaseoli*[a] | Uredospore (22 by 28) | 200 | 1.0 | 5 | 2.5 | 15 | 100 |
| *R. stolonifer* | Sporangiospore (8) | 200 | 0.5 | 10 | 5 | 30 | 30 |
| *D. discoideum*[b] | Asexual spore (3 by 7) | 12.5 | 0.25 | 5 | 1.25 | 15 | 50 |
| *A. nidulans*[c] | Conidia (3) | 500 | 0.17 | 10 | 3 | 30 | 15 |

[a] Conditions from H. Knoche.
[b] Conditions from D. Cotter.
[c] Conditions from R. Morris.

TABLE 2. *Effect of time and spore concentration on breakage of R. stolonifer and B. theobromae spores*[a]

| Spores per sample(mg) | Breakage (%)[b] at time (s): | | | | | | |
|---|---|---|---|---|---|---|---|
| | 5 | 10 | 15 | 30 | 45 | 60 | 90 |
| *R. stolonifer* | | | | | | | |
| 50 | 25 (39) | 64 (64) | 75 (91) | 99 (100) | 100 (100) | 100 (100) | 100 (100) |
| 250 | 34 (29) | 45 (50) | 74 (72) | 87 (98) | 100 (100) | 100 (100) | 100 (100) |
| 500 | 37 (22) | 65 (55) | 70 (60) | 79 (97) | 92 (98) | 100 (100) | 100 (100) |
| 1,000 | 25 (18) | 30 (29) | 33 (41) | 63 | | 74 (91) | 100 (100) |
| *B. theobromae* | | | | | | | |
| 50 | 22 (16) | 39 (33) | 66 (51) | 74 (78) | 83 (82) | 97 (96) | 100 (100) |
| 250 | 11 (17) | 25 (29) | 42 (41) | 80 (63) | 85 (84) | 89 (92) | 100 (100) |
| 500 | 22 (32) | 50 (48) | 64 (55) | 76 (65) | 80 (82) | 87 (90) | 100 (100) |
| 1,000 | 13 (19) | 14 (38) | 34 (37) | 58 (59) | 76 (72) | 80 (85) | 100 (100) |

[a] Samples contained 10 g of glass beads (0.5 mm for *R. stolonifer* and 1.0 mm for *B. theobromae*), 5 ml of buffer, and the amount of spores indicated.

[b] Numbers not in parentheses refer to the percent breakage determined microscopically. Numbers in parentheses refer to the percentage of total soluble protein after centrifuging the samples at 10,000 × *g* for 20 min. Protein was estimated by the procedure of Lowry et al. (3); 100% soluble protein was obtained by disrupting the spores in a Bronwill MSK mechanical homogenizer.

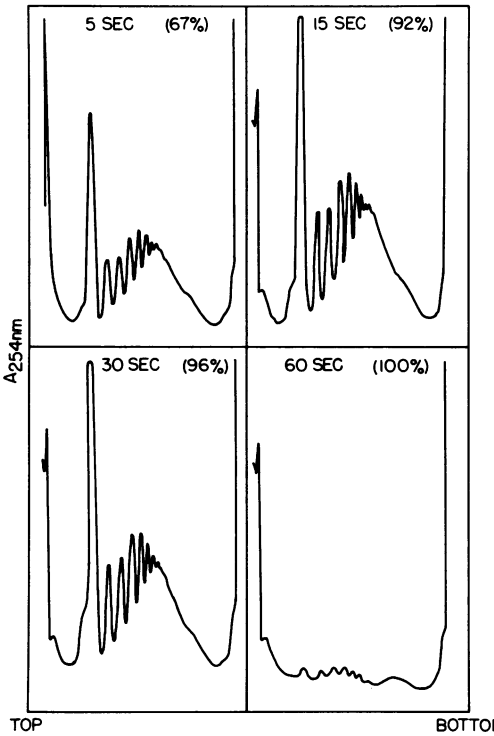


FIG. 1. *Polysomal profiles obtained from 50 mg of R. stolonifer spores germinated for 4 h. The spores were exposed to the breakage procedure for the time period indicated, and a polysomal-enriched fraction was isolated and subjected to sucrose density gradient centrifugation as described previously (1). The number in parentheses on each part of the figure refers to the percentage of* $A_{260\ nm}$ *(absorbance at 260 nm) material recovered after a low-speed centrifugation.*

spores, very short homogenization times might allow the isolation of intact organelles.

The procedure offers several advantages over other methods that are commonly used for disrupting fungal spores, such as the Bronwill MSK mechanical homogenizer and the Hughes, French, or Raper-Hyatt press. (i) Treatment is brief, usually less than 1 min; thus, many samples can be processed quickly. (ii) The method is suitable for small quantities of spores, since material is not lost in transferring samples. (iii) Because homogenization is brief, the sample temperature does not rise. (iv) By using very short homogenization times, i.e., a few seconds, one can get single cracks in the spore wall without totally macerating the spores. (v) The glass beads can be reused after soaking in concentrated HCl and extensive washing. (vi) The equipment is inexpensive.

We thank Calvin McLaughlin for calling our attention to the procedure used for breaking yeast cells and to Herman Knoche, David Cotter, and Ronald Morris for informing us of the optimum conditions required to disrupt *U. phaseoli*, *D. discoideum*, and *A. nidulans* spores, respectively.

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## LITERATURE CITED

1. **Freer, S. N., M. Mayama, and J. L. Van Etten.** 1977. Synthesis of polyadenylate-containing RNA during the germination of *Rhizopus stolonifer* sporangiospores. Exp. Mycol. **1**:116–127.
2. **Lipinski, C., A. J. Ferro, and D. Mills.** 1976. Macromolecule synthesis in a mutant of *Saccharomyces cerevisiae* inhibited by S-adenosylmethionine. Mol. Gen. Genet. **144**:301–306.
3. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265–275.