

## Improved techniques for assaying protein concentration in germinating *Neurospora* conidia.

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### Abstract

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are permeabilized for enzyme assays. This procedure eliminates many of the problems that are associated with accurately measuring the specific activity of enzymes.

Protein concentration of permeabilized conidia was measured using an adaptation of the "Bio-Rad Protein Assay" (Bulletin 1069, February 1979, Bio-Rad Labs.). The concentrated dye was diluted 1:4 with double distilled water and filtered. The protein standard (bovine gamma globulin) was diluted analytically to 1.4 mg/ml, and a standard curve was prepared using protein concentrations from 0.2 to 1.4 mg/ml. Permeabilized conidia were prepared for the protein assay by vortexing 300  $\mu$ l of the sample, containing 5 to 10 mg of conidia, with 300 mg of acid washed sand for two minutes. As soon as the sand settled, the conidial suspension was removed and stored at 4°C. The protein content of the sample was determined by mixing 20  $\mu$ l of the ground cells with 1.0 ml of diluted dye. After ten minutes, the tubes were gently mixed, the absorbance was measured at 595 nm, and the protein content calculated by comparison to a standard curve prepared at the same time. The assay is very sensitive; therefore, care must be taken to insure that all glassware is thoroughly clean.

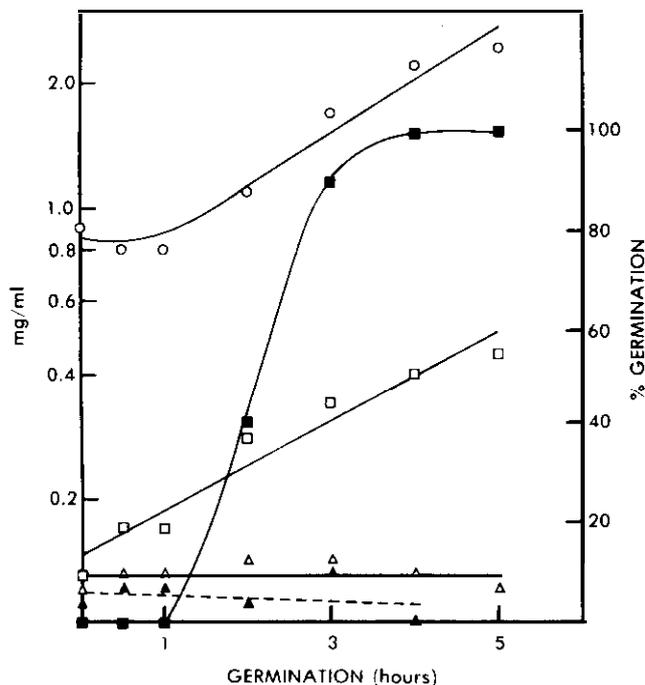


Figure 1. -- Protein and dry weight levels of germinating conidia. Conidia of *nada* strain (FGSC 2688) were dry harvested and then inoculated at 1.5 mg/ml in either Vogel's minimal medium (Vogel 1964 Am Nat. 98:435) with 2% glucose, or in distilled water. The cultures were shaken at 150 rpm at 24°C. The dry weight was measured in 1.0 ml samples that were harvested on a preweighed filter and dried at 90°C for 24 hours. The percent germination was determined (Schmit and Brody 1975 J. Bacteriol. 124:232). The protein levels were assayed as described in the text. Symbols: (○), dry weight; (■), percent germination; (□), protein concentration in minimal glucose medium; (Δ), protein concentration in distilled water; (▲), protein concentration in minimal glucose medium with 36  $\mu$ M cycloheximide.

Protein content increases during conidial germination with the same doubling time as the dry weight (Figure 1). When cycloheximide is added to the germination medium or when conidia are incubated in distilled water, there is no increase in protein content.

The protein assays were found to be very reproducible; duplicate samples of the same preparation of permeabilized conidia that were ground with sand and then assayed for protein varied less than  $\pm 5\%$ ; duplicate assays of the same preparation of ground cells varied less than  $\pm 3\%$ . The protein assay was linear up to about 0.70 optical density units. This corresponded to a maximum of 28  $\mu$ g of protein per assay.

The major cause of scatter in the data in Figure 1 is due to error in the initial sampling of germinating conidia. Conidia have a tendency to clump during germination, and it is difficult to remove uniform samples. The error due to clumping does not affect the specific activity calculations because both protein content and enzyme activity are assayed in the same sample of permeabilized cells. Minor errors in the protein assays of conidia incubated in distilled water or with cycloheximide are exaggerated in Figure 1 because the data are graphed on a logarithmic scale. School of Medicine and Department of Chemistry and Biochemistry, Southern Illinois University, Carbondale, Illinois 62901.