Fungal Genetics Reports

Volume 27 Article 15

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Recommended Citation

Christensen, R. L., D. Wolens, and J.C. Schmit (1980) "Improved techniques for assayinq protein concentration in geminating Neurospora conidia.," *Fungal Genetics Reports*: Vol. 27, Article 15. https://doi.org/10.4148/1941-4765.1678

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Abstract
Improved techniques for assayinq protein concentration in geminating Neurospora conidia.

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Improved techniques for assaying protein concentration in germinating Neurospora conidia.

The measurements of the specific activities of enzymes during developmental sequences, such as conidial germination, are directly related to the accuracy of the protein determinations (Schmit and Brody 1976 Bacteriol. Rev. 40:1). A permeabalization procedure (Basabe et al. 1979 Anal. Biochem. 92:356; Christensen and Schmit 1979 Neurospora Newsletter 26:13) can be used to assay various enzyme activities during conidial germination. We have developed a protein assay which uses the same samples that are peneabilized for enzyme assays. This procedure eliminates many of the problems that are associated with accurately measuring the specific activity of enzymes.

Protein concentration of permeabalized 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. Permeabalized conidia were prepared for the protein assay by vortexing 300 µl 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 µl 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.

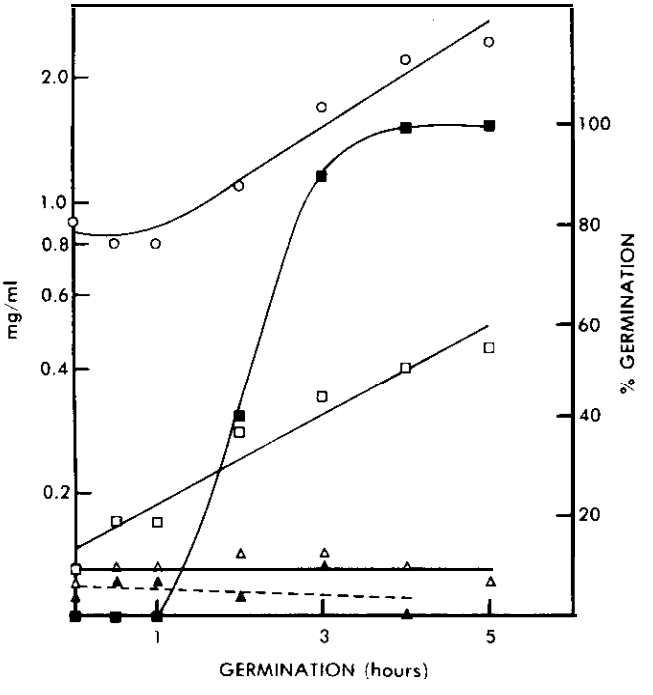

Figure 1. -- Protein and dry weight levels of germinating conidia. Conidia of nada strain (FGSC 2688) were dry harvested and then innoculated at 1.5 mg/ml in either Vogel's minimal medium (Vogel 1964 Am. Nat. 98:435) with 2% glucose, or in distilled water. The cultures were shaken at 150 rpm at 24°C. 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 clucose medium; (△), protein concentration in distilled water; (▲), protein concentration in minimal glucose medium with 36 µM 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 permeabalized conidia that were ground with sand and then assayed for protein varied less than ±5%; duplicate assays of the same preparation of ground cells varied less than ±3%. The protein assay was linear up to about 0.70 optical density units. This corresponded to a maximum of 28 µg 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 permeabalized 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.