View metadata, citation and similar papers at core.ac.uk

brought to you by CORE

provided by Kansas State University

Fungal Genetics Reports

Volume 30 Article 12

Procedure for preparing permeabilized hyphae for enzyme assays

V. P. Gujar

J. C. Schmit

Follow this and additional works at: https://newprairiepress.org/fgr

This work is licensed under a Creative Commons Attribution-Share Alike 4.0 License.

Recommended Citation

Gujar, V. P., and J.C. Schmit (1983) "Procedure for preparing permeabilized hyphae for enzyme assays," *Fungal Genetics Reports*: Vol. 30, Article 12. https://doi.org/10.4148/1941-4765.1627

This Technical Note is brought to you for free and open access by New Prairie Press. It has been accepted for inclusion in Fungal Genetics Reports by an authorized administrator of New Prairie Press. For more information, please contact cads@k-state.edu.

Procedure for preparing permeabilized hyphae for enzyme assays

Abstract

Procedure for preparing permeabilized hyphae for enzyme analysis.

This technical note is available in Fungal Genetics Reports: https://newprairiepress.org/fgr/vol30/iss1/12

Gujar, V.P. and J.C. Schmit

Procedure for preparing permeabilized hyphae for enzyme assays.

Permeabilization procedures have been developed for measuring many enzyme activities in dormant and germinating conidia (Basabe et at., 1979, Anal. Biochem 92: 356; Christensen and Schmit, 1980, J. Bacteriol., 144: 983). We have modified this procedure in order to measure enzyme activities in permeabilized hyphae. The protocol used to prepare permeabilized hyphae for glutamic acid decarboxylase (GAD) assays is given below.

Vegetative hyphae and aerial hyphae were obtained from standing cultures (Table I). The vegetative hyphae formed a pad on the surface of the liquid medium and, after a few days, aerial hyphae grew from the mycelial pad. The aerial hyphae and then the mycelial pad were removed from the test tube with a sterile spatula. Immediately the material was agitated on a vortex mixer for 2 min. with 5.5 ml of the permeabilization solution. The permeabilization solution contained 0.5 ml of toluene:methanol (1:4 v/v) and 5 ml of 20 mM potassium phos-

TABLE I.

GAD[a] activities in conidiating cultures[b]

Days[c]	Specific Activity nmoles CO_2 min^{-1} (mg lyophilized weight)$^{-1}$	
	Vegetative hyphae	Aerial hyphae
2	0.323 ± 0.005[d]	
4	0.885 ± 0.007	0.735 ± 0.02

[a]GAD was assayed by measuring the amount of $^{14}CO_2$ released from DL[1-^{14}C] glutamic acid (Christensen and Schmit, 1980 J. Bacteriol. 144: 983).

[b]Standing conidiating cultures of nada (FGSC #2688) were grown in test tubes (18 x 150) on 5 ml of liquid complete medium containing 1% casamino acids, 0.5% yeast extract, 2% sucrose, 1% glycerol and Vogel salts (Vogel 1964, Am Nat. 98: 435) at 25°C.

[c]Days after inoculation with 5 x 10^5 conidia/tube.

[d]The assays were done in duplicate.

phate (pH 6.8), 10 mM EDTA, 1 mM phenylmethylsulfonyl fluoride (PMSF) and 1 mM benzimidine hydrochloride (BHCl). The permeabilized cells were collected by centrifugation, transferred to preweighed disposable micro test tubes (1.5 ml) and frozen with liquid nitrogen. The frozen cells were lyophilized in a Speed-vac Concentrator (SAVANT). The lyophilized cells were weighed and ground to a fine powder. Small samples, 5-15 mg of cells, were ground on a cold spot plate with a glass rod whereas larger samples were ground with a cold mortar and pestle. The ground cells were suspended in a buffer (20 mM potassium phosphate (pH 6.8), 10 mM EDTA, 1 mM PMSF, 1 mM BHCl) to give a final concentration of 20 mg/ml of lyophilized mycelia.

Aliquots of the crude cell suspension were transferred directly to the GAD assay mixture with an automatic pipetter. The pipet tips were cut off to ensure that a uniform sample was transferred. The specific activities of GAD in vegetative hyphae and aerial hyphae were determined (Table I). Each assay was done in duplicate and the deviation between duplicates was less than 3%. This experiment has been repeated several times and the specific activities of duplicate assay; always varied less than 10%

We have found that this procedure for measuring enzyme activities in hyphae has the following advantages, as compared to preparing cell free extracts.

1. The permeabilization of the hyphae assures that all the enzyme activity, intraorganellar as well as cytosolic, is accessible for measurement.

2. Small molecules are removed rapidly during permeabilization; thus, dialysis is not necessary.

3. The cell interior can be rapidly exposed to EDTA and protease inhibitors minimizing enzyme degradation

4. Lyophilized cells can be weighed to caclulate specific activities.

- Department of Chemistry and Biochemistry, Southern Illinois University, Carbondale, Illinois 62901.