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Extraction of mycelial protein: some specific comparisons

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Abstract

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Various methods hove been described for extmction of soluble mycelial proteins of Neurospora, ond nine of these methods were compared by Stine, Strickland ond Barratt (1964 Con. J. Microbiol. 10: 29). These, and other methods in use in various laboratories, allow the selection of extmction conditions well suited to specific studies. We hove found that certain combinations of extmction methods ore especially suited to efficient and convenient extmction of both ß-glucosidase ond both ß-galactosidase enzymes of Neurospora. We hove also compared the extmction of there enzyme activities with extraction of alkaline phosphotose ond of total protein.

Induced mycelium was obtained by growing the "L5D" isolate on 1.5% lactose for 5 days at 30°C with rotary agitation, with cellobiose added at 2 days to a concentmtion of 0.001 M. Mycelium was harvested on a Buchner funnel and a 10.0 g portion was lyophilized, yielding 1.89 g dry weight. The remainder was sealed in Saran Wmp and frozen. The ratio of dry to wet weight allows comparison of extraction of wet and dry mycelium.

The following extraction conditions were compared:

I. Wiley Mill: lyophilized mycelium was ground and 0.40 g of the resulting powder extracted with 30 ml of buffer.

II. Omni-Mixer (Ivan Sorvall, Inc.): 3.18 g wet mycelium was extracted with 45 ml buffer with 15 g acid-washed fine gloss beads in a 50 ml chamber. Extraction was for 10 min at 60 volts input.

III. VirTis 45: 4.24 g (wet) + 60 ml buffer; 10 min at a setting of 68 volts with a 250 ml chamber, using sharp cutting blades.

IV. Virtis 45: 4.24 g (wet) + 60 ml buffer + 20 g fine gloss beads; 10 min at 68 volts using a serrated impeller.

V. Sonifier (Branson): 1.03 g (wet) + 15 ml buffer + 5 g glass beads, or, 15 ml of samples I, II, III or IV. Sonified 11/2 min at 6.3 amperes.

All extmction procedures were carried out with samples immersed in on ice water both, with the exception of the Wiley Mill procedure. The buffer used for all extractions was 0.01 M phosphate (Na), pH 7.4, containing 5 x 10^5 M dithiothreitol. Note that the ratio of buffer to mycelium was the some in all extractions. Immediately after the final step of each procedure, samples were sealed in screw copped tubes and shaken horizontally at approximately 70 cycles per minute while resting in ice on a reciprocal shaker. Samples were then centrifuged for 30 minutes at 27,000 x g in a refrigerated centrifuge. The resulting supernatant crude extracts were assayed using p-nitmphenyl-R-D-glucopyronoside, o-nitrophenyl-ß-D-galactopyranoside ond p-nitrophenyl phosphate ond using the protein assay method of Lowry, et al. (1951 J. Biol. Chem. 193: 265). Enzyme activities ore presented in arbitrary units.

The results of extmction of both ß-glucosidase enzymes, both ß-galactosidase enzymes, and of alkaline phosphotose ond total protein ore summarized in Table 1. From the data presented, specific activities moy also be calculated. For all enzymes studied, nd for total protein, the most effective total extmction is achieved by combining procedures II and V. The most effective single procedure is II. Expression of the results as specific activities greatly reduces the differences observed with different extraction procedures. Although differences remain, the relatively constant specific activities suggest that valid comparisons moy be mode between wet or dry samples.

Table 1. Effectiveness of various extraction procedures for the extmction of several enzymes.

Extraction procedure	Enzyme Activities (units/ml) aryl ß-glucosidase	cellobiase	ß-galactosidase pH 7	ß-galactosidase pH 4	alkaline phosphotow	Protein (mg/ml)
I	45	50	201	123	75	1.60
I + V	74	66	232	187	140	2.30
II	58	58	239	151	90	2.00
II +v	69	91	244	191	152	2.40
III	21	21	57	Bo	27	0.67
III + IV	49	48	178	154	90	1.90
IV	40	43	173	139	90	1.51
IV + v	66	44	201	165	120	2.05
V	55	48	166	156	120	2.00

These results demonstrate the value of procedure II, alone or in combination with V, in comparative studies of soluble proteins of Neurospora. It should be emphasized, however, that use of this, and certain other extmction procedures, has yielded erratic results if not followed by the gentle reciprocal agitation prior to centrifugation.

The origins of many extmction procedures ore very difficult to trace, ond we hove not attempted to provide a summary of appmpriate references. However, we suggest that, wherever possible, original descriptions be cited for each procedure.

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