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A method for disrupting conidia of Neurospora

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Abstract

Method for disrupting conidia

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The following method is routinely used by us for counting and disrupting the conidia for enzyme extraction. A conidial suspension containing 2 - 3 x 10^7 conidio per ml is prepared in buffer (phosphate or tris 0.05 M, 5 x 10^{-4} M in EDTA and 10^{-4} M in ß-mercaptoethanol). The number of conidia is estimated by determining the absorbance of the suspension with a Klett-Summerson photoelectric colorimeter, using a blue filter. A standard was prepared initially by correlating the Klett reading with the number of conidia, calculated from colony counts obtained by serial dilution followed by plating on sorbose media. A linear relationship between the number of conidio and Klett reading is realized up to on absorbance of 300 Klett units (equivalent to about 6 x 10^7 conidia). Suspensions at concentrations higher than thir do not fall within the linear portion of the standard curve. Our standard has been prepared for conidio harvested from cultures grown at 28°C, for 6 days, using strains pe (Y8743m) (FGSC#38) and wild type 79a (FGSC#533).

Conidio are disrupted by treating a suspension containing not more than 2.1 x 107 conidia per ml (Klett reading of 100) with a Branson, model S-75 sonifier cell disruptor. Figure 1 illustrates the quantity of soluble protein liberated as a function of time of insonation of conidia of strain Y8743m. The rigmoid shape of the curve is due to the release of one fraction of protein immediately following the disruption of the conidiol wall, while that associated with the separation of protoplasmic material from the wall is released at a subsequent stage. As much as 95% of the soluble protein is liberated within 15 minutes.

Conidial ATPase (Mg^{++}-requiring) is liberated within the first 5 minutes of insonation, whereas this treatment yields only about 40% of the total soluble protein.

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