

"In situ" changes in enzyme activity during Neurospora conidial germination

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Abstract

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TABLE 1.
In situ enzyme activities

Enzyme ^a	Specific Activity (nmoles/min/mg dry weight) ^b		
	Conidia ^c	Germinating Conidia ^d	Early Log-phase Mycelia ^e
Glutamic Acid Decarboxylase (GAD) ^f	32.0	15.0	2.8
Succinic Semialdehyde Dehydrogenase (SSADH)	1.8	2.4	1.9
Glutamate Dehydrogenase (NADP) (GDH)	2.0	36.0	
Malate Dehydrogenase (MDH)	410.0	280.0	610.0
Glutamate Oxaloacetate Transaminase (GOT)	28.0	22.0	34.0

^aGAD and GDH were from strain bd naga 2256 and SSADH, MDH and GOT were from strain naga 61-R-13.

^bCells were permeabilized with the toluene-ethanol procedure of Basabe et al. (And. Biochem. (1979) 92: 356). The permeabilized cells were washed with buffer three times to remove all tracer of ethanol.

^cConidia were dry harvested (Schmit and Brody, J. Bacteriol. (1975) 124: 232).

^dSamplers were taken after incubating for 3-5 hours in minimal glucose medium at 30° c.

^eSamples were taken after incubation for 8-12 hours.

^fGAD was assayed by measuring γ -aminobutyric acid production by "GABAse" (Sigma). All other dehydrogenases were assayed at 20° C with optimal substrate concentration by measuring changes in NAD(P) (H) concentrations. GOT was assayed by measuring oxaloacetate production using MDH.

Two enzymes of the γ -aminobutyric acid (GABA) bypass of the citric acid cycle, glutamic acid decarboxylase (GAD) and succinate semialdehyde dehydrogenase (SSADH) have been detected in conidia. Neither of these enzymes have been assayed previously in *Neurospora*. GAD and SSADH comprise part of a new pathway that may be responsible for metabolizing glutamic acid during conidial germination (Schmit and Brody 1975 J. Bacteriol. 124: 232). GAD appears to be stored at high levels in dormant conidia (Table 1). The specific activity of this enzyme decreases during germination and early log-phase growth. SSADH appears to be a constitutive enzyme. The activities of NADP glutamate dehydrogenase, malate dehydrogenase and glutamate oxaloacetate transaminase increase as conidia germinate and enter log-phase growth.

All of these enzymes were assayed "in situ" using cells permeabilized by the procedures of Basabe et al. (1979 Anal. Biochem. 92: 356). Strains containing the naga mutation (deficient in nicotinamide adenine dinucleotidase activity) (Nelson et al., 1975 J. Bacteriol. 122: 695) were used to eliminate the problems of NAD and NADP destruction that occurs with conidia from wild type strains. By combining the cell permeabilization techniques and use of the naga mutant strains, we have simplified the procedures for assaying enzymes during conidial germination. We are in the process of using these techniques to measure "in situ" changes in enzyme activities throughout the asexual cycle of *Neurospora*.