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"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]	Germinatin Conidia[d]	Early Log-phore Mycelia[e]
Glutamic Acid Decarboxylase (GAD)[f]	32.0	15.0	2.8
Succinic Semialdehyde Dehydrogenore (SSADH)	1.8	2.4	1.9
Glutamate Dehydrogenore (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

[a] GAD and GDH were from strain bd nada 2256 and SSADH, MDH and GOT were from strain nada 61-R-13.

[b] Cells were permeabalized 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.

[c] Conidia were dry harvested (Schmit and Brody, J. Bacteriol. (1975) 124: 232).

[d] Sampler were taken after incubating for 3-5 hours in minimal glucose medium at 30° c.

[e] Samples were token after incubation for 8-12 hours.

[f] GAD was arrayed by measuring γ-aminobutyric acid production by "GABAse" (Sigma). All other dehydrogenases were arrayed 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-aminobuturic acid (GABA) bypass of the citric acid cycle, glutamic acid decarboxylase (GAD) and succinate remioldehyde dehydrogenase (SSADH) have been detected in conidia. Neither of these enzymes hove been assayed previously in Neurospora. GAD and SSADH comprise part of a new pathway that may be responsible for metabolizing glutamic acid during conidiol germination (Schmit and Brody 1975 J. Bocteriol. 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 nada mutation (deficient in nicotinamide adenine dinucleotidase activity) (Nelson et al. 1975 J. Bacteriol. 122: 695) were used to eliminote 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 nada mutant strains, we have simplified the procedures for assaying enzymes during conidiol germination. We are in the process of usingthere techniques to measure "in situ" changes in enzyme activities throughout the asexual cycle of Neurospora.

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