Totten, R. E. and H. B Howe, Jr. Enzyme profiler during synchronous development of conidiophorer and conidio in N. crassq.

The following enzymes have been investigated previously during development of conidio: alcohol dehydrcgenare and glucose-6-phosphate dehydrogenase (Weiss and Turian 1966 J. Gen. Microbiol. 44: 407), invertase and trehalase (Hanks and Sussman 1969 Am. J. Botany 56: 1152), isocitrate lyase (Turian et al. 1962 Pathol. Microbiol 25: 737; Flavell and Fincham 1968 J. Bacteriol. 95:1063), NAD- and NADP-glutamic dehydrogenase (Sanwal and Lata 1961 Can. J.Microbiol.7: 319; Stine 1968 J. Cell Biol. 37:81), and NADH-oxidase (Stine 1968). We have used our method for obtaining synchronously-developing mycelial pads (1971 Biochem. Genet. 5:521) in examining the profiles of 12 enzymes to determine changes in their specific acitivities during development of conidiophorer and conidia.

Such pads were incubated in petri dishes containing 10 ml of 1% Difco Bacto-Agar a+ 35°C under fluorescent illumination for 0, 1, 4, or 7 hr, washed with deionized water in a Buchner funnel, pressed dry, and then frozen. Each frozen pod was ground intermittently with a Virtis 45 homogenizer for 15 min with 5 g of glass beads in 25 ml of 0.05 M phosphate buffer (pH 7), and the homogenate war centrifuged at 15,000 x g for 30 min. The precipitate was resuspended in 5 ml of phosphate buffer and sonicated intermittently for 2 min, the sonicate was centrifuged at 15,000 x g for 30 min. and this supernatant was combined with the first supernatant. All procedures were carried out in the cold. Seven-hour pads had developed conidiophores and conidia; 4-hr pads, conidiophores, but not conidia; and I-hr pads, neither of these structures. Dry weights (mg) and total soluble protein (mg) of the pads at 0, 1, 4, and 7 hr, respectively, were: 1,080 and 133. 1; 876 and 127,8; 818 and 129.6; 862 and 128.7.

The total units and the specific acitivites for the 12 enzymes assayed in the supernatants we given in Table 1, as are the percentage changes in specific activities between the six combinations of incubation times. Percentage change was apparently on unreliable indicator of a regulatory role for a given enzyme at a particular developmental stage. For example, isocitrate lyase increased in specific activity during each interval more than any other enzyme assayed, suggesting the operation of the glyoxalate bypass during those intervals; however, the preceding enzyme in the pathway, aconitase, decreased in specific activity in all but one interval, in which a slight increase occurred. Generally consistent trends were also not apparent in specific activities of other enzymes which could be pathway-related, such as fumarase and malate dehydrcgenare; urease and the two glutamic dehydrogenases; and invertase, trehalase, and glucose-6-phosphate dehydrogenase.

Although it was known that enzymatic activities in vitro may have little relationship to enzymatic activities in vivo, it was nevertheless anticipated that consistent trends might be found owing to our use of synchronous cultures and short incubation times. Even under these conditions, however, enzyme profiles seemed to have little functional significance, as previously found by others (e.g., Hess and Brand 1965 In Control of energy metabolism, Chance et al, (Eds.), Academic Press, New York.

Table 1. Total units and specific activites of 12 enzymes, extracted after four incubation times, and % change in specific acitivities.

Enzyme	Total Units				Specific acitivity				% change in specific activity					
	Incubation time (hours)				Incubation time (hours)				Combinations of incubation times					ours
	0	1	4	7	0	1	4	7	o-1	o-4	O-7	1-4	1-7	4-7
Aconitase	4.85	3.11	3.32	2.77	36.4	24.4	25.6	21.5	-33	-30	-41	+5	-12	-16
Acohol dehydr.	26.6	22.4	17.7	13.0	200	176	136	101	-12	-32	-49	-22	-42	-26
Fumarase	1.30	1.20	1.20	1.20	9.7	9.4	9.3	9.3	-3	-4	-4	-1	-1	0
G-6-P dehydr.	58.9	49.2	49.2	55.0	442	385	380	427	-13	-14	-3	-1	+11	+13
Invertase	25.2	22.8	26.4	25.9	189	179	204	201	-5	+8	+7	+14	+13	-1
Isocitrate lyase	0.87	0.98	1.53	2.39	6.5	7.7	11.8	18.6	+18	+82	+186	+53	+142	+58
Malate dehydr.	0.51	0 47	0.65	0.58	3.8	3.7	5.0	4.5	-3	+32	+18	+35	+22	-10
NAD-GDH	3.62	3.47	2.68	2.75	27.2	27.2	20.7	21.4	0	-24	-21	-24	-21	+3
NADP-GDH	107	113	113	116	809	888	871	a99	+10	+a	+11	-2	+1	+3
NADH-oxidose	2.03	1.98	1.91	1.23	15.3	15.5	14.8	9.6	+1	-3	-37	-5	-38	-35
Trehalase	4.11	3.69	3.27	3.13	30.9	28.9	25.2	24.3	-6	-18	-21	-13	-16	-4
Urease	22.4	18.7	21.3	20.6	168	147	164	160	-13	-2	-5	+12	+9	-2

• Expressed in millimicromoles of product/min/mg protein, except for fumarase, which is expressed as OD change/10 KC x 10^6. All enzymes were assayed from the same extract for each incubation time.

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