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Some characteristics of adaptation in *am1*

Abstract

Adaptation in *am1*

characteristics of adaptation in am₁.

Amination (am) mutants require a-amino acids for normal growth but will "adapt" after a lag period to give nearly wild type growth. The am mutants all produce defective varieties of the NADP-specific glutamic acid dehydrogenase (NADP-GDH) but the NAD-specific glutamic acid dehydrogenase (NAD-GDH) is normal.

The growth of am₁ (32213) and wild type (74-OR8-~~3~~) in liquid Vogel's minimal medium was compared. Mycelial pads were grown from conidial inocula (from 5-8 day old cultures) in 100 ml portions of medium. The cultures were shaken on a rotary shaker at 30°C; at various times four mycelial pads of each strain were harvested by filtration. Two of the four were dried and two were used to prepare crude enzyme extracts in which the NAD-GDH and NADP-GDH activities were assayed. This procedure was performed twice.

Growth curves: In both experiments, approximately 1×10^6 conidia were used for each inoculum of wild type and the results were almost exactly comparable. Other growth curves of wild type indicate that variations in conidial concentrations do not seem to affect the final growth curve obtained. The concentration of conidia, however, does affect the length of the lag period in am₁. When 2×10^7 conidia were used for each am₁ inoculum, a lag period of about 18 hours was observed; but when 1×10^6 conidia were used a lag period of 24-30 hours was present. In both cases, a wild type growth rate was observed after the lag period until a dry weight of about 550 mg was attained; the growth of am₁ levelled off at this point but that of wild type continued to increase for as long as observed (760 mg at 72 hrs.).

Enzyme assays: A reductive amination assay based on the decrease in absorption at 3400 Å attending the oxidation of NADH₂ or NADPH₂ was used. A unit of enzyme activity is defined as a change in optical density of 0.02/minute. Specific activity is expressed as units/mg protein. Protein determinations were made using the procedure of Lowry et al. (1951 J. Biol. Chem. 193: 265). As expected, activity for the NADP-GDH was absent in all am₁ cultures tested. Activity for the MD-GDH was similar to that of wild type if comparisons were made between cultures of equal dry weight.

The activity of the NAD-GDH does not seem to be enough to adequately explain the growth of am₁ since this activity never increases to any level higher than that of wild type. Assuming that the units of activity of the NAD-GDH and NADP-GDH are equivalent, am₁ cultures have 40-50 times less activity for glutamic acid dehydrogenases bring the period when they begin to grow rapidly. The NAD-GDH would have to be extremely efficient in building up a large amino acid pool. It is possible that this may be happening during the lag period. Crude enzyme extracts of wild type (STA4) conidia have been shown to possess activity for the MD-GDH (Tuveson, West and Barratt 1967 J. Gen. Microbiol. 48: 235). An alternative explanation of the adaptive growth of am₁ mutants on minimal medium may be that some enzyme or enzyme system other than the NAD-GDH is induced and that this accounts for the observed growth. Representative dry weights and enzyme activities for wild type and am₁ cultures are shown in Table 1, below.

Table 1. Dry weights and enzyme activities of wild type and am₁ cultures grown in minimal medium.

Culture	# of conidia in inoculum	age in hours	mg dry weight (avg. of 2 pads)	NADP-GDH sp. activity (avg. of 2 pads)	MD-GDH sp. activity (avg. of 2 pads)
Wild type	1×10^6	18	27	348.0*	5.30*
<u>am₁</u>	1×10^6	40	22	0.0*	8.10'
Wild type	1×10^6	24	102	264.0*	5.10*
'ml	1×10^6	48	97		
Wild type	1×10^6	30	226	222.2	5.20
<u>am₁</u>	1×10^6	60	220	0.0	6.10
<u>am₁</u>	2×10^7	48	240	0.0	6.6m
Wild type	1×10^6	36	372	207.3	9.55
<u>am₁</u>	1×10^6	66	388		
<u>am₁</u>	2×10^7	54	358	0.0	7.67
Wild type	1×10^6	48	528	144.3*	10.13'
<u>am₁</u>	1×10^6	84	540		
<u>am₁</u>	2×10^7	72	525	0.0	14.40

* Determined with one pad.

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