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Abstract

Adaptation in *am*$_1$

Shields, M. and W. N. Strickland. Some characteristics of adaptation in *am*1.

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** aid **dehydrogenase** (NAD-GDH) is normal.

The **growth** of *am*1 (32213) and wild type **(74-OR8-1**) 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** war performed twice.

Growth curves: In both **experiments, approximately** 1×10^6 conidia were used **for** each imculum 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 fiml **growth curve obtained.** The concentration of conidia, however, **does** affect **the length** of the lag **period** in *am*1. When 2 **x** 107 conidia were **used** for **each** *am*1 imculum, a lag period of **about** 18 **hours was** observed; but when 1 **x** 106 **conidia** were used a **lag** period of 24-30 **hours** was present. In both cases, **a** wild **type growth** mte was observed after the lag period until a **dry** weight of about 550 **mg was** attained; **the growth** of *am*1 **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 $NADH_2$ 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 *am*1 cultures tested. **Activity for** the MD-GDH **was** similar **to** that of wild type If **comparisons** were **made** between **cultures** of **equal** dry wieght.

The activity of **the** NAD-GDH does **not seem** to **be** enough to **adequately** explain the growth of *am*1 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, *am*1 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 *am*1 **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** *am*1 **cultures** are shown in Table 1, below.

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

Culture	# of conidio in imculum	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*
*am*1	1 x 106	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 x 106	30	226	222.2	5.20
*am*1	1 x 106	60	220	0.0	6.10
*am*1	2×10^7	48	240	0.0	6.m
Wild type	1×10^6	36	372	207.3	9.55
*am*1	1×10^6	66	388		
*am*1	2x107	54	358	0.0	7.67
Wild type	1 x 106	48	528	144.3*	10.13'
*am*1	1×10^6	84	540		
*am*1	2 x 107	72	525	0.0	14.40

• Determined with **one pad.**

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