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Arginaseless mutants

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Arginaseless mutants

Abstract

Arginaseless mutants

Morgan, D. H. Arginaseless mutants

of Neurospora

two respects: 1) I used Vogel's "N", with ammonia as nitrogen source, for the filtration and plating medium whereas Davis anticipated complications involving arginine uptake in the presence of ammonia and therefore used a nitrate minimal.

The selection procedure for arginase mutants described by R. H. Davis, (1968 Report of the Fourth Neurospora Information Conference, Neurospora Newsl. 13: p. 13) was arrived at independently by the author while working in N. H. Horowitz's laboratory at Pasadena. Our procedures differed in only

2) Filtration survivors were plated onto arginine plus proline by me but onto ornithine by Davis. Several vigorous colonies selected by this procedure after matagenesis with MNNG were crossed to wild type. Among the prototrophic progeny were several which had no detectable arginase on minimal or on minimal plus arginine. (Wild type grown on "N" has a specific activity of about 2 μ moles ornithine/mgm protein/minute, which is increased some two-fold on 3 mM arginine. The assay procedure would easily detect 0.1% of wild type activity.)

The uptake of arginine by one arginase mutant (aga-2.2) has been studied using C^{14} -arginine. Although the data do not permit the calculation of precise Km values for wild type and mutant strains, it is clear that there is no gross disturbance of arginine uptake in these mutants. As expected, however, the mutants will not utilize arginine as sole nitrogen source. Like those isolated by Davis, the mutants are strongly inhibited by arginine. The mean dry weight of triplicate 26-hour shake flask pads of aga-2.2 was reduced by 41% in 0.1 mM arginine, by 73% in 1.0 mM arginine and 90% by 3 mM arginine.

A further point of interest is that a five-fold induction of ornithine transaminase (OT) by arginine has been demonstrated in ago-2.2 (although no induction was observed in another aga mutant in a parallel experiment.) The induction of OT by arginine in the absence of arginase would preclude a sequential mode of induction via ornithine and would support the idea that arginase and ornithine transaminase function together in arginine catabolism.

One enzyme of arginine biosynthesis, acetylornithine transaminase, has been assayed in ago-2.2 grown in minimal with STA4 as a wild type control. Specific activities of 22-hour shake flask pads, 17.3 μ mole/mg/min for STA4 and 14.4 μ mole/mg/min for aga-2.2, were not significantly different, in view of the variation between cultures normally encountered in this assay.

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