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Valine sensitivity in N. crassa

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Abstract

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Inhibition of the growth of a number of bacterial strains by L-valine is well known. In some cases, the valine sensitivity of growth was shown to be caused by valine sensitivity of acetohydroxy acid synthetase (Leavitt and Umbarger 1962 J. Bacteriol. 83: 624; Armstrong and Ishiwa 1971 Genetics 67: 171). Acetohydroxy acid synthetase from N. crassa is also greatly inhibited by valine in the mitochondrial pellet suspension but is not in the pellet extract (Takenaka and Kuwana 1972 J. Biochem. 72: 1139). Working on the nature of regulation of the enzyme activity by valine, we intended to get valine-resistant acetohydroxy acid synthetase. For isolating valine-resistant mutants, we needed valine-sensitive conditions in N. crassa.

The growth of the wild type strain KG1967a (an offspring of 5256A x 5297a) of N. crassa, determined as mycelial weight, was not inhibited but, rather, was enhanced by L-valine (up to 40 mM) added to Vogel's minimal medium. The speed of hyphal elongation in Ryan's growth tube was not affected by valine at all. Finally we found three valine-sensitive conditions in N. crassa.

(1) Germination of the conidia of KG1967a, and hence the increase of optical density of the conidial suspension, was retarded by one to two hours by 1.5 mM L-valine in liquid Vogel's medium. L-Isoleucine at 1.5 mM reduced the valine effect to half, while isoleucine alone inhibited the germination to the same extent as valine. L-Methionine also inhibited the germination.

(2) Colony formation by KG1967a conidia was markedly inhibited in the presence of L-valine on the following medium: sorbose 1.0%), sucrose (0.05%) and Vogel's salt mixture with agar were mixed and then autoclaved for 60 minutes. On this agar in plates without valine, plating efficiency after incubation for two days at 34°C was about 100%. L-valine and the agar medium were autoclaved separately. In the presence of 0.015 mM L-valine, plating efficiency was 95 %; 0.05 mM - 67%; 0.15 mM - 40%; 0.5 mM - 10%; and 1.5 mM - 2%. L-Isoleucine and L-leucine at 1.5 mM did not inhibit colony formation, but counteracted the valine effect by 94% and 67% respectively. Among other amino acids, 1.5 mM L-histidine (plating efficiency = 22%), L-methionine (24%), DL-norvaline (34%), L-serine (38%), DL-norleucine (43%), L-cysteine (50%) and glycine (55%) were inhibitory. All the other natural amino acids showed little effect. The inhibition of colony formation by valine did not occur on sorbose (1.0 %)-glucose (0.02%)-salts agar plate.

(3) Acetoin accumulation in the medium by an iv-2 mutant (blocked at the reductoisomerase step) was decreased when excess valine was added to the medium. Concentration of acetoin in the medium where the iv-2 mutant 231a was cultivated with isoleucine and valine in the ratio of 1 (1.5 mM):1 was 471 μg/ml; 1:3 - 22.9 μg/ml; 1:3.5 - 6.0 μg/ml; 3:3 - 29.7 μg/ml; and 3:10 - 5.1 μg/ml. These differences can be detected by the Westerfeld reaction on a porcelain color reaction plate using several drops of the medium. Addition of L-lysine, L-glutamate, L-threonine or L-leucine at 1.5 mM to the medium containing 1.5 mM each of isoleucine and valine did not reduce the accumulation of acetoin, while addition of L-isoleucine or L-methionine at 1.5 mM inhibited growth.

The decrease of acetoin accumulation in iv-2 medium by excess valine may be due to feedback inhibition of acetohydroxy acid synthetase by valine, while (1) and (2) might be caused by other mechanisms.

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