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Wild type Neurospora crassa is inhibited by para-fluorophenylalanine in low concentrations. A number of mutants which are resistant to fluorophenylalanine have been isolated. One of these mutants, FS-188, was selected for study in elucidating the mechanism of resistance to fluorophenylalanine.

It was thought, initially, that these mutants were resistant to fluorophenylalanine because they possessed a low permease activity and were thus unable to transport the compound into the cell. Although some of the resistant mutants do exhibit a much lower permease activity, the activity of FS-188 is not lowered sufficiently to explain its resistance to fluorophenylalanine. The permease activity of FS-188 is 70-80% of that of wild type 74A when tested in Vogel's minimal salts medium without sucrose and approaches 100% of wild type in the presence of glucose or sucrose.

The inhibition of growth of 74A by fluorophenylalanine can be reversed competitively by phenylpyruvate, tryptophan, tyrosine and leucine. Previous studies (Biochem. Biophys. Acta, in press) of the phenylalanine permease system of 74A have shown that the above three amino acids inhibit the phenylalanine (fluorophenylalanine) permease and it is thought that these three amino acids relieve growth inhibition by interfering with fluorophenylalanine transport into the cell. The keto analog of fluorophenylalanine, fluorophenylpyruvate, is also inhibitory to strain 74A. Fluorophenylpyruvate inhibition is competitively relieved by phenylpyruvate and non-competitively by phenylalanine.

The rate of germination of 74A and FS-188 conidia in minimal and fluorophenylalanine medium was studied. It was found that FS-188 germinates somewhat more slowly than 74A on minimal medium. In the presence of fluorophenylalanine FS-188 germinates at approximately 60% of its normal rate. Only about 10% of 74A conidia germinate and these conidia do not continue to grow.

Flasks of medium containing 10 μM/25ml fluorophenylalanine were inoculated with 74A. After 24 hrs. the conidia were plated onto minimal agar medium and it was found that 100% of the conidia survived. No appreciable growth lag was observed when fluorophenylalanine-exposed conidia were transferred to minimal medium. Using C^{14}-fluorophenylalanine it was found that 70% of the fluorophenylalanine present in the free amino acid intracellular pool of 74A can be removed by transferring the cells from fluorophenylalanine medium to minimal medium.

When the intracellular free amino acid pools of 74A and FS-188 conidia exposed to fluorophenylalanine were chromatographed it was found that the number and concentration of amino acids from 74A conidia exposed to fluorophenylalanine were much lower than in the control conidia. The amino acid pool of FS-188 is unaffected by the presence of fluorophenylalanine. Manimetric studies of 74A and FS-188 showed that phenylalanine and phenylpyruvate stimulate oxygen uptake of both strains, while fluorophenylalanine and fluorophenylpyruvate inhibit. The inhibition of fluorophenylalanine can be overcome by the addition of phenylalanine or, better yet, a mixture of amino acids.

74A and FS-188 were exposed to C^{14}-labeled phenylalanine and fluorophenylalanine either by growing the mycelium in the presence of small amounts of high specific activity compounds or by incubating the conidia with larger amounts of less highly labeled compounds. The exposed cells were fractionated into free amino acid, ethanol-soluble, nucleotide, and protein fractions and the distribution of radioactivity determined. The protein fraction of the mutant contained about 30% less C^{14}-fluorophenylalanine than did that of the wild type. When protein fractions were hydrolysed and chromatographed, the radioactivity was recovered as unaltered fluorophenylalanine. The amount of C^{14}-fluorophenylalanine in the free amino acid pool of FS-188 was about 30-40% less than that in 74A. The distribution of C^{14}-phenylalanine was the same in the two organisms.

Therefore, both the resistant and sensitive strains were able to incorporate fluorophenylalanine into cellular proteins although there is an indication that the activating or incorporating enzymes of the mutant possess a different affinity for the two compounds. Studies on the amino-acyl-sRNA synthetases are currently in progress.

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