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A pleiotropic mutation in Neurospora conferring sensitivity of analogues of amino acids, purines and pyrimidines

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Abstract

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Much of this work has already been reported (Catcheside 1971, Austral. Biochem. Soc. 4:17) and is described here only because of the potential utility of the mutant and the restricted accessibility of the original abstract.

Wild type Neurospora is capable of growing in the presence of high concentrations of structural analogues of a number of cellular metabolites. This handicaps the genetic dissection of metabolic control processes since the direct selection of analogue resistant mutants may be impracticable. For example, although anthranilate synthetase and chorismate mutase, the allosteric enzymes concerned in the control of chorismate utilization for tryptophan synthesis, are sensitive to 5-methyltryptophan (5MT) in vitro, whole cells are able to grow on media saturated with 5MT ($\pm 10^{-2}$M). In order to obtain material from which allosteric mutants affecting the tryptophan sensitive enzymes might be selected, mutants with increased sensitivity to 5MT were sought using filtration enrichment in the presence of 5MT followed by plating ungerminated conidia on medium free of 5MT (Catcheside (1966) Ph.D. Thesis, Univ. of Birmingham, U.K.). Twelve 5MT sensitive mutants were isolated, all map close to *ylo-1* on linkage group VI and on the basis of recombination frequency are probably alleles of one gene: *ttI*.

One allele, mts MN1(s), has been further characterised. The absence of any qualitative change in 5MT catabolism prompted testing for sensitivity to analogues of other metabolites: mts MN1(s) is more sensitive than wild type to analogues of all tested aromatic, neutral and basic amino acids and is also more sensitive to analogues of purines and pyrimidines. Where wild type is inhibited and comparisons can be made, mts MN1(s) is inhibited to a similar degree by between one-tenth and one-hundredth of the analogue concentration effective with wild type. The mutant is not more sensitive to cold, salt or detergent, and the cellular complement of lipids, membrane structural protein and ATP appears normal. The permease systems for 5MT, phenylalanine and arginine ore not derepressed, the K_s for phenylalanine uptake is not grossly affected and efflux is not abolished though significantly larger intracellular pools are maintained following uptake of phenylalanine.

The nature of the change in mts mutants is not clear though alteration to an external or internal permeability barrier seems likely. Like mod-5 (St. Lawrence et al. (1964) Genetics 50: 1383) which also maps on linkage group "I, mts enabler trp-3 A78 to grow well on complex media. However unlike mod-5, mts doer not enable pyr-1 H263 to grow on complete medium. mod-5 and mts have not been tested for allelism.

The mts mutation has provided a genetic background enabling the isolation of 5MT resistant mutants with altered regulation of tryptophan biosynthesis. There mutants excrete tryptophon and anthranilic acid (Catcheside (1969) Proc. Austral. Biochem. Soc. 2: 67). mts has also enabled the selection of 8-azaadenine resistant mutants which overproduce and excrete purines (Jha (1972) Molec. Gen. Genet. 114:168). It is likely that the mts mutation will prove useful in probing a wide range of cellular processes, particularly where wild type is not sufficiently sensitive to structural analogues of cell metabolites to enable direct selection of resistant mutants.

Stocks of mts MN1(s) have been deposited in the Fungal Genetics Stock Center: A FGSC #2746, a FGSC #2747. The mutation is conveniently scored in tuber. Strains containing mts fail to grow at 25° in 72 hours on slopes of Vogel's minimal agar supplemented with 350 ng ml^{-1} DL-SMT or the appropriate concentration of another amino acid, purine or pyrimidine analogue. Emerson A or a wild type, such as FGSC #691 and 692, is an appropriate mts^+ reference strain. The approximate concentration of analogue which reducer growth yield of mts in liquid Vogel's medium by 50% in 72 hours is: 5-methyl-DL-tryptophan 4×10^{-4}M, 8-azaadenine 4×10^{-5}M, L-ethionine 2×10^{-6}M, L-canavanine 5×10^{-7} M. - - - School of Biological Sciences, Flinders University, Bedford Pork, South Australia, 5042, Australia.