Guanine-requiring mutants

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Abstract
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Mutants with increased sensitivity to caffeine.

Three caffeine-sensitive mutants have been isolated following LN-mutagenesis and filtration enrichment in high-sorbegalose minimal medium containing caffeine at 0.1 mg/ml. The mutants, designated cfs(OY305), (OY306), and (OY307), grow slowly on minimal medium and are morphologically distinct. On minimal containing caffeine at 0.2 mg/ml, cfs(OY306) grows little or not at all, cfs(OY305) fails to grow for 2 to 3 days but may grow very slowly thereafter, and cfs(OY307) grows only slightly for 1 to 2 days but then grows to the same extent as in absence of caffeine. The growth of cfr(OY305) is stimulated by adenine (or by complete medium), whereas cfs(OY306) and cfs(OY307) are inhibited by adenine, and are not stimulated by complete medium. cfs(OY305) is not sensitive to caffeine if adenine is present, whereas cfs(OY306) and cfs(OY307) are as sensitive to caffeine in the presence of adenine as in its absence.

On both minimal or minimal plus caffeine media, cfs(OY305) complements cfr(OY306) and cfs(OY307) complements cfr(OY307), with respect to growth rate, morphology and sensitivity. All three mutants map in linkage group 1; cfs(OY305) and cfs(OY307) are very closely linked to each other and are about midway between mating type and ad-8, and od-9. Recombination among the three mutants is not sensitive to DNA repair, and because work with the mutants, begun at Stanford University, will not be continued in this laboratory, Stocks of cfs(OY305), cfs(OY306), and cfr(OY307) (A and a for each) have been deposited with FGSC.

Guanine-requiring mutants of Neurospora have not been available, although they were once reported for N. crassa by Woodward _et al._ (Proc. Nat. Acad. Sci. 40: 192). Fries (J. Biol. Chem. 200: 325) obtained guanineless mutants of Ophiostoma multitubulatum and found that they were inhibited by adenine. This suggests that the nucleic acid component of complete medium, which is usually used to select auxotrophs, would prevent recovery of guanine auxotrophs. To test this possibility, conidia were mutagenized with UV. Survivors were concentrated by filtration enrichment in high-sorbegalose minimal medium, and mutants were selected on minimal medium containing guanine (0.2 mg/ml). Four survivors (out of 191) grew up promptly on guanine and not on minimal or complete medium.

Three of these mutants behaved identically. Although initially clearly negative, they started growing after 3 to 4 days on minimal or complete medium and within 2 additional days achieved growth comparable to that of wild type. Subcultures behaved like the original isolates. The mutants grew more readily on guanosine than on guanine, presumably because guanosine is the more soluble; the threshold level of guanosine required for normal growth was 1 to 2 μg/ml. Growth on guanosine-medium was inhibited by adenine, adenosine or adenylic acid. The inhibition was apparently competitive since there was complete inhibition when adenosine and guanosine were at equimolar concentrations but inhibition was abolished if the guanosine concentration exceeded that of adenosine. Cytidine was also inhibitory but at a concentration 5-fold higher than that of guanosine. Other compounds were not inhibitory: thymine, hypoxanthine, xanthine, uracil, thymidine, uridine, cytosine, xanthurenic acid. Adenine (1 mg/ml) prevented adaptation to minimal or complete medium.

In heterokaryon tests on minimal medium, the three mutants did not complement each other, but all three complemented the fourth mutant. Recombination among the three was < 0.1% and they were therefore assigned to a locus designated gua. Results of five crosses indicated that gua is linked to mating type (probably left) in linkage group 1. Stocks of gua have been deposited with FGSC and assigned Nos. 3524 (A) and 3525 (a).

The remaining mutant, gua(OY304), grew slowly on guanosine-medium and not at all on minimal or complete; it did not adapt. In crosses, it did not act as a female parent but was highly fertile as a male. Progeny (54) of Oak Ridge X gua(OY304) were all fast growing prototrophs. gua(OY304); at-2 formed a fast growing, orange heterokaryon when combined with A+ on minmal medium. Most colonies derived from plated conidia of the heterokaryon were orange, but a few were white. Ten white and three orange colonies were isolated and transferred to minimal, complete, and minimal plus guanosine. Of the white isolates, 1 was fast-growing gua+, 2 were slow-growing gua, 1 was slow-growing gua+, and 6 grew at an intermediate rate; 4 of these were gua and 2 were gua+. All three orange isolates grew moderately fast; 2 were gua and 1 was gua+. The gua(OY304) mutant appears to display quantitative effects and may be cytolytic. The guanine mutants, obtained at Stanford University, will not be studied further in this laboratory.