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Guanine-requiring mutants

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Abstract

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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 multiannulatum 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-sorbose minimal medium, and mutants were selected on minimal medium containing guonine (0.2 mg/ml). Four survivors (out of 191) grew up promptly on guonine 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 guonine, presumably because guanosine is the more soluble; the threshold level of guanosine required for normal growth was 1 to 2 μM. Growth on guanosine-medium was inhibited by adenorine, adenine 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 adenorine. 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 on minimal or complete.

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 hove 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 adopt. In crosses, it did not act as a female parent but was highly fertile as a mole. Progeny (54) of Oak Ridge X gua(OY304) were all fast growing prototrophs. gua(OY304); al-2 formed a fast growing, orange heterokaryon when combined with in on minimal 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 on intermediate rote; 4 of these were gua and 2 were gua$^+$. All three orange isolates grew moderately fort; 2 were gua and 1 was gua$^+$. The gua(OY304) mutant appears to display quantitative effects and may be cytoplasmic. The guanine mutants, obtained at Stanford University, will not be studied further in this laboratory. - - - Deportment of Plant Pathology, Cornell University, Ithaca, NY 14853.