Fungal Genetics Reports

Volume 6 Article 10

Growth and allelism of arg-11 and adg

D. Newmeyer

Recommended Citation
Newmeyer, D. (1964) "Growth and allelism of arg-11 and adg," *Fungal Genetics Reports*: Vol. 6, Article 10.
https://doi.org/10.4148/1941-4765.2087

Growth and allelism of arg-11 and adg

Abstract

Growth and allelism of *arg-11* and *adg*

Newmeyer, D. Growth and allelism of arg-11 and adg.

The mutant arg-11 (30820) was initially reported to require arginine (or citrulline) plus either a purine or a pyrimidine (Srb 1950 Botan. Gaz. 111:470). It was subsequently mapped in linkage group VII, proximal to arg-10 (Perkins 1959 Genetics 44:1185). Mutant adg (44601), originally listed as "unknown requirement", was shown by Houlahan et al. (1949 Genetics 34:493) to segregate as a single gene unlinked to sex (21 asci). Subsequently J. Mauron (cited in G. Dubes 1953 Ph. D. Thesis, California Institute of Technology) found adg to grow sub-optimally on arginine plus either adenine or a pyrimidine. This result suggested allelism with 30820, which proved to be the case (see below). However, flask assays with these strains gave unexpected results. These results have been largely superseded by the recent report of Charles and Broadbent (1964 Nature 201:1004) that 30820 will grow on minimal in the presence of 30% CO_2, and that when CO_2 is excluded both purine and pyrimidine become essential. However, as the relation of 30820 to the other

CO_2 mutants is still far from clear, our fragmentary results may be useful.

Flask assays: (All results are based on 3 days growth at 25°C in 20 ml of Vogel's Neurospora minimal.) It was found that the growth requirements of 30820 are abnormally dependent on inoculum size. With very small inocula there is negligible growth unless arginine, adenine and uridine are all present, and optimal growth when all three are added. (Alternative purines and pyrimidines were not tested.) As the inoculum size is increased, first uridine can be omitted; with a further increase in inoculum either adenine or uridine can be omitted; with still larger inocula both adenine and uridine can be omitted. (In the one experiment where conidia were counted, all three compounds were essential at or below 2.2×10^5 conidia per flask.) The growth of control cultures of arg-1 (B369) and arg-3 (30300), which is also a CO_2 mutant, was essentially unaffected by a similar range of inoculum sizes. In preliminary tests mutant 44601 (adg) behaved much like 30820, except that 44601 appeared to be a more extreme departure from wild type.

Although with small inocula the requirements for adenine and uridine are absolute, the concentrations required are very low. With 30820, essentially maximal growth was obtained with only 0.005 mg/ml each of adenine and uridine, compared with values in the literature of ca. 0.05 mg/ml for ordinary adenine mutants and ca. 0.075 mg/ml for ordinary pyrimidine mutants. The arginine requirement is also rather low (0.05 to 0.1 mg/ml). It may be significant that the concentration of pyrimidine required by 30820 is similar to that which represses or inhibits the synthesis of pyrimidine-specific CAP, according to Charles's hypothesis (1964 J. Gen Microbiol. 34:131).

Allelism tests: A cross of 30820 x 44601 gave no wild types among 268 random isolates and one true wild type out of an estimated 4,000 ascospores plated (scored by early growth on minimal sorbose, as in Lein et al. (1948 Proc. Natl. Acad. Sci. U. S. 34:435). However, the results are complicated by the fact that roughly 10 to 15% of the ascospores also failed to grow on glycerol complete medium or on supplemented minimal. When isolated to complete slants, about half of these grew up in a few days; these will be called slows, and are not uncommon. The other half either did not grow at all, or grew so slowly that it took weeks to make a macroscopically visible culture; these will be called near-lethals. Near-lethals were also found among the random isolates. Since it was possible that wild type recombinants could occur preferentially among the near-lethals, via a pair of balanced lethals, four near-lethals from this cross were revived via prolonged growth and repeated subculturing. These eventually attained a normal growth rate, and were then tested; all failed to grow on minimal. (All slows tested also failed to grow on minimal.) In addition, the balanced lethal hypothesis seems unlikely because a cross of wild type x 44601 also produced slows and near-lethals. (A cross of wild type x 30820 produced slows but no near-lethals among the small sample examined.)

Allelism of 30820 and 44601 was also indicated by complementation tests. Both mutants were backcrossed until strains were obtained which appeared fully compatible, in that each gave wild type heterocaryons with an arg-1 tester. These strains did not complement each other. It is therefore concluded that 30820 and 44601 are alleles.

It also seemed desirable to test for a functional relationship between arg-11 and the rather closely linked arg-10 locus, despite their biochemical differences. Both arg-11 alleles were therefore tested with a compatible strain of B368, a non-complementing arg-10 allele (J. Rice, personal communication via O. Gillie). In each case, heterocaryons with wild type growth rate were obtained.

Phenotypic variation: Among single-ascospore isolates from crosses of wild type x 44601, there is much variation in growth rate and morphology, even among the 44601^+ segregants; the near-lethals appear to be merely the most extreme examples of this variation. The variation does not appear to be genotypic, since (a) it tends to diminish on subculture, and (b) even after four generations of back-crossing to wild type (each time selecting the most vigorous 44601^- segregant), essentially the same range of variation was found. Preliminary attempts to determine whether the variability is due to an unrelated gene with low penetrance or to a semidominant effect of 44601 (e.g., via threshold level(s) of limiting compound(s) in the ascospores) have been unsuccessful.

Mr. Oliver Gillie at the University of Edinburgh has obtained much more extensive data on the variable growth of both arg-11 alleles. - - - Department of Biological Sciences, Stanford University, Stanford, California.