Growth of conidia of adenine-dependent mutants of Neurospora crassa

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Abstract
Growth of conidia of adenine-dependent mutants
This experiment showed, therefore, that od-8, ts and pan-2 mutants do not require adenine for germination and will not be limited in their growth by the presence of the mutation. If the evidence from asco, ts and pan-2 proves to be of general application, then all spore color mutants in Neurospora will have reduced ascospore germination and will be of limited value as markers for the selection of aberrant tetrad for intragenic recombination studies. "--- Department of Biology, Queen's University, Kingston, Ontario, Canada.

**Ahmed, M., A. Dar, M. R. Khan and M. N. Huda.**

Improving fertility in crosses of *N. crassa* lys-5 mutants.

Ascospores from crosses involving lysine-5 mutants show delayed maturation and in many cases do not mature at all. During fine structure studies on the lys-5 locus, means had to be found to improve the formation and shedding of spores in interallelic crosses. The following media and methods were found to improve fertility: (1) Repeated back-crossing to the wild type parent. (2) Crossing in Suyama's medium (Suyama et al., 1958 Microbial Genet. Bull. 14: 29). (3) Subculturing mutants every fourth day for five times in Vogel's minimal medium (Vogel 1956 Microbial Genet. Bull. 13: 42). (4) Increasing the concentration of phosphate to 0.4-0.6% in Westergaard's medium (Westergaard and Mitchell 1947 A. M. J. Bot. 34: 573). Not all strains responded to this treatment but in some cases it was helpful. (5) Supplementing Westergaard's medium with a few drops of an extract prepared from the mycelia of a highly fertile cross.

The latter approach was tried on the assumption that one or more hormones required for sexual reproduction might be lacking or produced rub-minimally in the lys-5 mutants. Culture filtrates and mycelial extracts of two highly fertile strains, both singly and from crosses, were tried. The two strains used were the wild type Em (5297) and the mutant strain leu-1 (33757) A. Sterile filtrates of Vogel's medium in which the two strains had been grown either separately or together for 14 days at 25°C with shaking were used in place of distilled water to prepare Westergaard's crossing medium. There filtrates did not increase the fertility of triple-point crosses involving 12 lys-5, ad-8 double mutants and asco (37402) on appropriately supplemented medium.

Next, mycelia of the two strains, grown separately and together, were ground in culture filtrates for 1 hour in a mortar with powdered glass. These homogenates were then filtered and a few drops were added to slants of Westergaard's medium supplemented with 5 mg lysine and 10 mg adenine per 100 ml. After the drops of mycelial extract were absorbed by the medium, 12 triple-point crosses of lys-5, ad-8 double mutants and asco were made in duplicate.

All the 12 crosses proved to be sterile in slants containing the extracts of Em and leu-1 A grown separately. The use of the mycelial extract from the cross Em x leu-1 A improved the fertility of the lys-5 crosses. Addition of 4 mmol of this extract to the slants permitted shedding of spores in one-third of the crosses. "--- Department of Botany, University of Dacoo, Dacoo-2, East Pakistan.


Differ in their ability to germinate on adenine-deficient medium. Their growth on adenine-supplemented medium is greatly enhanced. Germination of washed macroconidia of ad-3B, ad-3A, nit-2 (38701, 43002) A (FGSC #142) and ad-3B, thi-1, a1-2 (35203, 56501, 15300) a (FGSC x259) and the ad-8 mutants ad-8, ylo-1 (E6, Y30539y) A (FGSC #448) and ad-8, ylo-1 (E6, Y30539y) a (FGSC #449) are not different from that on adenine-supplemented medium but there is a 1-2 hour log phase on adenine-deficient medium which is not apparent on supplemented media. Narrower diameter germ tubes are formed than on supplemented media. These may reach lengths varying between 10 and 400 μ after 24 hours incubation. Thus a certain amount of growth, accompanied by nuclear division, occurs. The number of nuclei correlates with increase in germ tube length. The germination of washed macroconidia of ad-8, ylo-1 on adenine-deficient medium was much lower, about 15% after 6 hours' incubation at 30°C; no further germination occurred after 24 hours' incubation and the narrow germ tubes were very short. Some nuclear divisions occurred.

A possible explanation for the differences in germination on adenine-deficient medium is that stock cultures of ad-3B, thi-1, a1-2 maintained on supplemented media, that is, with adenine, may store ATP and various imidazole compounds, as well as hypoxanthine and xanthine by a reversal of the adenine synthesis pathway, since these occur in the pathway after the ad-3 blockage. These compounds may be used to synthesize adenine when the spores are placed on adenine-deficient medium. Whereas ad-8, ylo-1 mutants supplied with adenine may only store ATP and adenylosuccinic acid ribotide, and this may be insufficient to support growth of the germ tubes.

After transfer of conidia of ad-3B, thi-1, a1-2 and ad-8, ylo-1, which had been incubated on the surface of sterilized 1 cm sq dialyzing membrane on adenine-deficient medium for 24 hours, to adenine-supplemented medium, swelling of the hyphal tips
occurred in those spores which had germinated. Three hours later further growth of the germ tube beyond the point of swelling was observed. The width of this portion of the germ tube was greater than that formed on adenine-deficient medium and was equivalent to germ tube width of spores grown under optimal conditions. Ungerminated spores began to germinate and many of these showed some welling prior to germination. Swelling was not observed in the hyphal tips of wild type spores germinated on adenine-deficient medium and then transferred to adenine-supplemented medium. The relationship between adenine uptake and synthesis and growth and nuclear division is being investigated further.

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