

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

er. 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 Gene+. Bull. 14:29). (3) Subculturing mutants every fourth day for five times in Vogel's minimal medium (Vogel 1956 Microbial Gene+. Bull. 13:42). (4) Increasing the concentration of phosphate from 0.4 to 0.6-0.8% 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 sub-minimally in the lys-5 mutants. Culture filtrates and myceliol 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. These 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 filter-sterilized and a few drops were added to slants of Westergaard's medium supplemented with 50 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 a and leu-1 A grown separately. The use of the myceliol extract from the cross Em a x leu-1 A improved the fertility of the lys-5 crosses. Addition of 4 drops of this extract to the slants permitted shedding of spores in one-third of the crosses. - - - Department of Botany, University of Dacca, Dacca-2, East Pakistan.

Ascospores from crosses involving lys-5 mutants show delayed maturation and in many cases do not mature at all. During fine structure studies on the lys-5 locus, therefore, means had to be found to improve the formation and shedding of spores in interallelic cross-