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Abstract

Staining Neurospora nuclei

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A method of staining Neurospora nuclei.

A procedure is described for the cytological preparation of vegetative and meiotic nuclei of Neurospora crassa with Schiff's reagent. The main objective behind developing this technique was to determine microspectrophotometrically the relative DNA content at various stages of meiosis in order to follow the timing of DNA synthesis pertaining to recombination. The details of the microspectrophotometric analysis will be published elsewhere (Iyengar et al. 1977 Genet. Res. Camb. (In press)).

Stains of both mating types of N. crassa CBS 285-62 were obtained from Central Bureau voor Schimmelcultures, Baarn, The Netherlands. The technique for crossing these stains was essentially the same as that of Barry (1966 Neurospora Newsl. 10: 12).

Fixation: a) Perithecia: The fixative, containing 9 parts of n-butyl alcohol, 6 parts of glacial acetic acid, and 2 parts of 10% aqueous chromic acid (Lu 1962 Can. J. Bot. 40: 843), was directly poured into cultures containing perithecia. The cultures were then placed in a vacuum dessicator to ensure complete infiltration with the fixative. After 24 hours, strips of agar bearing perithecia were cut out of the cultures and washed in running water for an hour. The strips were then immersed in small vials containing Newcomer's fluid (Newcomer 1953 Science 118: 161) consisting of 6 parts isopropyl alcohol, 3 parts propionic acid, 1 part acetone, 1 part petroleum ether, and 1 part dioxane. After 24 hours, the strips were transferred to the vials containing 70% ethanol and stored at 4° C. The fixed perithecia can be stored for 3-4 months without any deterioration in the quality of preparations.

b) Hypha and conidia: Hyphae and conidia were scraped from growing cultures and directly fixed in 3:1 ethanol-acetic acid for 24 hours, transferred to 70% ethanol, and stored at 4° C.

Staining: a) Meiotic nuclei: Perithecia were scraped from agar strips, hydrolysed in 1 N HCl for 8 minutes at 60° C, washed in distilled water, and treated with methanol for 2-3 minutes. The perithecia were then immersed in the conventional Schiff's reagent prepared according to Graumann (1953 Z. wiss. Mirk. 61: 225) with the modification that 0.1% pararosanilin (Merck, Dormstadt) was used instead of 0.5%.

c) Vegetative nuclei: Hyphae and conidia were hydrolysed in 1 N HCl at 60° C for 12 minutes and stained with Schiff's reagent according to the method as described for perithecia.

Preparation of Slides: Perithecia were placed on a clean slide in a drop of 45% acetic acid and mycelial fragments and remnants of agar were removed with a needle and pair of forceps under a dissecting microscope. The asci were expressed through the ostiole by carefully pressing the perithecium with a flattened needle. Perithecial wall and other debris were removed and the clumps of asci were teased apart and then carefully covered with a glass coverslip. The slide was exposed to steam for a few minutes and then the asci were flattened by pressing the coverslip with a thumb. This helps to increase the contrast between the cytoplasm and the chromosomes. For good results, exposure to steam should not be too long but should sting when touched with the back of the hand. Also, pressure should be applied to the coverslip in a single steady motion, otherwise the asci may get distorted or overlap. The excess acetic acid was then blotted out with a filter paper and the corners of the coverslip sealed with transparent nail polish. The slides thus prepared can be stored for 3-4 days at 4° C. The preparation of slides with hyphae and conidia was the same as that described for asci.

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