Srb, A. M. and Sister A. M. Infanger. Formation of heterokaryons by fusion of isolated hyphal tips on solid medium in petri plates.

This precise method for forming heterokaryons permits visualization of hyphae as initial fusions occur between strains and isolation of hyphal fragments from different regions of a young, heterokaryotic mycelium. The strains to be fused are each grown in a petri plate containing a minimal medium lacking sucrose and solidified with 4% agar. Sucrose is omitted to decrease hyphal branching. The high concentration of agar makes the medium firm enough for easy cutting. Individual hyphal fragments, approximately 0.6 mm long, are cut by means of a sterile platinum or stainless steel microspatula. The blade should be thin and have a smooth cutting surface. Observations and transections of hyphae are aided by a binocular, dissecting microscope with 45 x or 60 x magnification. At these magnifications hyphae are easily observed and cut. The "peg" connections between overlapping hyphae, however, cannot be seen. Members of a pair of hyphal tips, one tip from each strain, are inoculated less than 0.5 mm apart near the edge of a petri plate containing minimal medium solidified with 4% agar. Pairs of hyphae can be seen as they grow from the inocula. In favorable cases, hyphae can be seen to fuse and to form a single hypha. Certain compatible strains of Neurospora sitophila, when incubated at 28° C, have hyphal fusions after 6-8 hours of incubation and have numerous heterokaryotic hyphal tips after 24 hours. The heterokaryotic cultures are obtained by isolating fragments of the hyphae into individual culture tubes.

This method allows one to observe the fused hyphae and estimate grossly the cytoplasmic contribution of each strain to a heterokaryon. A report of experiments that employ this method to study nucleo-cytoplasmic interactions will be published elsewhere.

This work was supported in part by Public Health Service Research Grant No. C-1256 to A. M. Srb from the National Cancer Institute. - - - Department of Plant Breeding, Cornell University, Ithaca, New York.