A Rapid Slide-Mount Technique for Agar-Grown Fungal Cultures

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ABSTRACT

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A rapid slide-mount technique was developed that used the melting of the agar substrate on the slide in a drop of suitable stain or mounting medium. The technique facilitated routine examinations of conidium-conidiophore relationships and submerged mycelial structures.

For many plant diseases, the determination of causal fungi requires plating the diseased materials onto culture media. Once the fungi have emerged onto the agar medium, they must be mounted for microscopic examination. This step often disturbs the fragile conidium-conidiophore relationships and causes difficulties in interpretation. We have developed a rapid slide-mounting technique that can minimize this problem.

MATERIALS AND METHODS

Small, thin, surface sections of agar with fungal growth were removed with a finely sharpened microspatula (5) and immersed on a slide in a small drop of the selected stain or mounting medium. After 15–20 sec, a coverslip was gently placed on the material. The mount was flattened by warming it over a low flame (alcohol lamp, etc.) so that the agar barely melted 1–2 sec after removal from the flame. Too vigorous heating of the mount caused the medium to boil and degraded the final product. The timing and amount of heat needed was learned best by experience.

RESULTS AND DISCUSSION

The critical aspect of this technique was the *gentle* melting of the agar substrate. This method allowed the mount to flatten with minimal disruption of the conidium-conidiophore relationships and provided, on cooling and resolidification of the agar, a more vibrationally stable and desiccation-resistant mount. The resolidified agar did not degrade the optical quality of the preparation (Fig. 1).

The technique differed in several respects from those previously described. The Riddell slide culture method (6) depends on the growth of the fungi in situ on the slide, and the agar block substrate is removed before the final mounting step. The glycerine and glycerine jelly method (4,5) is based on gradual dehydration of the mount and final embedment in the melted glycerine jelly. Both methods require more time than our technique, and only the glycerine-glycerine jelly method includes the agar substrate in the mount, usually in a squashed rather than a melted and resolidified state. Neither method is particularly well suited to routine direct

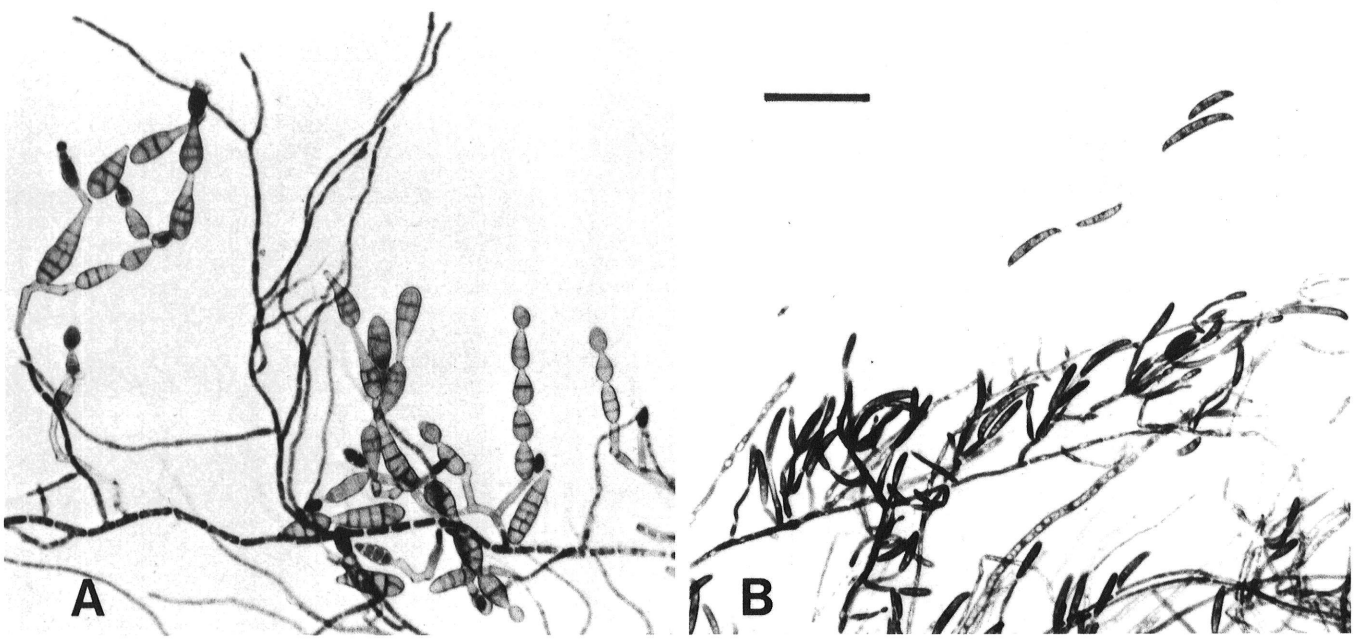

Fig. 1. Conidia and conidiophores from agar-grown cultures mounted in 0.05% trypan blue in lactophenol. **A,** *Alternaria alternata.* **B,** *Fusarium* sp. Scale bar represents 50 μm.

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examination of fungi from isolation plates. Elimination of trapped air bubbles by gently heating the mount has been advised (7), but no recommendation to heat the mount specifically to melt the agar has been described.

Of previously published methods, the use of Shear's mounting fluid most closely parallels our technique (1–3). In that procedure, the mount is heated to boiling to drive off the water and alcohol and to swell any hygroscopic structures. This method would also coincidentally melt any agar in the mount, but such drastic heating frequently causes cytoplasmic disorganization and disrupts the fragile conidium-conidiophore relationships that are needed for identification of fungi.

An additional advantage of our technique is its compatibility with many mounting media and stains that have a boiling point above 100 C. We have had good to excellent results with various stains in lactophenol and with various nuclear stains followed by mounting in 50% glycerol, but we prefer 0.05% trypan blue in lactophenol. A 0.05% concentration provided excellent stain differentiation when the mount was heated, but higher concentrations of trypan blue tended to overstain the cytoplasm. With 0.05% concentration, the nuclei stained dark purple-blue and the cytoplasm and hyphal cell walls stained light blue.

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