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Tergitol-induced colonial growth without inhibition of conidiation

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Abstract

The cloning of genes from *Neurospora crassa* usually involves transformation of a mutant with genomic library DNA and the subsequent identification of transformants showing wild-type phenotype (Vollmer and Yanofsky 1986. Proc. Natl. Acad. Sci. 83:4869- 4873). This can be problematic in the case of morphological mutants, because the sorbose- containing medium that is typically used to promote colonial growth of the transformants tends to interfere with conidiation, and sometimes inhibits aerial hyphal growth altogether. The medium described below uses the nonionic detergent tergitol (Tatum et al. 1949. Science 109:509-511) to induce colonial vegetative growth without affecting aerial hyphal growth or morphology.

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This medium is based on standard Vogel's minimal medium plus 1.5% sucrose and 1.5% agar. Tergitol NP-10 (available from Sigma) is added to autoclaved bottom agar medium at a concentration of 0.005%. Medium containing 0.001% tergitol allows some spreading growth, while viability suffers at concentrations of 0.01% and above. Sterilization of the concentrated tergitol has not been necessary. Care should be taken when mixing the detergent into the medium to avoid excessive foaming. It is normal for the resulting medium to be slightly turbid.

Plating transformants directly in top agar containing tergitol will kill the spheroplasts. Instead, the top agar should consist of Vogel's minimal medium plus 1.5% sucrose, 1.5% agar, and 1 M sorbitol for osmotic stabilization. Tergitol medium has successfully been used with both benomyl and hygromycin to differentiate wild-type transformants from aconidial mutant transformants.

Unlike sorbose, which restricts both mycelial and aerial hyphal growth, tergitol only restricts the growth of hyphae that are in contact with the medium. Aerial growth, which commences about one day after plating, proceeds vigorously until aerial hyphae and conidiophores completely fill the space between the agar and the lid of the Petri dish. The colonies must therefore be checked about twice a day to avoid overgrowth. For this reason, standard sorbose medium is still preferable for colonial growth where the morphology of the transformants is inconsequential. However, the preservation of aerial morphology makes tergitol medium quite useful for the study of morphological mutants.

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