Tergitol enables the rapid and inexpensive scoring of nutritional and drug-resistance markers in the progeny of Neurospora crassa genetic crosses

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After cultures from the progeny ascospores of a genetic cross containing nutritional markers are obtained, it is often necessary to test them on a variety of media. Testing using individual culture tubes may be ideal, but is time consuming. In contrast, inoculation of plates with conidial suspensions is relatively quick.

Tatum originally reported that medium containing tergitol results in an initial phase of colonial growth (Tatum et al. 1949. Science 109:509-511), and Springer reported the use of tergitol as a substitute for sorbose in N. crassa transformation procedures (Springer, Fungal Genet. Newsl. 1991 38:92). The limitations of sorbose are that it adds expense and that it takes longer to score phenotypes on sorbose-containing media due to reductions in cell growth rate.

In crosses testing the segregation of arg-12s, pyr-3, and an arg-2-hph fusion gene, we found that the sensitivity of arg-12s pyr-3 progeny to arginine in the absence of uridine, the uridine requirement of pyr-3 progeny, and the hygromycin-resistant phenotype of progeny containing an arg-2-hph fusion gene could be examined using plates containing tergitol. Tergitol containing media may be useful to other investigators for large scale nutritional screening.

Our procedure was as follows: sucrose-containing plating media (containing Vogel's Medium N (Vogel, 1956. Microbiol. Genetics. Bull. 13:42-43)) and appropriate supplements were prepared and autoclaved in 100 ml aliquots in milk dilution bottles for 20 minutes. After cooling media to 50oC following autoclaving, Tergitol NP-10 (Sigma) was added to a final concentration of 0.005% by volume. Tergitol was not sterilized prior to use. Tergitol clings to the inside of the pipet tip and flushing of the tip with the sterile medium was essential in dispersing the entire volume of tergitol. Bottles of media were then gently mixed to avoid foaming and poured into standard 100 mm diameter plastic petri dishes at approximately 20 ml of medium per plate. Plates were dried prior to inoculation.

Grids were printed on acetate transparency film and taped to the bottom of the petri dishes. The grids consisted of four rows of five columns (20 squares); individual squares in the grid were approximately 13 mm x 13 mm.

Conidia were collected with a wet inoculating loop, transferred to 1 ml of sterile water, and briefly mixed with a vortex mixer. Six microliters of each suspension were applied as a spot to a

square in the same grid position on each of the media involved in the screening process. Once all positions were inoculated and all liquid absorbed by the media, plates were incubated at 32oC. Growth was checked after 12, 15, 18, 24 and 48 hours of incubation. Scoring of a few progeny could be done after 15 hours, but reliable screening required 24 hours. Growth remained in the form of colonies even on extended incubation, but the aerial spreading along the lid of the petri dish which occurred was seen as potentially compromising the integrity of data collected after 48 hours.

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