Optimal light for conidiation

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Abstract
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Exposure to light is often employed in Neurospora studies as an inducement for conidiation. For example, one standard procedure for agar flask culturing is to incubate newly inoculated flasks at 34°C in the dark for 2-3 days, followed by exposure to light at room temperature. Various experiments in our laboratory have necessitated the large scale production of agar flasks each containing a uniform and maximum number of viable conidio. Since light exposure is a factor in the production of conidio, experiments were performed to determine whether or not increased exposure to light, beyond 24 hours at 24°C, would increase the production of viable conidio. Results have indicated that light exposure beyond 24 hours at 24°C does not lead to an increase in numbers of viable conidio and may, in fact, contribute to a loss of viability.

For these experiments 125 ml Erlenmeyer flasks were employed, each flask containing 15 ml of Vogel's minimal medium supplemented with 1.5% (w/v) agar and 1.5% (w/v) sucrose. A uniform inoculum of strain STAA was placed in each of several such flasks, which were then incubated in the dark at 34°C for 64 hours. After this time, the flasks were exposed to light (100 watt incandescent light bulb with average intensity of 278.2 foot-candles measured at the surface of the flasks) at 24°C. Determinations of the total numbers of viable conidio per flask were made at 0, 24, 48, 72, 96, and 168 hours of light exposure. For these determinations 20 ml of sterile water was added to each flask, the contents were suspended by swirling and the resulting suspension was poured through two layers of sterile cheese cloth. Appropriate dilutions of this filtered suspension were plated on sorbose minimal agar plates (minimal medium + 1.5% agar, 1.0% sucrose and 0.1% sucrose, all w/v). The plates were incubated at 34°C in the dark and the resulting colonies were counted after 4% and 72 hours.

The averaged results of duplicate experiments gave the following total number of viable conidio per flask (x 10^6) per stated hours of exposure to light: 6.5 for 0 hours exposure, 9.6 for 24 hours, 8.1 for 48 hours, 5.6 for 72 hours, and 4.8 for 168 hours. It was concluded that 24 hours of light exposure at 24°C is sufficient for maximum production of viable conidio under the conditions here employed. — Department of Biology, University of Minnesota, Duluth, Minnesota 55812.


The study of sexual differentiation in Neurospora, as with other developmental phenomena, could be greatly aided by having large numbers of mutants blocked at various developmental stages. Although unwanted female-sterile mutants often appear at times when one is searching for other kinds of mutants, a systematic search for such strains has been hampered by the absence of a good selection procedure. In lieu of such a selection technique, we have developed a rapid screening procedure, several steps of which may be useful for other purposes as well.

Agar plates are prepared with a medium of Westergaard and Mitchell's salts containing 0.5% sorbose and 0.1% sucrose. This sorbose/sucrose ratio induces colonial growth of wild type strains without inhibiting formation of protoperithecia. Sterile filter paper (Whatman #1) is placed over the solidified agar. Mutagenized conidio are suspended in 10 ml of overlay agar (0.8% plain agar; held at 45°C) at a concentration of about 50-100 viable conidio per 10 ml. This suspension is immediately poured over the filter paper and the agar is allowed to solidify.

Within 3-4 days colonies are visible. A day later the filter paper with the colonies on it is transferred to a second petri dish containing plain agar (1.5%) to reduce further growth of the colonies and to increase starvation. Within 2 days after transfer, the colonies contain visible protoperithecia, which are then fertilized. Within 3-4 more days, block perithecia are visible and colonies without perithecia can be easily distinguished. When unmutagenized wild type (74A) conidio are used, better than 95% of the colonies develop perithecia. To isolate the colonies without perithecia, the filter paper is lifted off the agar and the appropriate colonies are picked from the replica mode in the plain agar underneath by growth of the hyphoe through the filter paper.

By this technique we have isolated, so far, 11 strains after UV mutagenesis which do not form perithecia on either normal Westergaard and Mitchell's medium or on corn meal agar. All are male fertile and all develop protoperithecia, although some are quite slow in doing so. It also appears that on adaptation of this technique would be useful for replica plating nearly any strain of Neurospora, since colonies always grow through the filter paper and make replica in the bore agar. We have not yet done extensive study on this, however. — Department of Biological Sciences, State University of New York at Albany, Albany, New York 12203.