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Quantitative determination of perithecium formation

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Abstract

Quantitative determination of perithecium formation

DeSha, C.M. and R. Fuerst. A technique for the quantitative determination of perithecium formation.

This precise method permits quantitative evaluation of damage induced in protoperithecia-forming nuclei by either chemical or physical treatments. A three-day-old culture of N. crassa Em5297a grown on an agar slant of complete medium is washed with 5 ml of sterile distilled water and filtered through 2.5 cm of sterile cotton in a disposable 10 ml syringe in order to remove hyphal fragments from the conidial suspension and to obtain microconidia. Sterile water is used to dilute this suspension until an absorbance reading of 0.05 is reached cm a Bausch and Lomb Spectrophotometer 20, set at 500 nm. One ml of the suspension is diluted 1:100 with sterile water. Sterile 35mm Brewer plates with metal tops holding absorbent discs are filled with 15 ml of sterile CoLab's Oxoid cornmeal agar, which is mode according to the specifications on the label with 1.5% agar. The Brewer plater are used since the discs absorb the water that condenser in the plater. Fewer perithecia ore formed when other brands of cornmeal agar, such as Difco's or BBL's are employed. Westergaard's medium (Westergaard and Mitchell 1947 Am. J. Botany 34:573) can not be used since conidial growth is enhanced, thereby making it more difficult to count the perithecia.

After the plates are cool, 0.05 ml aliquots of the Em 5297a suspension, diluted 1:100 and containing less than 150 but more than 100 microconidia, are delivered to the centers of the plates, using a Schwartz BioResearch Autopipettor. The disposable tips for the pipettor are sterilized in a petri plate for 24 hr before use with several drops of ethylene oxide, since the tips cannot be autoclaved. The automatic pipettor is used since it allows fast, accurate dispension of the conidial suspension onto the plates. Inoculated cornmeal plater are left at 25°C until protoperithecia develop, after which 0.05 ml of N. crassa St. Lawrence 74A inoculum (with the same microconidial concentration and obtained in the same manner as described for Em5297a and with an absorbance of 0.05, is pipetted onto the surface of the agar in the inoculated plate. This time the conidial suspension is spread over the surface of the agar with on autoclaved 5 x 7.5 cm glass slide. The slide is used for the spreading procedures, instead of a rod, since the inoculum can be applied faster and more evenly, according to our experience in the laboratory, by this method.

Dark perithecia form in seven days on CoLab's cornmeal agar. The spreading technique allows for even distribution on the surface of the agar, so that the perithecia can be more easily counted with the aid of a bacterial colony counter. Before counting, however, the plater are washed with 2% Clorox to inactivate the conidiospores which have formed. A control plate, one in which the maternal strain has not been treated, is found to contain approximately 250 perithecia.

The 1:100 dilution of Em5297a conidia used in this technique might be varied by the investigator depending on the perithecia producing capacity of the strains used. This method has been found in our laboratory to be applicable to several strains that were tested. It may be concluded that this method is excellent for measuring changer in the ability of the chemically or physically-treated strain to produce perithecia when mated to an untreated paternal strain, as compared to on untreated control cross.

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