How to use spot tests to determine mutagen sensitivity

Alice L. Schroeder

Background

Mutations that are defective in DNA repair are recognized because they confer sensitivity to physical or chemical mutagens (for review see Schroeder *et al.* 1998). Spot tests provide a rapid method for detecting mutagen sensitivity and for determining the general range of doses to be used for obtaining survival curves.

Procedure

The following description is based on Schroeder (1988):

Small tubes (10 or 13 × 75mm) containing 1 ml distilled water are plugged with cotton and autoclaved. Plates with a grid of labeled squares on the bottom are prepared, containing 12–20 ml of the appropriate sorbose medium. Up to 36 cultures can be tested on one plate (6 × 6 grid).

For each culture to be tested, flame a platinum-iridium needle, cool in a water tube, and gently touch the conidia. Remove a just-visible amount of conidia. Large clumps of conidia can be shaken off by hitting the needle on the side of the tube.

Put the conidia in the water tube and shake the needle vigorously. The suspension should be slightly turbid when held up to a bright light. Conidia will survive many hours in water. Thus, all suspensions can be made before plates are spotted.

Vortex the suspension. Flame a platinum-iridium loop, cool it in the suspension and shake off the drop of suspension. Take another drop and carefully place it on the test medium in the appropriately numbered square, being careful not to break the agar surface. Also, spot a drop on a control plate that will not be irradiated or that lacks the agent to be tested. In general a dose that gives 5–10% survival in wild type gives a clear distinctions between wild type and sensitive mutants.

Gamma ray sensitivity: Expose suspensions held on ice to 65 rad, then spot. This dose gives 5–10% survival in wild type..

UV sensitivity: Spot the suspensions on sorbose medium and expose to 550–600 J/m^2 UV light within an hour of spotting. Do not break the agar surface when spotting, since the agar will shield conidia from the UV. Work in yellow or red light and wrap irradiated plates in foil to prevent photoractivation.

Chemical sensitivity: Spot on sorbose medium containing the agent. A rough survival curve may be obtained by treating conidia in liquid with increasing doses of a chemical and then spotting on sorbose medium.

Clear tests are obtained after incubating plates at 30°C for 36–48 hr. However, 25° may be used for temperature-sensitive mutants.

Turn plates upside down and score under a dissecting microscope (20–30×). Under these

conditions, individual conidia can be seen and the degree of germination and growth noted. Note that agents such as UV, MMS, and gamma rays kill conidia before or at germination, while histidine and hydroxyurea are different in their effects, resulting in slow, limited growth.

References

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