Ascospore-viability on glass spreaders treated with alcohol

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Abstract
Ascospore-viability on glass spreaders treated with alcohol

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by standing it in a beaker of ethanol, flaming off any alcohol clinging to the spreader and returning it to the alcohol after use. When only one cross has been plated at a time, this method has always appeared to give reliable results. Recently, however, we have been plating many different crosses in rapid sequence and have found that, under these circumstances, some ascospores can survive this method of sterilization. An unexpected colony type was found on two out of 15 plates where it could have been detected; on uninoculated control plates, spread immediately after a long series of inoculated plates, produced four colonies.

Rough tests on the viability of ascospores in alcohol, on samples of about 1,000–2,000 spores, showed that the majority of ascospores were killed within three minutes in either 70 or 95% ethanol; however, from 0.1 to 8% remained viable even after 30 minutes in alcohol. No spores survived standing overnight in either concentration of alcohol.

Mitchell et al. did not describe how they spread their spores. Our procedure was based on instructions that were obtained indirectly and therefore may have differed from the procedure used by these authors. However, it seems advisable to mention our results in case others are using a similar technique. We have now replaced the glass spreader by a platinum-iridium spreader that can be sterilized by direct flaming.


(83201(t); Y30539y) as the parental strain. This strain requires by incubation in an inositol-less medium at 35°C but grows well on the same medium at the lower temperature.

A suspension of inos; yo-1 conidio is mutagenized, plated on sorbose minimal medium and incubated at 35°C for 36-48 hrs. The plater ore then shifted to an incubation temperature of 25°C. Colonies may be picked 2-5 days after the temperature shift. Isolates may then be tested for failure to grow on inositol supplemented minimal medium at 35°C. This method provides a strong selection for temperature-sensitive mutants, which will not begin to grow on minimal medium at 35°C (and therefore will not be killed in the absence of inositol) but which can grow on minimal medium at 25°C. The parental strain is killed by incubation at 35°C in the absence of inositol, and auxotrophic mutants that are not temperature-sensitive should not be able to grow on minimal medium at either temperature and therefore should not develop on the plates. Overlaying of plates with supplementary inositol-containing medium is unnecessary since inos; yo-1 doer not require inositol at 25°C.

Preliminary screening of 65 isolates picked indiscriminately from plates incubated for 36 and 48 hours at 35°C, and then at 25°C until colonies formed, in one experiment indicates that 7 are temperature-sensitive auxotrophs, 10 are temperature-sensitive unknowns and 6 are morphological variants, which gives a temperature-sensitive mutant frequency of 0.26. (Survival frequency after UV irradiation was 0.45; number of viable conidio per plate was approximately 4.5 x 10^5; survival frequency after 36 hours incubation at 35°C was 9 x 10^5, and after 48 hours, 2 x 10^5. This temperature-sensitive version of the inositol-less death technique simplifies and shortens the old procedure by eliminating the agar overlaying step and greatly reducing the inositol-less incubation time.

In the ascospore plating method of Mitchell, Pittenger and Mitchell (1952 Proc. Natl. Acad. Sci. U. S. 38:569), a drop of spore suspension is placed on an agar plate and is then spread over the surface. In using this method, we have routinely used a glass spreader sterilized

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