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viability on glass spreaders after alcohol treatment.

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 agar surface.

In using this method, we have routinely used a glass spreader sterilized 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 plate, 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. - - - Department of Biological Sciences, Stanford University, Stanford, California 94305.