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Details for collection of asci as unordered groups of of eight projected ascospores

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Abstract

Collection of asci as unordered groups of ascospores

Perkins, D. D. Details for collection of asci as unordered groups of eight projected ascospores.

Asci that ore shot spontaneously from the perithecium of Neurospora have been used successfully for tetrad analysis in several laboratories. Other workers have experienced difficulty, which may indicate that more explicit instructions we needed in order to achieve optimal results.

Our general procedure follows Strickland (1960 J. Gen. Microbiol. 22: 583), but we do not use bis because perithecio are produced more abundantly and asci are shot more quickly (IO-12 days) when a wild type or one of the fluffy muting-type tester stocks (Fungal Genetics Stock Center Nos. 295, 297) is used as protoperithecial parent. (fl is convenient because conidial scatter is eliminated and no conidial masses block the path of ejected asci.) Crosses are made in petri dishes, which are kept inverted throughout incubation and during the collection of asci.

Groups of eight ascospores are collected on 4% agar-water slabs placed on microscope slides which are built up so that the collecting surface is within 1 mm of the ostioles, under the inverted cross plate. Each slab is exposed only once, for a period ranging from a few seconds to several minutes, depending on the rate of shooting. Ascospore scatter is a function of distance from the ostiole.

Groups of eight that ore judged to be adequately distinct from other ascospores are removed in situ under 40-60x magnification using a flattened platinum-iridium plate to lift out a piece of agar bearing the group. Fifty such asci are stored per plate in petri dishes containing 4% agar-water, the surface of which has previously been treated by spreading a drop of 50% diluted 5% hypochlorite solution *using a glass spreader*.

Storage plater are allowed to ripen at least 5 days at 25° or 30°C (not 34°), b efore spores are isolated to individual 75 mm tubes and heat-shocked 30 minutes in a 60° water both. (Improved germination is obtained after longer incubation, with at least some genotypes. Storage plates con be kept to 30° for 1 month if wrapped in plastic to prevent evaporation.)

Cross plates incubated in the dark shoot asci slowly when first brought into the light. The rote of projection accelerates for the next hour or two, and moy be extremely rapid after two hours. • • • Department of Biological Sciences, Stanford University, Stanford, California. 94305.