ONE LINERS

We have found that a remarkably simple procedure for isolating DNA from Aspergillus nidulans (Oakley et al. 1987 Gene 53:293-298) involving no organic extraction, works well for Neurospora. H. Foss and E. Selker, Institute of Molecular Biology, University of Oregon, Eugene, OR 97403-1229.

The following three one-liners are from R.L. Metzenberg:

Keeping ascospores in suspension for even plating.

It is often desirable to plate Neurospora ascospores for quantitative counts. This is made more difficult because they are so large and dense that they settle out of suspension very quickly. Often this is partly remedied by suspending them in 0.05% agar instead of water. In my experience, however, the agar often coagulates into small irregular masses, giving an unpredictable viscosity, and also a "noisy" field of view under the microscope. A 1% solution of polyvinyl pyrrolidone (PVP-360, Sigma Chemical Co.) provides a good viscosity and appears to be completely non-toxic. It is conveniently made up and autoclaved as a 10% solution (w/v), and then one part is added to about nine parts of ascospores suspended in water.

Separating conidia from hyphae (as in filtration enrichment).

Suspensions containing conidia and growing hyphae may be separated by filtration through glass wool, cheesecloth, Miracloth, or absorbent cotton, but in my hands, the separation is erratic. The filter may either contain a few oversized holes so that everything passes through, or it may plug up so that even non-growing conidia are removed. I find "Thermolam Plus", a felted synthetic, to be very much superior to the traditional filtration materials. It is distributed by Stacy Industries, Inc., P.O. Box 395, Wood Ridge NJ 07075-0395 (Cat. # 970), but it can be bought very inexpensively in small amounts at ordinary fabric stores. The Thermolan Plus is cut to the desired shape, put into the filter holder of choice, and autoclaved. The suspension to be separated is poured onto the filter. Non-growing conidia (or ascospores) in suspension pass through readily without clogging the filter, while those that have grown substantial hyphae are retained.

Separating ascospores from conidia

The heat shock treatment which induces the germination of Neurospora ascospores is generally sufficient to kill contaminating conidia. Under my conditions of heating, however (60°C oven with a fan), some of them survive the heat shock. This has mainly been a problem when the conidia have been aged so that they are very dormant, or when they are present in great excess. Ascospores may be enriched from ordinary aqueous suspensions by their faster sedimentation, but the degree of purification leaves much to be desired. In my experience, isopycnic sedimentation from salt solutions such as strong solutions of potassium tartrate has been unsatisfactory, apparently because the density of conidia is a function of salt concentration and because strong salts themselves sometimes induce premature germination of the ascospores. These difficulties can be avoided with aqueous solutions of sodium diatrizoate (Sigma Labs or Winthrop). The powder (10 g) is dissolved in 29.3 ml of water to give 33.3 ml of a 30% (w/v) solution (sp. gr.=1.18). The solution is best filter-sterilized, which also removes any dust particles, or it may be autoclaved for 10 minutes. Autoclaving should be done in a screwcap vial so that the specific gravity of the solution is not changed by gain or loss of water. A mixture of ascospores and conidia suspended directly in about 1 ml of this solution or layered carefully on top of it as an aqueous suspension are separated by five minutes' centrifugation in a clinical centrifuge, or they can be allowed to settle by gravity for about an hour (ascospores sink, conidia float). The ascospores may be retrieved from the bottom with a Pasteur pipet and then heat-shocked. Supported by NIH Grant GM 08995. - - - Dept. of Physiological Chemistry, University of Wisconsin, Madison, WI 53706.