How to separate conidia from hyphae.

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Background

Agitation in water often fails to separate conidia from each other, conidiophores, and aerial mycelium. When preparing macroconidia routinely for plating or when selecting for mutants by filtration enrichment, it is important to remove multicellular agregates and fragments of mycelial or conidiophore hyphae. To obtain suspensions of mainly single conidia, filtration after agitation is often necessary This can be accomplished by passage of a suspension through filters of various types. For noncritical applications, especially in large-scale procedures where many filters are needed and volumes are small, graduated pipettes can be adapted for use as filters.

Procedure

Large volumes: Cotton or glass wool in thistle tubes has commonly been used, with mixed results. Metzenberg (1989) has found a material that is convenient and reliable. The following description is from that source:

"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 Thermolam Plus is cut to the desired shape, put into the filter holder of choice, and autoclaved. The suspension to be separated is poured into 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."

Small volumes: When numerous small samples are to be plated, filtering through cotton plugs in "upside down pipettes" offers a quick and convenient alternative to conventional filters (Newmeyer 1990). The following is from that source:

"This method was in common use in the Tatum lab in the 1940's, but was apparently never published. One simply sucks the suspension through the cotton plug of a pipette, used upside-down. Graduated pipettes from 1 to 10 ml work well.

Plug the pipettes at what is normally the top (nonpointed) end, but leave 1 cm of cotton protruding. Plugs should be tight enough to stay in securely, but should not be difficult to pull out. Twist the protruding cotton from each pipette into a point with moistened fingers, and cut off a few millimeters from the end of it, so that it will lie straight and not tangle with the cotton protruding from the other pipettes. Sterilize pipettes in a canister with the plugged ends at the bottom.

To filter, put the plugged end of an upside-down pipette into the conidial suspension and press it against the bottom of the tube or flask so that the protruding cotton is bent to one side and held in place, to prevent its being sucked into the pipette with the suspension. If the suspension is hard to suck into the pipette, the plug is too tight. In that case, use a different pipette; otherwise most of the conidia will get stuck on the cotton. After sucking up the suspension, remove the cotton plug with sterile forceps. (Have a receptacle handy to receive the contaminated plug and forceps.) Let the filtrate run into a sterile tube without allowing the pipette to touch the tube, because unfiltered material is present on the outside of the pipette. Don't blow the filtrate out, because there is no plug in the pointed end of the pipette."

References

Metzenberg, R. L. 1989. Separating conidia from hyphae (as in filtration enrichment). Fungal Genet. Newslett. 36: 82.

Newmeyer, D. 1990. Filtering small quantities of conidial suspensions to remove mycelial fragments. Fungal Genet. Newslett. 37: 27.