How to measure and monitor linear growth rate.

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Background

Linear growth of *N. crassa* mycelia can exceed 5 mm/hr on solid medium -- perhaps the most rapid on record. This is a great asset for such purposes as determining optimal growth conditions (Ryan *et al*. 1943), assaying growth responses to metabolites (Ryan *et al.* 1943) and inhibitors (Newmeyer 1984), analyzing heterokaryons (Davis 1966), and studying complementation, heterokaryon incompatibility (Jacobson *et al*. 1995), circadian rhythms (Sargent *et al.* 1966, Feldman and Dunlap 1983), senescence (Sheng 1951, Bertrand *et al.* 1976, Rieck *et al.* 1982), genome instability (Schroeder 1986), species differences (Perkins and Pollard 1986), and transmission of retrotransposons between nuclei (Kinsey 1993). Most studies of linear growth have employed long glass tubes bent up at each end ('race tubes'), devised by Ryan *et al*. (1943). Sterile 50 ml plastic pipettes are a convenient substitute for the reusable glass race tubes (White and Woodward 1995). Large petri dishes can also be used (Brody and Harris 1973).

Procedure

Glass tubes: Laboratories that routinely use large numbers of race tubes have usually employed custom-made glass tubes as pictured by Ryan *et al.* (1943) and Feldman (Fig. 32 in Perkins *et al.* 2001) Specifications for glass race tubes and detailed instructions for their preparation and use are given by Ryan *et al.* (1943) and Ryan (1950).

Vogel's minimal medium in 2% agar is used for most purposes, with or without supplements. (When conidial banding is timed in studies of circadian rhythms, Vogel's medium N salts are used with 0.3% glucose, 0.5% arginine HCl.)

Depth of agar medium in the leveled race tube should be slightly below the maximum diameter of the tube. For uniform growth rates, the depth of agar should be similar in all tubes. About 13 ml medium is required for glass race tubes ~30 cm long and 13 mm internal diameter.

Tubes are conveniently filled with molten agar before autoclaving. Precautions must be taken to keep tubes level so as to avoid wetting the cotton plugs. (A generous supply of sterile plugs should be kept on hand for use if replacements are needed.) While transporting race tubes that contain molten agar, or when putting them in the autoclave, wetting can be minimized by orienting the tubes at right angles to the direction of motion to prevent sloshing. The bent-up tube ends are conveniently kept upright by wrapping two or three tubes together using autoclave tape. Alternatively, clamps can be used. After the sterilized agar is solidified, individual tubes are conveniently held upright with spring-type wooden or plastic clothespins. Tubes should be allowed to harden for 1 to 3 days before use, to avoid slippage of agar when the tube is handled and to allow evaporation of condensate on the wall above the agar.

Alternatively, race tubes may be sterilized before filling, and the desired volume of molten agar medium poured into each cotton-plugged race tube from an individual test tube in which the medium has been sterilized.

Tubes are inoculated at one end. After a 6 to 12 hour period to allow initiation of steady growth, the position of the advancing mycelial front (leading hyphae) is marked periodically on the tube (usually twice daily; the time intervals need not be equal.). If feasible, mark at 6 and 12 hours after inoculation so as not to waste part of the linear growth. Times of marking are recorded, and when growth is complete, distance is plotted against time to obtain the linear growth rate. If accuracy is required, tubes are run in triplicate.

Washing glass race tubes is a nuisance. Used tubes are autoclaved and the contents are poured out while still liquid. Tubes are immediately rinsed with a strong stream of running water, and washing is continued using a pipette-washing routine. Dried-down tubes are best autoclaved while submerged in water.

Plastic pipettes: Preparation is simpler when commercially produced disposable sterile 50 ml plastic pipettes are used in lieu of glass race tubes, and there is no problem with cleaning. (White and Woodward 1995; Jacobson *et al.* 1995). This is a boon especially for workers who don't possess glass race tubes and who plan to use the tube method only occasionally or on a small scale. The following details are adapted from White and Woodward:

"A 25 ml sterile disposable plastic pipette is used (almost any brand will work). First, draw up enough sterile molten medium to nearly fill the pipette. Do this slowly to prevent bubbles. Then release all but 13 ml. (The initial filling wets the tube and prevents subsequent condensation on the wall.) Close the open pipette tip with a gloved finger of your free hand. The gloved finger can be sterilized by dipping with ethanol and used immediately; the small amount of ethanol has not been a problem. Keeping both ends closed, lay the pipette down slowly onto a level surface, starting with the tip and slowly lowering the cotton- plugged end so as not to wet the plug. Tubes can be kept from rolling by first putting down two strips of laboratory tape, sticky side up and turned under at the ends, then sticking the tubes down on the tape. Work quickly because the air in the closed pipette will rapidly heat and expand, causing leakage and bubbles."

"Allow the medium to cool and harden. When you are ready to use the tubes, snap off the pointed tip of the pipette, cap it with a sterile test-tube cap, and secure the cap with a piece of tape. We have found that a wrench with a tapered hole is the best tool for breaking off the tip. Any box wrench or pliers can be used that will fit part way up the tip. Insert the tip into the hole of the wrench and snap it off quickly; this minimizes fracturing the straight part of the pipette."

"To inoculate, remove the cap at the broken end of the tube, and replace it after inoculation. Record growth by marking the graduated bottom of the tube. The volumetric graduations can often be used as a built-in scale to measure linear growth rates. The conversion factor from ml to mm can be determined for any particular brand by measuring one pipette."

Large petri plates: For slow growing mutants, growth rate can conveniently be measured by inoculating the strain to be tested in the center of a 15 cm diameter petri plate. Plates have been used effectively by Stuart Brody in experiments timing the rhythmic formation of conidial bands (see for example Brody and Harris 1973). Care should be taken to avoid conidial scatter.

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