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A continuous-flow system for the growth of Neurospora

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A continuous-flow system for the growth of Neurospora

Abstract

Continuous-flow system for growth

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A method has been developed for growing Neurospora in a continuously flowing liquid medium. The system was designed 1) to constantly expose the mycelium to fresh medium, and 2) to allow a rapid shift of the growth medium without disturbing the mycelium. We intend to use the system to investigate the effects of sudden chemical perturbations on the circadian rhythms of Neurospora. In essence, liquid medium is pumped from a reservoir, through a length of dialysis tubing, and then into a waste receptacle or back into the reservoir. The Neurospora grows along the outside surface of the dialysis tubing "growth tube".

The system is composed of dialysis tubing (Union Carbide, Dialysis Membrane, 0.4 in. diam., 5 in. long), polyethylene quick-disconnectors (size A, 1/4 in. to 3/8 in.) at each end of the dialysis tubing, and rubber-hose sleeves (3/16 in. i.d., 1/16 in. wall, 1/4 in. long) to secure the dialysis tubing to the disconnectors. The disconnectors must be sandpapered to fit into the dialysis tubing. Tygon tubing (1/8 in. i.d., 1/8 in.wall), with rubber hose adapters (1/8 in. i.d., 1/32 in. wall, 1/2 in. long) to the disconnectors, carries the medium to and from the "growth tube". The "growth tubes" are supported on a frame of chicken wire (1 in. mesh) inside of a Pyrex baking dish (19 x 30 cm) that is covered with plate glass (strips of cotton secured to the baking dish with masking tape provide support for the cover while allowing aeration and preventing contamination). We use a mesh support for the "growth tubes" to reduce the seepage which occurs wherever the dialysis tubing contacts another surface.

The assembled dialysis tubing, disconnectors, and rubber sleeves are sterilized by boiling in water for 10 min. The rest of the apparatus is assembled and then sterilized by autoclaving. Final assembly and inoculation is done in a sterile transfer hood. The "growth tubes" are inoculated at one end with a small amount of dry conidia, and the whole apparatus is put under appropriate growth condition.

Clamps, Y-tubes, and valves can be used to run several cultures simultaneously, and to control the origin and disposal of the medium. We have found that four-channel peristaltic pumps (Buchler Polystaltic Pump, Model #2-6100) provide a suitable and constant flow of growth medium (2-3 ml/min.). Fifteen min. sufficed to completely flush the "growth tube" with new medium.

The linear growth rate and phenotypic expression (e.g. good bands with the bd strain), of the few strains we have tested is similar to growth on agar medium under similar conditions, provided that the concentration of the carbon source is decreased by about half. With normal carbon-source concentrations growth is so luxuriant that one obtains a cylinder of Neurospora up to 5 cm in diameter.

The one serious problem is the occurrence of leaks in the dialysis tubing after a few days of incubation, presumably a result of tubing degradation by cellulases. Since cellulases can be induced by cellulose-breakdown products (Mandels and Reese 1960 J. Bacteriol. 79: 816), we have used the highest flow rate possible (excessive flow rates cause tube bursting) and a medium lacking glucose (0.15% mannose, 0.3% arginine-HCl, 1 x Vogel's salts; cf Sargent and Kaltenborn 1972 Plant Physiol. 50: 171). A minimum of 4-5 days of growth (occasionally considerably longer) usually can be obtained before leakage becomes serious. Use of Singapore-2 (FGSC #436), a strain of N. intermedia with low levels of cellulase under certain conditions (Eberhart, unpublished), has not consistently increased the lifetime of the system.

Possible methods for increasing the system lifetime include 1) "growth tubes" in series with their ends overlapping so that the mycelium could grow from one tube to the other, or 2) transfer of portions of the growth front to more conventional substrates after a chemical pulse. The latter is based on the concept (Brody, unpublished) that such transfers do not phase shift the biological clock. Other conceivable tactics are the use of 1) a non-cellulose membrane, 2) a cellulase inhibitor or 3) a cellulaseless mutant. To the best of our knowledge none of these is available.

Although the system has a shorter lifetime than desirable, it is proving useful for our rhythm experiments and could be beneficial for other studies where precise control and alteration of the growth medium are required. - - - Departments of Botany, and Genetics and Development, University of Illinois, Urbana, IL 61801.