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Temperature-sensitive, protoplast-forming os-1 variant of Neurospora - a few tricks.

Protoplasts of Neurospora can be formed by treating hyphae with one of a number of "cell-wall'ases". However, resulting protoplasts often have residual cell-wall and will not grow and divide. The slime variant of Neurospora will, under certain conditions, grow as a population of protoplasts. Unfortunately, such cultures will not participate in crosses thus making genetic manipulation difficult. In efforts to overcome this and other difficulties, we have used a temperature-sensitive osmotic mutant (os-1 (NM233t)) in order to form protoplasts. The general method has been previously published (Selitrennikoff et al., 1981 Experimental Mycology 5: 155-161) and included here are some details.

Mycelial isolates of os-1 (NM233t) are available in either mating type from the Stock Center and can be used as a male or female parent in order to construct any desired genotype. Progeny can be grown on agar slants at either 25° or 37°C.

Macroconidia are inoculated (1-10 x 105 cell/ml) into 250 ml flask containing 50 ml of the following medium: 10% (w/v) Sorbose; 2% (w/v) Sucrose; 1 x Vogel's salts, 400 µg/ml Polyoxin B. Polyoxin B is an inhibitor of chitin synthetase activity and is purified from Polyoxin AL Wettable Powder (available either over-the-counter in Japan or from the Kaken Chemical Co., Ltd., 28-8 2-Chome, Honkomagome, Bunkyo-Ku, Tokyo, Japan). One hundred grams of powder are dissolved in 1 liter of water and insoluble material is removed by filtration using Whatman #1 paper. The brown filtrate is applied to a Norite column (granular form the powder packs to a very slow (100 ml/day) column). The column is washed with water and the Polyoxin eluted with 60% aqueous acetone. The dirty-brown fractions are pooled and the acetone is flushed out. The resulting brown "goo" or powder is dissolved in water and applied to a Dowex 50 (8X,H+ form) column. The Polyoxin is eluted with 0.3N NH_4OH and the resulting solution freeze-dried twice (to insure complete removal of NH_4OH). Typical batches result in a product - 70% pure based on U.V. absorption with yields of 25.35%. Polyoxin powder can be stored desiccated at 4°C (or -20°C) for long periods of time.

Cultures are grown at 37°C with shaking (140 rpm) and are filtered daily through sterile glass wool into fresh medium. After seven days a stable culture of protoplasts results. It is important that during the initial transfers that the cell density be maintained at 5 x 10^5 cells/ml or greater. Cultures are then filtered into flasks containing medium lacking Polyoxin and transferred daily (with filtration) to fresh medium for another seven days. These, then, are filtered to medium in which Sorbose has been replaced by 7.5% Sorbitol and growth allowed to continue for yet another week (with, of course, daily fitration). The population of protoplasts that results is stable for up to one year of growth and protoplasts can regenerate a mycelium (including macroconidia) upon a temperature shift to 25°C. However, far reasons that are not clear, the resulting mycelium is different morpholocially from the original os-1 strain. Protoplasts may be stored at -70°C in their medium for at least two years. These temperature-sensitive protoplasts are very useful for a number of studies, including hosts for exogenous DNA's, (Supported in part by NSF grants to CPS.)

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