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Cryopreservation of slime mutants of Neurospora crassa.

Cryogenic storage in the frozen state at liquid nitrogen temperatures is the most satisfactory method to date for long-term preservation of living fungi with maximal viability and genotypic stability. The American Type Culture Collection (ATCC) has employed this technique for conservation of a wide variety of fungi since 1960 (Hwang, S.-W. 1966 Appl. Microbiol. 14: 784-788). Three slime mutants of Neurospora crassa, originally described by Emerson (1963 Genetica 34: 162-182), were deposit-the ATCC and were designated ATCC 26187 (fz;sg;os-1), ATCC 32313 heterocaryon: (fz;sg;arg-1, cr-1 aur or-1 + al-2, nic-1, lys-3 or-1) and ATCC 32360 (fz;sg;os-1, arg-1, cr-1 aur).

Since ATCC 26187 has survived ten years (the length of the experimental period) in liquid nitrogen at -196 C, details of the procedure used at the ATCC are given below.

Cultures are grown on Difco Neurospora culture agar (ATCC medium 331) plates for one week at room temperature. Three agar discs containing the slime cells are removed with a 5-mm sterile cork borer and placed in a sealable borosilicate ampoule of 1.2 ml capacity. A volume of 0.4 ml of 10% (v/v) glycerol in distilled water is added as a cryoprotective agent. The ampoules are cooled to +5 C to prevent overheating during sealing, after being sealed, the ampoules are placed onto prelabeled aluminum canes in boxes which are then placed into the freezing chamber of a programmed freezer. The initial coaling is carried out at rate of 1° centigrade per minute from room temperature to -35 C; subsequent cooling to below -100 C is rapid and uncontrolled. Then the ampoules are immediately transferred to storage in liquid nitrogen at -196 C or in liquid nitrogen vapor (temperature about -150 to -180 C).

Vapor-phase liquid nitrogen refrigerators are used for routine storage of fungi at ATCC. They can be used for both sealed and unsealed ampoules. Ampoules immersed in liquid nitrogen require proper sealing as an improperly sealed ampoule permits entry of liquid and will explode at the time of thawing due to the sudden expansion of the nitrogen into gas. The operator should wear a face mask to avoid possible injury from exploding vials.

For recovery of the cultures frozen in liquid nitrogen, the frozen ampoules are thawed rapidly in a 37C water bath with moderate agitation until the last trace of ice is dissipated. This usually takes about 40-60 seconds. The culture samples are aseptically transferred to appropriate medium.

Recently Butterfield, Jong and Alexander (1978 Mycologia 70: 1122) reported the use of screw-cop polypropylene vials for storage in liquid nitrogen of many problem strains of fungi, which did not survive other freezing procedures. Like unsealed glass ampoules, they must be stored in the vapor phase of liquid nitrogen refrigerators because, if immersed, liquid nitrogen can enter the vial around the threads of the cap. The slime mutants ATCC 32313 and ATCC 32360 have been successfully stored in polypropylene vials since 1975. - - - Mycology Department, American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD 20852.