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 deposited at ATCC and were designated ATCC 26187 (fz;sg;arg-l,c,-I), ATCC 32313 heterocaryon: [fz;sg;arg-l,c,-I] sur. or+1. [+ pl-2,nic-l.lys-2. or-I] and ATCC 32360 (fz;sg;ss-l, arg-l, c,-l or-r).

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 sealed 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 cooling is carried out at rate of 1°C 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°C 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, so on improperly sealed ampoules 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 37°C 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-cap 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 con enter the viol around the threads of the cop. 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.

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Preservation of Neurospora stock cultures with the silica gel method for extended periods of time.

The silica gel method for preservation of Neurospora stock cultures has now been in use for almost 25 years. Perkins (1962 Can. J. Microbiol. 8: 591-594) reported survival of silica gel cultures for six years. It is of interest to collect information about long term survival to know approximately how long it is safe to keep stocks before transfer to new gels.

At our laboratory we have preserved 22 cultures with this method since October 1968. The method described by Brockman and de Serres (1962 Neurospora News 1: 8) was generally followed. Pyrex tubes (12.5 x 1.5 cm) with rubber lined screw caps were used. The caps were tightened soon after the conidial suspension in skim milk had been added at ice water temperature. The tubes were stored at 2-4°C and each strain was maintained in duplicate.

To test survival, one tube of each strain was sampled after 5, 7 and 10 years. Some of the tubes have also been opened for transfers on other occasions. Upon sampling in November 1978, it was found that all 22 germinated after transfer to Fries' minimal medium with appropriate supplementation.

The 22 cultures represent a rather restricted sample with regard to their genetic composition. All have wild type morphology with good conidial formation. They are reisolates of the mutants ure-1 (9) and ure-2 (47) from crosses with the standard wild types 74-OR8-1 A and 74-OR23-1 A, as well as combinations of these urease-defective mutants with the closely linked markers am (32213) and his-1 (C91). The wild types and the separate marker stocks were also preserved.

It is reassuring that the silica gel method, besides being very convenient, also allows a satisfactory survival over periods of many years. - - - Department of Genetics and Plant Breeding, 750 07 Uppsala, Sweden.