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Abstract

Viability of macroconidia after liquid nitrogen refrigeration

Wellman, A. M. Viability of Neurospora macroconidia after cryogenic storage by liquid nitrogen refrigeration.

Survival of air dry conidia of Neurospora after freezing in liquid air for 1 hour was reported by Faull (1929 Mycologia 22: 2881. Marc recently the successful preservation of a wide range of biological materials at ultra-low temperatures has stimulated interest in liquid nitrogen refrigeration (-165 to -196°C) as an alternative to freeze-drying or silica gel or soil methods for storing fungi (Hwang 1966 Appl. Microbiol. 14: 784; Mazur 1966 In Meryman (ed.), Cryobiology. Academic Press; Wellman and Walden 1964 Can. J. Microbiol. 10: 585).

Low temperature storage: The following procedure for the storage of Neurospora strains has been developed in this laboratory during the past 4 years. The fungi are grown on agar slants (on Fries minimal or supplemented medium) in plugged 2 ml cryogenic ampules (T. C. Wheaton Co., Millville, N. J.) for 7 days at 25°C. The ampules are then heat-waled, placed immediately on aluminum canes (Arnold Nasco Ltd., Guelph, Ontario), loaded into canisters and rapidly frozen (1-15°C/sec) by direct immersion into a liquid nitrogen refrigerator (Linde LR -35 -9). After various storage periods frozen cultures are wormed rapidly by transferring from the refrigerator to a water both at 35-40°C for 2 min and then left at room temperature for 1/2-l hour before testing for viability. On each occasion four ampules were sampled.

The results recorded here ore port of an investigation of the effects of low temperature storage on several strains of fungi over a ten-yew period. Viability of macroconidia of Neurospora was estimated as percentage germination. In order to distinguish between freeze/thaw injury and the effects of storage, cultures frozen and immediately warmed were compared with unfrozen control cultures; thereafter frozen cultures stored for up to 30 months were compared with 7-day-old control cultures which had been maintained routinely on agar slants by successive transfer.

Germination tests: 0.5 ml of spore suspension (3×10^4 spores/ml) was spread on the surface of each agar plate (4 plates per treatment) ond the plates were incubated at 30°C. Discs were removed from the plates from l-8 hours after incubation; a drop of 10% formlin was added to each disc and two randomly-chosen fields/disc were examined under a 40x high dry objective and scored for germination. Each field (16 fields/treatment/time interval) was recorded on 36 mm film using a Leitz Ortholux camera, so that on analysis of germ tube lengths under different treatments could be made from the projected negatives.

Conidio were also germinated on squares of sterilized dialyzing membrane on the surface of agar plates. The spores on the membrane con be fixed (in Helly's) and stained for more extensive morphological investigations. Some microorganisms are metabolically injured during freezing and thawing such that their nutritional requirements are altered. The nutritional requirements of one strain of N. sitophila, in which 27% non-germinating spores are present after 6 hours' incubation on minimal medium following freeze/thawing, are being investigated by transfer of spores on dialyzing membrane to supplemented media to determine whether non-germinating spores are non-viable or whether they have more demanding nutritional requirements.

Viability:s shown in Table 1, there is a slight decline in viability of frozen and thawed spores (significant at 1% level $F = 18.56$) but no significant decrease occurs with increased storage time ($F = 2.09$). Equally good recovery has been obtained with wild type conidio of N. crassa strains 79a (FGSC#533) and 74-OR23-1A (FGSC#987) after 18 months storage and with 16 wild type and mutant strains after 3 months storage (in press).

Table 1. % germination of Neurospora strain UWO 913 conidia after 6 hours incubation at 30°C (average of 4 sub-samples).

Treatment	1 hr	1 month	6 months	12 months	18 months	24 months	30 months
Stored at -196°C	93.43	87.07	91.31	90.31	92.65	84.85	96.73
7-day-old control grown at 25°C	95.78	96.45	95.34	97.81	93.58	95.73	97.20

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