Liquid nitrogen storage of yeast cultures
I. Survival, and literature review of the preservation of fungi at ultralow temperatures

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All 20 yeast strains of 17 species tested survived 75 days (the length of the experimental period) in liquid nitrogen at −196 C. The components of the more protective of the two freezing media used were (w/v) malt extract 2.5 %, yeast extract 0.25 %, peptone 0.5 %, calf serum 15 % (v/v) and dimethyl sulfoxide 10 % (v/v). Viability of the cells in this medium after rapid uncontrolled freezing and thawing in sealed plastic ampoules ranged from 2 % to 98 % (average 67 %) compared with the viability of the cultures before freezing. In only 4 strains was survival lower than 50 %. (90 references).

INTRODUCTION

Liquid nitrogen (LN) refrigeration is now used widely in the preservation of microorganisms (see Swoager, 1972). For example, at the ATCC many fungal cultures are routinely stored in LN since 1965 (Hwang, 1966; Alexander, 1973; Cunningham, 1973; Butterfield, Jong and Alexander, 1974).

Spores of filamentous fungi (Aspergillus, Penicillus, Neurospora, Mucor, Phycomyces, Rhizopus and various Basidiomycetes) were reported to survive in temperatures below −170 C in liquid air, hydrogen or helium (Becquerel, 1910, 1950; Buller, 1912; Faull, 1929; Kärcher, 1931; Lipman, 1937; Joshi, Wilcoxson, Gera and Chatterjee, 1974). LN was used first for conservation of viable rust spores in the dry state (Loegering, McKinney,

Mycelial fungi suspended in various freezing media, with or without cryoprotective agents, were successfully frozen in LN by Doebbler and Rinfret (1963), Bugbee and Kernkamp (1965), Hwang (1966, 1968), Bromfield and Schmitt (1967), Goos, Davis and Butterfield (1967), Hwang and Howells (1968), Barnhart and Terry (1971), Hasegawa (1973), Butterfield et al. (1974), Gale, Schmitt and Bromfield (1975) and Homolka (1976).

Yeasts and yeast-like organisms are also able to survive at ultralow temperatures. Goetz and Goetz (1938) observed 85% viability of *Saccharomyces cerevisiae* after the culture had been immersed in isopentane at −160°C. *S. cerevisiae* (Kärcher, 1931; Graievski and Medvedeva, 1948) and *Endomyces magnusii* (Rumyantseva, 1963; Rumyantseva and Tribis, 1965) survived in liquid air at −180°C. Udelnova (1957) studied various factors influencing the survival, cytological and physiological changes after freezing in LN and thawing of *E. magnusii* and *S. cerevisiae*. This was apparently the first use of LN freezing in fungi, published 4 years before the paper of Loegering et al. *S. cerevisiae* was reported to survive in LN by many other authors (Doebbler and Rinfret, 1963; Moor and Mühlethaler, 1963; Wellman and Walden, 1964; Mazur and Schmidt, 1968; Davies, 1970; Albrecht, Orndorff and MacKenzie, 1973; Alexander, 1973; Bank and Mazur, 1973; Daily and Huggens, 1973; Souzu, 1973b; Wellman and Stewart, 1973). Various other yeast species were successfully stored in LN by Wellman and Walden (1964), Sokolski and Staperet (1965). Tsuji (1966), Albrecht et al. (1973), Wellman and Stewart (1973) and Butterfield et al. (1974). The yeast-like forms of the dimorphic human pathogenic fungi *Histoplasma capsulatum* and *Blastomyces dermatitidis* retained high viability after LN storage (Hwang, 1966; Butterfield and Jong, 1975), as did the protoplasts of *Entomophthora* (Tyrrell, Sohi and Welton, 1972).

Yeasts were used as a model in numerous studies of the mechanisms of cryo-injury. Morphological and physical effects of freezing and/or thawing were examined in *S. cerevisiae* by Graievski and Medvedeva (1948), Hansen and Nossal (1955), Mazur (1960a, 1960b, 1961a, 1961b, 1963, 1966), Nei (1960, 1973), Mazur and Miller (1967), Mazur and Schmidt (1968), Bank and Mazur (1973), MacKenzie, Rasmussen and MacAulay (1973), Ushiyama, Cravalho, Diller and Huggins (1973), and in *E. magnusii* by Udelnova (1957), Rumyantseva (1963), Rumyantseva and Tribis (1965). It was observed that