

Long-Term Preservation of a Nonsporulating Strain of *Claviceps paspali*

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We have been working for the last three years with a strain of *Claviceps paspali* isolated in Israel from the sclerotia of *Paspalum distichum*. This strain produces neither ascospores nor conidia in saprophytic culture. A. Tonolo et al. (Sci. Rept. Ist. Super. Sanit. 1:404, 1961) and P. Bianchi et al. (Nature 202:312, 1964) previously noted that strains of *C. paspali* giving high alkaloid yields did not produce conidia. When nonsporulating strains are maintained on Potato Dextrose Agar (Difco) slants with successive transfers, productivity gradually decreases. Although fungal spores have been successfully preserved by freeze-drying, mycelium does not survive this treatment (S. W. Hwang, Appl. Microbiol. 14:784, 1966), and a different storage method is required for nonsporulating fungi.

J. W. Carmichael (Mycologia 48:378, 1956) successfully stored stock cultures of fungi on Sabouraud's agar at -20 C, and E. Meyer (Mycologia 47:664, 1955) preserved dermatophyte cultures in human blood serum at -22 to -52 C. D. D. Perkins (Can. J. Microbiol. 8:591, 1962) held strains of *Neurospora crassa* that do not produce conidia on anhydrous silica gel at 4 C for several years; S. W. Hwang (Appl. Microbiol. 14:784, 1966) successfully maintained many fungal strains, including *C. paspali*, under liquid nitrogen at -165 to -196 C.

Several storage methods were included in this study. In method 1, storage was on Potato Dextrose Agar slants at -70 C. Mycelial transfers were made to agar slants that were incubated for 7 days at 24 C. Cultures were then frozen in a deep freezer at -20 C for 24 hr and stored at -70 C.

In method 2, storage was on agar B of H. Kobel et al. (Helv. Chim. Acta 47:1052, 1964) containing beer wort and corn steep liquor. Cultures were prepared, frozen, and stored as in method 1.

In method 3, storage was in medium of R. M. Fry and R. I. N. Greaves (J. Hyg. 49:220, 1951) consisting of calf serum supplemented with

nutrient broth and glucose. Portions (2 ml) of sterile medium were dispensed into tubes, and 0.5 ml of submerged mycelial culture was added. The suspension was frozen and stored as in method 1.

In method 4, storage with anhydrous silica gel was at 4 C. In accordance with the procedure of D. D. Perkins (Can. J. Microbiol. 8:591, 1962), tubes were filled to two thirds with anhydrous silica gel (6 to 16 mesh; Fisher Scientific Co., Pittsburgh, Pa.) and sterilized in a dry sterilizer at 160 C for 90 min. To each tube, 0.5 ml of submerged mycelial culture or 0.5 ml of submerged mycelial culture suspended in 0.5 ml of sterile skim milk was added. Tubes were closed with sterile cork stoppers, sealed with hot paraffin, and stored at 4 C.

To test for viability, cultures stored at -70 C were placed at -20 C for 24 hr and then thawed at 24 C. Transfers were performed to Potato Dextrose Agar plates, and incubation was for 5 to 7 days at 24 C. In the case of dehydrated mycelium, the silica gel granules were placed directly on the Potato Dextrose Agar plates.

Cultures on Potato Dextrose Agar slants (method 1) died after a few days of storage at either -20 or -70 C, and those on Kobel's agar B medium at -70 C (method 2) remained viable for at least 2 years. Mycelial suspensions frozen in the Fry and Greaves medium at -70 C (method 3) or dried with anhydrous silica gel at 4 C (method 4) remained viable for at least 6 months.

All subcultures obtained after these storage periods produced alkaloids at the same level as the parent cultures before preservation. Tests for alkaloid production were performed in shake flasks according to the method of F. Arcamone et al. [Proc. Roy. Soc. (London) Ser. B. 155:26, 1961].

A preservation method for industrial cultures should be simple and should ensure viability of the strain and stability of productivity. Suppression of growth appears to be the method of

choice for the prevention of strain degeneration (F. Reusser, *Advan. Appl. Microbiol.* **5**:189, 1963). Repeated transfers should be avoided, and cultures should be stored under conditions in which no growth takes place and in which the

structural and metabolic characteristics are preserved. In the case of *C. paspali*, drying of the mycelium with silica gel and storing at 4 C or storage in a deep freezer at -70 C in suitable liquid or solid medium was satisfactory.