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Method for freezing slime

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Abstract

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To overcome the necessity of constant passage in either liquid or solid medium of the strain FGSC#326 (fz; sg; os-1, arg-l, cr-I, aur), a slime variant of N. crassa, freezing was studied as a holding method.

Eight-day cultures, grown on Vogel's agar medium (1.5% agar) with arginine (50 mg/100 ml medium) was washed down with a stream of Vogel's arginine SSP medium (Vogel's arginine medium plus 2% sucrose, 10% sorbose, Penicillin G Sodium 100U/ml of medium, dihydrostreptomycin sulphate 100µg/ml of medium) with a sterile Pasteur pipette. The milky liquid was transferred to a sterile tube and centrifuged 15 minutes at 2500 rpm.

The supernatant was discorded and the residue resuspended in the freezing fluid (Vogel's arginine SSP plus dimethyl sulfoxide, 4:1), until a four-fold dilution of this suspension gave on absorbance reading of 1.0 at 280 nm on a Beckman spectrophotometer. 2 ml of the concentrated suspension was placed In sterile vials, sealed and quick-frozen, using liquid nitrogen. The viols were stored at -70°C until used. To date they have been held up to three months, but longer periods of holding time ore being tested. Thawing was done in warm water and the contents of the vial were emptied into a liter of Vogel's medium plus arginine and antibiotics (Penicillin and Streptomycin in the concentrations noted above). No retardation of growth has been noted when the frozen and thawed cultures have been compared to others maintained by continual passage.

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