Fungal Genetics Reports

Volume 20 Article 35

Freezing as a convenient method for preserving vegetative stocks

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Recommended Citation
Perkins, D. D. (1973) "Freezing as a convenient method for preserving vegetative stocks," *Fungal Genetics Reports*: Vol. 20, Article 35. https://doi.org/10.4148/1941-4765.1843

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Abstract

Preservation of vegetative stocks by freezing

Perkins, D. D. Freezing as a convenient method for preserving vegetative stocks.

A convenient method that avoids the need for frequent transfer and minimizes chances of accumulating mutational changes is simply to store slant cultures at -20°C in a deep-freeze or the freezing comportment of a refrigerator. Conidiating strains survive at least a few years. (Long-term tests have not been mode.) They con be repeatedly sampled and refrozen. Nonconidiators ore more of a problem: survival of stocks such as fluffy is erratic and cannot be trusted, especially if thawing and refreezing occur and the mycelium is flat on the surface so as to become wet. Ascospore survival is said to be poor after freezing.

Domestic freezer models present a freezing-thawing problem because of the automatic defrost cycle. This is controlled by a timer in series with the internal light, in models with which I am familiar, and the cycle can be stopped simply by removing the light bulb. Dehydration of slants con be minimized by using sealed plastic boxes for storage. We routinely use 10 x 75mm tubes in plastic sandwich boxer. Sampling is by transfer with an inoculating needle, just as for a normal culture.

The Fungal Genetics Stock Center has routinely maintained oconidial stocks on agar slants in screw-copped culture tubes in the freezer. To prevent the accumulation of mutations and to ovoid the risk of inviability, frozen aconidial stocks ore replaced annually by stocks preserved in liquid nitrogen. They report that vegetative transfers of oconidial stocks con readily be made without thawing the culture. A wealth of information on survival of fungi after freezing is given by Mazur in Chapter 14 of The fungi, on advanced treatise, Vol. 3 (Ainsworth and Sussman, eds. 1968, Academic Press, New York and London.)

Deep-freezing takes no more effort than storage at 5'. where contaminating mites or molds moy proliferate, and where mutation, dessication, and death occur. An added advantage is that mites and mite eggs ore killed at -18° (Subden and Threlkeld 1966 Neurospora New. 10:14). For this reason, we routinely freeze all cultures collected from nature before they ore introduced into the laboratory. Because of the disadvantages of freezing - vulnerability to power failures, erratic viability of nonconidiators - silica gel stocks or lyophils are preferred for long-term preservation of important strains. But deep-freezing is ideal for stocks awaiting permanent preservation, for working stocks, for strains held in reserve pending the outcome of experiments, or for isolates so numerous as to make the other methods unfeasible.

We continue to use anhydrous silica gel for the preservation of all permanent stocks (1962 Can. J. Microbiol. 8: 591; 1963 Neurospora Newsl. 4: 21). The silica gel technique gives long-term survival without the necessity of freezing, and it is applicable both to conidiating and nonconidiating strains. Repeated samples can be taken from the some tube. A silica gel stock is much easier to make than a lyophil, which con be used only once.

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