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LIQUID NITROGEN CRYOPRESERVATION OF *PARACOCCIDIOIDES BRASILIENSIS* IN FAVA'S NETTO MEDIUM

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The aplicability of Fava's Netto medium in the liquid nitrogen cryopreservation technique of Paracoccidioides brasiliensis *cells was demonstrated.*

The storage of pathogenic microorganisms in liquid nitrogen (LN) is a standard practice in many laboratories of experimental biology, because, besides its relative simplicity, this method prevents biologic variations of stored parasites, providing a basis for critical comparative studies under standardized conditions of subsequent experiments and in different research centers.

Paracoccidioides brasiliensis (Pb) has been recognized as being able to loose its virulence when cultivated in laboratories for long periods (Rezkalla-Iwasso, 1981), and to increase its pathogenicity by successive inoculation in animals (Fava Netto, Brito & Lacaz, 1961; Brito & Fava Netto, 1963). This labil behaviour of *Pb* when subcultured in laboratories has been referred to as one of the main obstacles in comparing different experimental results in the literature (Defavieri, Rezkalla-Iwasso & Franco, 1982; Moscardi & Franco, 1980).

Mayer (1955), probably the first author to test the practicability of the deep freeze storage of fungi in laboratories, emphasized the ability of this technique in preventing biologic changes of stocked cultures. *Pb* cells have been LN frozen and stored in a few foreign reference centers, although Fava's Netto medium (Fava Netto, Vegas, Sciannamea & Guarnieri, 1969), has not yet been tested in LN cryopreservation of this fungus.

In order to investigate the possibility of recovering viable fungi after LN freezing in Fava's Netto medium, 6 steril (10 x 25 mm) screw-capped plastic vials (Nunc, Intermed, Denmark) were filled at room temperature (28-30°C) with 1,5 ml of a 2% *Pb* viable yeast cell suspension in liquid Fava's Netto culture medium, containing 10% (v/v) of glycerol, sealed and dropped in a -79°C etanol-dry ice mixture. After 3 h, vials were placed in a canister and immediately immersed in the liquid nitrogen of a LINDE-XR 16 container for 24 h. Thawing was accomplished by transferring vials directly from nitrogen to a 37°C water bath for 10 min, and the viability of cells were checked by incubating at 37°C, 0,4 ml of the content of each vial in a fresh Sabouround's agar slant.

Our preliminary results did not provide quantitative data on the number of recovered viable fungi, but all transfers were observed to grow readily from all parts of the inoculum, suggesting that the Fava's Netto medium is suitable for use in cryopreservation of yeast *Pb* cells, not only in "cryobanks", but also in laboratories interested in storing yeast *Pb* cell stabilates for experimental biology. Research on the most suitable freezing-thawing method, new tubes and cryoprotectors, as well as the evaluation of the biologic characteristics of parasites preserved for different periods are currently under way in our laboratory.

RESUMO

Foi demonstrada a viabilidade do uso do meio de Fava Netto na técnica de criopreservação de células de *Pb* em nitrogênio líquido.

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