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Technical Information Sheet No. 5

Cryopreservation of fungi

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Cryopreservation in liquid nitrogen (LN) is a reliable method for long-term storage of microorganisms. Different protocols have been published (Elliott 1976; Smith & Onions 1983; Kirsop & Snell 1984; Stalpers *et al.* 1987), varying with equipment, special needs, preference of materials or the type of organism. A simple method is described here, which is applicable to a large variety of microorganisms such, as mycelial fungi, molds, and yeasts.

Materials and Methods

Equipment

Different types of storage tanks which accommodate 1.0 to 1.8 ml cryotubes (e.g. Intermed NUNC, Denmark) may be employed. The 'canister and cane system' (e.g. COSMOS L40, Messer Griessheim, Germany) (Figure 1 I,K), if available, is preferred because it allows the removal of only one 'cane', holding several ampoules at a time (Figure 1 I). The storage capacity of tanks employing the 'drawer system', is greater (e.g. BT 55, Air Liquide, France), however, LN evaporation rates are higher and removal of one ampoule requires lifting of a whole set of drawers out of the storage container.

PVC (polyvinylchloride) or polypropylene 'straws' with a diameter of 2–3 mm are cut to a length of approximately 25 mm on a paper-cutting machine. One end of the PVC straws is immersed in acetone for a few seconds and is heat sealed at a temperature of approximately 280°C; other materials may be sealed in a gas flame. The sealed straws are sterilized by autoclaving (15 min, 121°C). PVC straws not treated with acetone usually reopen during sterilization.

For sealing the straws a commercial household sealing machine with adjustable temperature may be used. Forceps with specially designed tips will greatly facilitate handling of the straws.

A block accommodating the frozen cryotubes is recommended, if opening of the tubes outside of the container is necessary and thawing of the remaining straws is to be avoided. The block is made of aluminum, brass or copper and is surrounded by a styrofoam carrying case. It will keep the temperature of the cryotubes below −120°C for about 15 min.

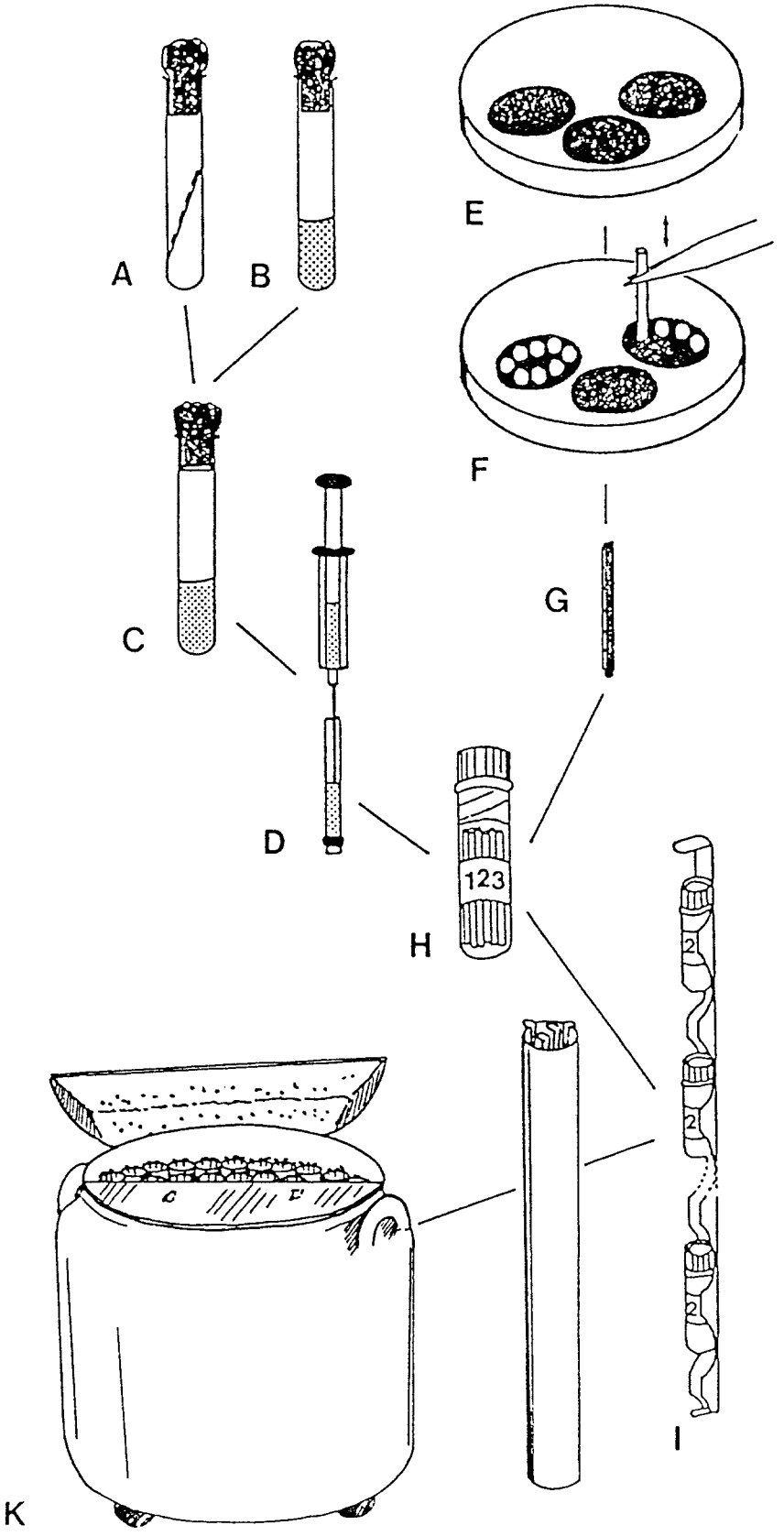

Figure 1. Cryopreservation of fungi and yeasts.

Preparation of Organisms

A schematic outline of the procedure is given in Figure 1. Yeasts are grown in liquid culture (Figure 1 B) or on a suitable solid medium (Figure 1 A) to a colony size of approximately 2 mmϕ. Two colonies of the strain are removed from the agar with a loop, carefully suspended in 1.5 ml sterile glycerol (10% w/v in water) (Figure 1 C) and put into the sterile straws (sealed at one end) with a disposable syringe or Pasteur pipette (Figure 1 D). The straws may then be sealed completely and transferred aseptically to a sterile cryotube (Figure 1 H).

Sporulating fungi are grown on solid media until conidia develop. A heavy conidial suspension is prepared in glycerol (10% w/v) which is treated as in the case of yeasts.

Mycelial fungi are grown in media supplemented with 5% (w/v) glycerol (Figure 1 E). Strains that do not tolerate the lower water activity caused by the cryoprotectant may be grown without glycerol and flooded with a 10% (w/v) glycerol solution shortly before processing. A sterile straw, open at both ends, is now used to punch the mycelium with the agar from near the margin of the colony (Figure 1 F). This procedure is repeated until the straw is completely filled (Figure 1 G). The straw is either left open at both ends and transferred aseptically to a sterile cryotube or it may be sealed.

Freezing

To obtain a freezing rate that is close to the theoretical optimum of 1–10°C per minute, the cryotubes are either transferred to a mechanical deep freezer at −70°C for 2 h in a styrofoam box with a wall thickness of 2 cm or placed in the gas phase of a liquid nitrogen tank for about 40 min.

Thawing

For revival, one straw at a time is removed from the frozen cryotube; the sealed straws are transferred into a 50 ml glass beaker with warm water (30°C), open straws filled with mycelial fungi are thawed directly on agar slants at room temperature (22 to 25°C). Sealed straws may be surface sterilized by immersion in 70% ethanol (v/v), before they are opened with sharp, sterile scissors or pincers. The cell suspension is withdrawn with a fine Pasteur pipette. Incubation is carried out at appropriate temperatures until growth is visible.

References

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