Long-term preservation of yeast cultures by liquid-drying

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Various selected strains from about 20 species of yeasts, which are reported to be sensitive to freeze-drying and liquid-drying, were successfully dried directly from the liquid phase without freezing using a simplified liquid-drying method. All tested cultures proved viable and the majority of the tested strains showed good survival rates after drying. However, different survival levels for different yeasts were observed; generally the sensitivity to drying appeared to be strain-specific. After 1 year's storage at 9°C, no further loss in viability was observed. Accelerated storage testing, for 1 week at 45°C, resulted in further loss of viability to various degrees. Yeasts that were filamentous, osmotolerant or psychrophilic appeared to be sensitive to liquid-drying and had relatively lower survival levels than the others. Growth and liquid-drying under microaerobic conditions resulted in improved survival. The dried yeast cultures proved stable and no mutation or loss in desirable characters was detected. The method can be used for the drying and long-term preservation of nearly all yeast genera.

Key words: Liquid drying, preservation, reduced conditions, survival, yeasts.

Yeasts are generally preserved in liquid N₂ and are also sometimes maintained as living cultures; simple drying has seldom proved successful (Rodrigues de Miranda & Yarrow 1972; Hieda 1981; Kirsop 1991). However, regular sub-culturing of yeasts on selective media for prolonged periods is not desirable as various studies have shown that morphological, physiological, industrial and genetic characters may be unstable under these conditions (Kirsop 1974; Kirsop & Kurtzman 1986). Although preservation of yeasts in liquid N₂ gives a high survival rate and good strain stability, the method requires a high initial capital outlay and running costs are high, particularly in tropical countries.

Although freeze-drying has been used for the preservation of yeasts for some time, reported survival rates are low and some genetic damage and mutations have been observed (Rodrigues de Miranda & Yarrow 1972; Kirsop 1991). Liquid-drying (drying directly from the liquid phase without freezing) is a convenient and effective method for the preservation of microorganisms. It has several advantages over freeze-drying and has been effectively applied for the preservation of large collections of fragile microorganisms in various culture collections (Annear 1962; Mikata et al. 1983; Malik 1990a). Liquid-drying appears successful for some species of yeasts but reported survival rates for filamentous, osmotolerant or psychrophilic strains are low (Ban no et al. 1979; Mikata et al. 1983). A simplified liquid-drying method, used for the successful preservation of microorganisms sensitive to freezing and freeze-drying (Malik 1990a) has now been adapted for the preservation of yeasts, using various protective agents and under various oxygen tensions. This paper describes the liquid-drying and long-term storage of various sensitive yeast cultures.

Material and Methods

Organisms and Growth Conditions
All yeast cultures used were strains from the German Collection of Microorganisms and Cell Cultures (DSM). The cultures were grown on appropriate media, as described in the literature or in the catalogues of strains of major culture collections (Malik 1992b).

Preparation of Protective Agents
Solutions of the most effective protective agents, such as adonitol (5% w/v), meso-inositol (5% w/v), honey (10% w/v), sodium glutamate (5% w/v) and trehalose (5% w/v), were prepared in distilled water, filter sterilized and stored at 4°C. For the preservation of osmophilic or halophilic yeasts, sugars or NaCl were used in addition to the protective agents. For the preparation
of carrier material discs, 10% (w/v) neutral, activated charcoal (medicinal grade) with 20% (w/v) skim milk was mixed with a protective agent and freeze-dried as described previously (Malik 1990a, 1992a).

Preparation of Cell Suspensions for Liquid drying

The cultures were grown until they showed good growth, old cultures were avoided. Growth of sensitive cells on a low-nutrient medium may improve survival levels (Malik 1991). A thick cell suspension (at least 10⁶ cells/ml) was prepared in an appropriate protective medium. Cells in liquid media were harvested by aseptic centrifugation for 30 min at 4000 x g and the pellet resuspended in a protective medium to yield a heavy cell suspension. The cells were equilibrated for about 20 min at 20°C with the cryoprotective agents.

Filling of Ampoules with Cell Suspensions

The ampoules, each containing a thin disc of carrier material, were kept at about 25°C for a few minutes to warm to this temperature. About 30 µl (one drop from a Pasteur pipette) of cell suspension was carefully and aseptically applied to a thin disc so as not to touch the sides of the ampoules. The ampoules were then quickly placed in metallic lids, transferred into a metallic jar maintained at 20°C in a water bath and, 20 to 30 min later, subjected to drying under vacuum (Malik 1990a). For liquid-drying under anaerobic conditions, a syringe was used to apply about 30 µl of equilibrated cell suspension to a freeze-dried disc or a mass of carrier material in a screw-capped vial (see Malik 1992a).

Liquid-drying Procedure

Liquid drying (L-drying) was according to Malik (1990a). Using a centrifugal, freeze-drying machine (Freeze-dryer, Edwards Modulyo), the primary drying was achieved in two stages without external freezing. When the condenser temperature had dropped to below -50°C, the metallic anaerobic jar, with evacuation tube, was connected to the freeze-drying chamber, the vacuum pump switched on and the air admittance valve slowly and partially closed to achieve vacuum (30 to 40 mbar). If available, a vacuum controller can be attached between the anaerobic jar or vacuum pump and the freeze-drying machine to ensure the proper vacuum.) First-step drying continued for about 2 h at about 20 to 30 mbar, followed by second-step drying at 0.1 to 0.05 mbar for about 1 h, both at about 20°C. The vacuum in the system was then replaced with sterile air (preferably N₂ gas). For determining the effect of various protective agents (Table 1), the cultures were dried to this stage. For prolonged storage (Table 2), the dried-cell preparations were subjected to secondary drying and were sealed under vacuum as double-vial preparations (Malik 1988, 1990a). For L-drying under anaerobic conditions (Table 3), the liquid-dried cultures in small gas-tight screw-cap glass vials were maintained under N₂ or Ar gas and no sealing under vacuum was necessary (Malik 1992a). Accelerated storage testing (at 45°C for 1 to 2 weeks) was used to predict the long-term storage capacity of the dried yeast cells.

Estimation of Viability and Stability Testing

Viability counts were made before L-drying, immediately after L-drying, and after storage and the percentage survival at each stage was determined. For the estimation of viability in some selected strains, a definite volume (30 µl) of cell suspension was liquid-dried and then used to make serial decimal dilutions in the appropriate liquid medium. From each serial dilution 100 µl volumes were also placed in parallel on agar plates and viability counts performed. For reproducible results, thorough mixing of the dilution tubes was important so as to eliminate clumps, especially in the case of filamentous yeasts, which would result in inaccurate counts of colony-forming units (c.f.u.). The stability of various characters was tested in some strains after L-drying and after storage, using API 50 CH. The revived cultures were also observed for mutation or changes in pigmentation or colony morphology.

Results and Discussion

Various protective agents, screened from a large number of compounds, have shown their ability to provide good protection during drying of difficult and fragile bacteria (Malik 1976, 1988, 1991). The protective effect of the most successful additives, such as adonitol (5% w/v), glutamate (5% w/v), honey (10% w/v), meso-inositol (5% w/v), and trehalose (5% w/v), was compared using a few selected strains and the new liquid-drying method of preservation (Malik 1992a). The results showed that all additives proved effective to various degrees (Table 1), efficacy varying with the yeast involved; no conclusions could be drawn as to the best protective agent for all the yeasts. Generally, viability of dried specimens after storage at low temperatures can be rapidly predicted by the accelerated storage test (Banno & Sakane 1981; Malik 1988, 1991). Dried cells were therefore kept in mini-vials (in this case, without sealing under vacuum) for 1 week at 45°C (Table 1). As Malik (1992a) observed that, at such elevated temperatures, losses in viability of dried cultures stored without sealing under vacuum are higher than those stored under vacuum, the present results probably underestimate storage life at lower temperatures.

In further studies, a wide variety of strains were liquid-dried using meso-inositol as a protective agent, a compound which has shown much success during drying of bacteria (Malik 1988, 1990a). The ampoules were sealed under vacuum and kept for accelerated storage testing. All tested strains proved viable after drying (with 10 to 100% survival) and no drastic loss of viability occurred after storage at 9°C for a year. The survival of a few selected yeasts after L-drying and during subsequent storage is shown in Table 2. The parallel viability counts showed that counts of the same samples were sometimes higher in liquid media than on agar. This phenomenon has also been observed in sensitive bacterial cultures and is presumably due to the higher surface tension of the agar media (Malik 1991).

Yeasts that were filamentous, osmotolerant or psychrophilic appeared to be more sensitive to aerobic L-drying, with relatively low survival rates. As L-drying under anaerobic conditions has proved beneficial for some aerobic microorganisms (Malik 1992a), a few sensitive yeast cultures, representing various species, were liquid-dried in the presence of meso-inositol using the anaerobic method (Malik 1992a). In almost all cases, L-drying in screw-cap