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Investigating alternative yeast storage methods

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Abstract

Several methods are used in the storage of yeasts. Storage of yeasts is important for identification studies in the laboratories. Also the stored yeasts can be used to prepare starter cultures which can be used in the manufacture of bread, wine and other alcoholic beverages. The method of storage should be such that the microorganism should be viable for a long period. There should not be any change in the genetic and morphological characteristics. In this study yeasts were stored by using different methods. Periodic subculture method on plates and slants showed that the yeasts were viable for three months and sub culturing was required after three months. The yeast cells showed higher percentage of viability in distilled water storage after six months. This is a cheap method which provides reasonably long term storage. Yeast cultures stored under mineral oil remained viable for one year. It was found to be the cheapest method which can be recommended for storage of yeasts in laboratories with limited resources for a very long time.

Keywords: yeast, storage, viable.

Introduction

There are several methods used in the storage of yeasts. It is important to preserve and maintain yeasts because they are used as starter cultures in the manufacture of bakery products and alcoholic beverages. So it is preferable to maintain a pure source of starter culture which is a valuable brewing ingredient. Yeasts also play a major role in the preparation of traditional fermented products in which starter culture is not added. Maintenance of stock cultures of yeast is essential in laboratories to use in various studies involving identification of strains and protein expression etc. Among several methods devised for maintaining yeast cultures, periodic subculture on agar slants or plates is the most common one. It is important to note that the method used should be such that the viability and the biochemical properties of the yeast culture are not lost. Previous studies done by Smith et al (1983), Smith et al (2006) and Ravimannan (2006) showed the feasibility of storing fungi and some yeasts (Kali et al, 2014) in distilled water for a few months. This is the least expensive method. Covering fungal cultures on agar slants under mineral oil was studied by Onions (1971). Though it is an old method it was found that the cultures were viable for many years. Liquid nitrogen storage is considered to be the optimum procedure for long term preservation of yeast samples in a viable state (Kockova-Kratochyflova et al, 1986). This method is very expensive. Therefore there is a need to find a long term storage method which is cheap. This study deals with finding methods to store yeast cultures for laboratory studies and starter cultures of yeasts for brewing which are most appropriate in a developing country like ours. The objective is to isolate and identify yeasts from natural sources like grains and fruits and to maintain yeasts in laboratories

easily available for various studies. This will also help produce starter cultures of yeasts whenever required for the preparation of bakery products/alcoholic beverages

Materials and Methods

(I) Isolation and identification of yeasts from natural sources

(i) Preparation of isolates from Grains/ Fruits

50g grapes were soaked in water for a few days. The isolate was prepared by taking the inoculum from the outer layer of the skin of grapes and the soaked water. These were plated on peptone yeast extract agar after preparing appropriate dilutions. Identification was based on morphological and biochemical properties using Lodder's manual (Lodder, 1970). Pure cultures were prepared by subculturing on the same media.

(ii) Preparation of isolates from Potato

50g potato was boiled and 25g sucrose was added to the mashed potato. This was allowed for a few days. Isolates were prepared by taking the inoculum from the surface of the mashed potato as well as from the water. These were plated on peptone yeast extract agar after preparing appropriate dilutions. Identification was based on morphological and biochemical properties using Lodder's manual (Lodder, 1970). Pure cultures were prepared by subculturing on the same media.

(II) Different methods of storage

(i) Periodic subculture on plates

Peptone yeast extract agar (23g/l) was prepared under sterilized conditions. The medium was poured (15ml each) into sterile petridishes. After setting, the inoculum of yeast was streaked on the surface of the medium. The plates were kept in an inverted position at 27 °C for a few days and observed for the growth of yeast. These plates were kept in a sealed plastic bag and stored in a refrigerator. The cultures were checked monthly for three months for purity, viability and morphological and biochemical properties. Viability was tested by using pour plate method. Morphological and biochemical properties were tested by following the Lodder's manual (Lodder, 1970). Subculturing was done at 3 months interval.

(ii) Periodic subculture on agar slopes

Peptone yeast extract agar (23g/l) was dispensed in Mac Cartney bottles and after the usual sterilization procedure, was allowed to set at an inclined angle to form a slope. Duplicates were prepared for each yeast strain. The yeast cultures were inoculated following the standard procedures for yeasts (Kirshop et al, 1984) and stored at 4 °C. The cultures were checked monthly for three months for purity, viability and morphological and biochemical properties. Viability was tested by using pour plate method. Morphological and biochemical properties were tested by following the Lodder's manual.(Lodder, 1970). Subculturing was done at 3 months interval.

(iii) Storage in distilled water

Mac Cartney bottles were sterilized after filling with distilled water. The discs were cut by using a cork borer from peptone yeast extract agar plates. A loopful of inocula were added onto the surface of the media discs and stored at 4 °C after labeling. This can also be done by taking a small amount of yeast colony from a plate and transferring into the Mac Cartney bottle such that the suspension inside the tube become only slightly blurred and not milky. The cultures were checked monthly for three months for purity, viability and morphological and biochemical properties. Viability was tested by using pour plate method. Morphological and biochemical properties were tested by following the Lodder's manual (Lodder, 1970). The transferring of the discs was done at few months interval depending on the observations made on viability, purity etc.

(iv) Storage under mineral oil

Peptone yeast extract agar (23g/l) was dispensed in Mac Cartney bottles and after the usual sterilization procedure, was allowed to set at an inclined angle to form a slope. Duplicates were prepared for each yeast culture. Each of the culture was inoculated onto peptone yeast extract agar slant by using a sterile wire loop separately in zig-zag manner. The oil was sterilized by heating at 121 ˚C, 15 lb/inch^2 followed by drying at 170 ˚C for 1-2 hrs. The cultures were covered by mineral oil (liquid paraffin) up to 1 cm level. To prevent the contamination of oil from spores blown out from cultures while pouring oil into the cultures, small quantities of the oil in individual containers were autoclaved and added as single doses. These were stored at room temp. Retrieval was done by removing a small piece of yeast colony with a

sterile loop, draining off the oil and streaking the inoculum into a plate or tube containing peptone yeast extract agar medium whenever required.

(III) Preparation of starter cultures

Starter culture of yeast was prepared from agar plates under storage. Sterile wort was prepared (volumes 70ml, 500ml, 3.8L). A single colony was taken by a sterile loop from the surface of the plate and immersed in the sterile wort and swirled well. The conical flasks were capped loosely and kept at 27 °C for 48hrs. It was observed for the sedimentation of yeasts at the bottom of the conical flasks. This is used as the source for successive starters.

Results and Discussion

Four different *Saccharomyces* sp were isolated and pure cultures were made. Shelf life is limited when storing yeasts on petridishes or slants in the refrigerator. It has to be subcultured after 3 months. The viability of the yeasts stored under distilled water was tested after 6 months and the yeast count showed that percentage of viable cells varied between 65-70% in all the subcultures. Cultures stored under mineral oil require transfer after a long period of inoculation due to reduced growth and metabolic activities. As it is covered by mineral oil there is reduced oxygen tension. The sterilized mineral oil does not allow oxygen or moisture to penetrate. Thus, sterilized condition is maintained. Some difficulties arise while subculturing in a tropical country like ours. In the tropics, contamination of culture media during isolation, subculturing etc is often more likely to occur than in temperate areas, partly because of the large amount of dust often present in the air and also due to air movement which may occur because of open windows. All the yeast cultures stored under mineral oil appeared in good condition after one year. Many of them showed a white to tan filamentous growth extending from the surface of the slant into the mineral oil. When observed under the microscope, this growth was found to be made up of some single cells. Upon subculture on peptone yeast extract agar medium, growth appeared within 24 hrs and almost all the subcultures showed abundant growth in 48 hrs. The microscopic examination of the yeast cultures and the biochemical tests agreed with the observation made before. Distilled water storage appears promising, fairly stable and more pleasant to handle though survival is for a shorter period when compared to mineral oil storage. This study will significantly reduce the cost of yeast storage which is appropriate for a developing country like ours.

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