Review Article

STABILITY OF CANDIDA ALBICANS OVER LONG AND SHORT TERM STORAGE IN A RESOURCE-LIMITED SETTING

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ABSTRACT

BACKGROUND: Candida albicans are widely isolated fungal yeast agents from clinical samples. Several storage methods for fungi have evolved overtime and they are not without setbacks. Preservation method is critical for research, training and teaching. In resource–poor setting, the method to employ must be cheap and easy to maintain with minimal risk of contamination as well as degeneration of the organisms. We thus, set out to study the stability of Candida albicans over long and short term storage in a resource-limited setting.

METHODS: One hundred Candida albicans strains isolated from patients with vulvovaginal candidiasis and oral candidiasis were preserved in triplicates using sterile distilled water, Chromagar plate, mineral oil overlay and brain heart infusion broth plus 10% glycerol at -20OC. Recovery rates were determined at six months, 12 months and 18 months by sub-culturing onto sabouraud dextrose agar.

RESULT: The recovery rate of C. albicans was 100% for all the preservation methods used during the six months storage, mineral oil overlay and brain heart infusion broth plus 10% glycerol for the 12months storage, and only the brain heart infusion broth plus 10% glycerol during the 18months storage.

CONCLUSION: Candida albicans can be preserved over long period of time in resource- limited setting where power supply is erratic using brain heart infusion broth plus 10% glycerol at -20OC and mineral oil overlay technique whereas for short term preservation, sterile distilled water and taped culture plates technique can be used. Preservation of Candida albicans isolates in resource- limited setting over short or long term period is possible and affordable depending on the technique employed.

KEYWORDS: Candida albicans, preservation, sterile distilled water, Chromagar plate, mineral oil overlay, brain heart infusion broth.

NigerJMed2017: 163-166

INTRODUCTION

Some fungal agents are emerging pathogens in several parts of the world[1]. Fungi could be moulds or yeasts. Yeasts can easily be lost due to poor storage techniques[2-4]. Candida albicans are the most widely isolated of all the yeasts and well studied. It is implicated in many clinical infections to include vulvovaginitis[5]. Cultures when properly done, will determine the starting point of isolating fungal agents. The importance of having a fungal culture collection is not only for preservation of endangered fungal agents but also as a principal source of material for teaching and research. Serial subcultures requiring transfer from staled to fresh media were used in the past but this method is not without its setbacks[3,4]. It is not practicable for storing large number of samples, it is labourious, prone to contamination, and does not prevent fungal cell degeneration[3,4]. Several storage methods evolved over time and these are not without their problems. Their main feature is suppression of growth and metabolism to almost negligible level[3,4]. This study sets out to look at the stability of Candida albicans over long and short term storage in a resource-limited setting.

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Methods:
The studied yeasts were 100 Candida albicans isolated from patients with vulvovaginal candidiasis and oral candidiasis. They were preserved in triplicates using distilled water, chromagar plate, mineral oil overlay and brain heart infusion broth plus glycerol at -20^{o}C. Recovery rates were carried out at six months, 12 months and 18 months of storage using a positive recovery to be a minimum of 500 colonies per plate or colony count of minimum 500 per plate.

Sterile Distilled Water:
Three hundred bijou bottles containing five milliliters (5ml) of sterile distilled water were inoculated with three colonies of Candida albicans (C. albicans). These were incubated for three hours and then stored in the locker. Recovery rate was determined at six months, 12 months and 18 months by sub-culturing onto plates of sabouraud dextrose agar (SDA) and incubating at 37OC for 18hours after which colony count was carried out and results documented (Figure I).

Chrom Agar plate:
Each plate of Chromagar media was divided into four and the organisms were inoculated onto the media, and incubated at 37^{o}C for 48 hours following manufacturer's instructions. The edge of the plates were taped using masking tape and they were kept safely in the locker. At six months after inoculation a set of 100 were sub-cultured onto SDA and they grew well. At 12months the media had dried so the set of 100 were obtained by using sterile blades and cutting piece of the medium and putting in cryovials containing brain heart infusion broth. The cryovials were incubated for 18hours at 37^{o}C and the broth sub cultured onto SDA and incubated at 37^{o}C for 18hours. The colony count was then carried out to determine the recovery rate. The process was carried out on the last 100 set of organisms on the Chromagar plate at 18months of storage and result documented (Figure I).

Mineral Oil Overlay:
Sabouraud dextrose agar slants were prepared in three sets of 100 each of bijou bottles and inoculated with Candida albicans. These were incubated at 37^{o}C for 18hours after which mineral oil was poured to a level above the agar medium and they were stored safely in the locker. A set of 100 each were sub-cultured at six months, 12 months and 18 months respectively to determine the recovery rate using colony count of 500 and above as positive recovery.

Brain heart infusion broth plus 10% Glycerol at -20^{o}C:
Glycerol was added to brain heart infusion broth at a ratio of 1:10 and dispensed into 300 cryovials. Each of the cryovials was inoculated with three colonies of C. albicans and incubated for five hours at 37^{o}C. They were then stored in the freezer at -20^{o}C. A set of 100 each were thawed and inoculated onto SDA at six months, 12 months and 18 months of storage in the freezer. Recovery rate was determined based on colony count of 500 and above.

RESULTS:
In the chart for the recovery rate of C. albicans during the storage periods of six months, 12 months and 18 months, colony counts of 500 and above was used to determine the recovery rate which accounts for the viability of the organisms.

Based on the result shown on the chart, the recovery rate of C. albicans during the six months storage was 100% for all the preservation method used. For the 12 months storage only the mineral oil overlay and brain heart infusion broth + glycerol gave 100% recovery rate whereas chromagar plate method was the least (92%). During the 18 months storage of C. albicans, only the brain heart infusion broth + glycerol had 100% recovery rate whereas the chrom agar plate method had the least (89%). C. albicans isolates can be preserved over long period of time using brain heart infusion broth plus 10% glycerol at -20^{o}C and mineral oil overlay technique. For short term preservation, sterile distilled water and taped culture plates can be used.

It was also observed that phenotypic characteristics of C. albicans were maintained throughout the period of storage.

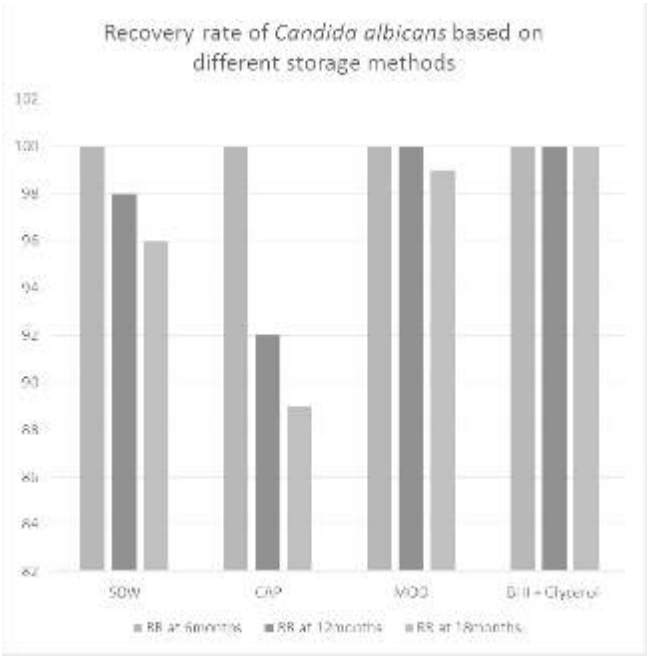

Figure I:
SDW= Sterile distilled water
CAP= Chrom agar plate
MOO= Mineral oil overlay
BHI= Brain heart infusion broth
RR= Recovery rate.

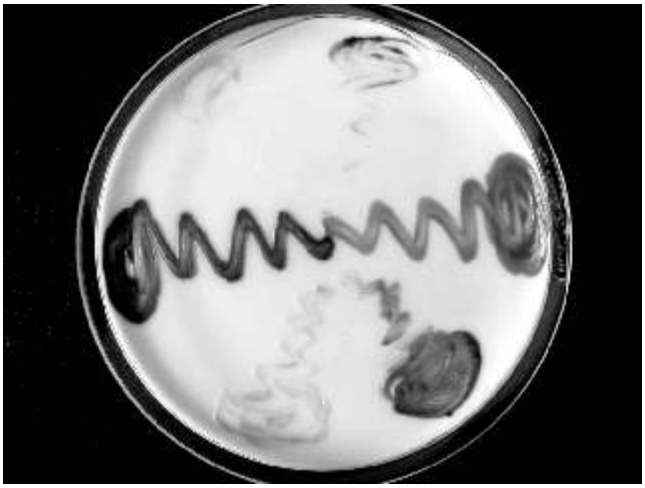

Plate I: shows different species of Candida on a Chromogenic medium

DISCUSSION:

Most yeasts to include C. albicans grow very fast in culture media when compared with molds. The growth may be contaminated with other fungi or even bacteria thus affecting the preservation of colonies[3-6]. Preservation of yeasts is very important for long time use particularly in teaching, training and research, so it is essential to develop an easy and cheap technique for its preservation. Most of the previous work for long term preservation of fungi was done through cryopreservation method using liquid nitrogen and commonly 5-10% of glycerol was used as cryoprotectant[6].

In this study, brain heart infusion broth, Chromagar candida plate, mineral oil overlay and sterile distilled water was used for C. albicans preservation.

Long term preservation and frozen effect on C. albicans cultures, can result in loss of their regeneration ability leading to loss of recovery or viability[6&7]. The addition of glycerol plays very significant role and acts not only as cryoprotectant for C. albicans but also maintains viability and regenerative potential [7].

This study found preservation at -20OC plus 10% glycerol to be very effective for C. albicans for a period of 18 months. This also supports the findings by other documented studies [7-10]. The glycerol thus protects the organism from degeneration as a result of power outage, hence supports the fact that this method can be used in resource limited countries where power supply is very poor.

The mineral oil overlay technique is equally good for C. albicans preservation in resource limited environment but has the following disadvantages: requires large space to keep the bottles, it may be contaminated by other fungi or even bacteria. In this study, only one C. albicans was lost; hence this method if properly carried out is very efficient for long term preservation of C. albicans. Similar findings was reported in other studies[11&12].

The Chromagar plate can be used for short term preservation of C. albicans. The major setback with this method is drying of the media on the plates resulting in loss of viability of the organisms. The finding in this study was corroborated by studies by kitamoto and colleagues [13].

Sterile distilled water method is also good for preservation of C. albicans in resource poor setting because it is cheap and easy to carry out but requires large space to keep the bottles, and can become contaminated during addition of top up distilled water to the bottles [14].

Preservation of C. albicans isolates in resource poor setting over short term or long term period is possible and affordable depending on the technique employed. The organisms in this study maintained their phenotypic characteristics during the six months, 12 months and 18 months period of the study.

CONCLUSION

Based on this research, C. albicans can be preserved over short or long periods in resource poor settings without alterations in their phenotypic characteristics. The use of sterile distilled water, mineral oil overlay, CHROMagar Candida plate, and Brain heart infusion broth plus 10% glycerol are very efficient for C. albicans preservation in resource-poor setting.

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