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A COST-EFFECTIVE TECHNIQUE OF YEAST STOCK CULTURE MAINTENANCE IN LABORATORY

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ABSTRACT

Maintenance of stock cultures of yeast is essential in all mycology laboratories for quality control. Among several methods proposed for maintaining yeast culture collections, periodic subculture on agar slopes is most commonly adopted. In an attempt to reduce cost of preservation of yeast cultures, we evaluated a simple modification of agar slope stock culture technique where 20 strains of yeasts were inoculated in 20 separate quadrants on Sabouraud Dextrose agar plates. In this study, performance of conventional and modified method were compared for 93 yeast strains. We found this modified method allowed simultaneous short term preservation of 20 isolates without compromising purity, viability and classic properties and showed results comparable to conventional agar slope stock culture. This approach will significantly reduce the cost of yeast culture maintenance which is especially relevant in developing countries.

KEYWORDS: **Candida; Stock culture; Yeast preservation**

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INTRODUCTION

Maintenance of yeast stock cultures is a critical and essential task in mycology laboratories. It has crucial role in diagnostics as well as in research for validation of new methods and quality control procedures for culture media and antifungal susceptibility tests.[1] However, it is often neglected. Among several methods of yeast stock culture maintenance currently in use, lyophilisation and cryo-preservation in liquid nitrogen are best options.[2] Since most small and medium laboratories in developing countries cannot afford costly equipment, infrastructure and skilled personnel required for these techniques, serial subculture on agar slopes is used most commonly.[3] However, it has inherent limitations. Apart from altered characters, contamination and loss of viability, it also has potential limitations like labeling errors pertaining to the complexity of handling several glassware and cultures.[4] Hence, there is need of a novel method which can overcome these limitations. In this study we describe a cost-effective simple modification of yeast culture maintenance on agar medium.

METHODOLOGY

This study was carried out in the department of Microbiology of a tertiary care hospital in south India. Six standard laboratory strains and eighty seven clinical isolates of yeasts recovered during June, 2010 - March, 2011 from respiratory samples *viz.* sputum, broncho-alveolar lavage & endotracheal tube aspirate, were included in this study. We have maintained a culture collection of 93 yeast isolates comprising of *Candida albicans, Candida tropicalis, Candida glabrata, Candida parapsilosis, Candida krusei, Cryptococcus neoformans* and *Geotrichum candidum* in duplicate by periodic subculture on both Sabouraud Dextrose agar (SDA) slopes and plates over 8 months. Both sets of stock cultures were checked monthly for 3 months for purity, viability and morphological as well as biochemical properties.

1. Isolation & identification of yeasts

All respiratory samples were cultured on blood agar, MacConkey's agar and SDA. Yeast like colonies were processed in the laboratory following standard procedures. *Candida* isolates were identified up to species level by gram stain, germ tube test, colony morphology on SDA, cornmeal agar, HiCrome Candida agar (HiMedia, Mumbai, India) and carbohydrate fermentation tests. Demonstration of capsule in India ink preparation, urease test and brown pigment production in sunflower seed agar were the fundamental properties utilized for identification of *Cryptococcus neoformans.* Characteristic gram stain, colony morphology on SDA and demonstration of hyaline arthroconidia without disjunctor cells helped in differentiation of *Geotrichum candidum* from other yeasts.

2. Modified method of yeast culture maintenance

SDA medium of 5 mm thickness was prepared in 90 mm diameter glass petri plates. The petri plates were demarcated with glass marking pen into 20 quadrants and different strains of yeasts were inoculated in each of these quadrants with proper labeling (Figure 1). A gap of minimum 5-7 mm was kept between two strains inoculated in adjacent quadrants. The petri plates were incubated at 30^{O}C for 2-5 days to obtain optimum growth and stored at 4^{O}C after sealing it with plastic paraffin tape (ParafilmM sealing film, SPI Supplies, West Chester, USA).

Yeast culture maintenance on SDA plate

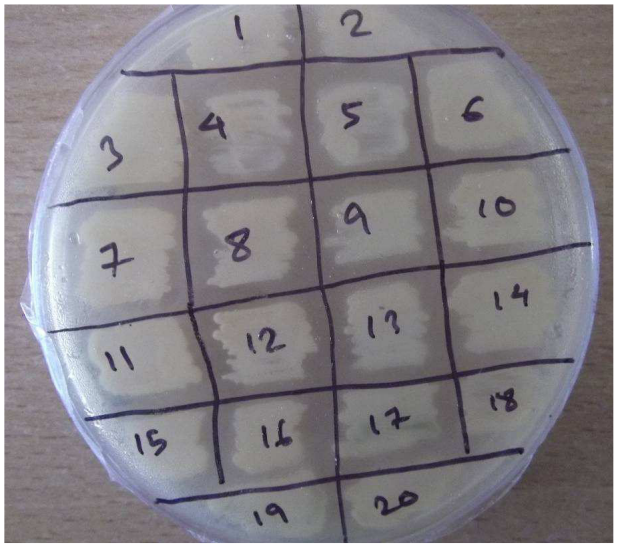

Figure 1
SDA plate with confluent growth of yeast isolates which were preserved at 4^{O}C.

3. Conventional method of yeast culture maintenance

Same isolates were also maintained by the conventional method of culture on SDA slopes in 18 × 150 mm glass test tubes. All isolates were inoculated in separate SDA slopes which were properly labeled, incubated and preserved at 4^{O}C after sealing.

4. Quality Check for purity, viability and morphological and biochemical properties

Viability and purity of yeast isolates were tested by subculture on SDA following overnight incubation in Sabouraud Dextrose broth. In addition to this, the number of viable yeast cells was determined by pour plate method as suggested by Nyanga *et al*.[5] One loopful of colony suspended in 1 ml of sterile saline was used for pour plate method with appropriate dilutions. After 48 hour incubation at 30^{O}C, the number of colonies in inoculated plates was counted and the viable count was noted in terms of colony forming units per ml (CFU/ ml). The Morphological as well as biochemical properties were evaluated by gram stain, urease test, growth characteristics on cornmeal agar and carbohydrate fermentation.

5. Quality Control

C. albicans ATCC 611098 and *C. tropicalis* ATCC 13803 were used as controls for yeast growth characteristics.

RESULTS

We found, 100% viability without contamination in both methods in first 2 months. Although the cultures were pure, at the end of 3rd month, three *C. tropicalis*, one *C. albicans* and one *C. glabrata* strain failed to grow from stock culture on SDA plate. Whereas, two strains of *C. tropicalis* and *C. albicans* each were not viable from SDA slope cultures. The survival of the yeast cells was determined by pour plate method immediately before storing the optimum growth of yeast at 4^{O}C and then once in a month for 3 months. There was a constant reduction in the number of viable cells over 3 months of storage in both methods of yeast culture maintenance (table 1). No substantial alterations of morphological as well as biochemical characters were noted in these isolates.

Table 1
Change in mean number of viable yeast cells over 3 months of preservation at 4^{O}C

	Before storing at 4^{O}C (CFU/ml)	After 1 month of preservation at 4^{O}C (CFU/ml)	After 2 months of preservation at 4^{O}C (CFU/ml)	After 3 months of preservation at 4^{O}C (CFU/ml)
Yeast stock cultures on SDA slopes	4.2×10^4	3.7×10^4	2.3×10^4	8.6×10^3
Yeast stock cultures on SDA plates	4.8×10^4	3.9×10^4	2.1×10^4	7.9×10^3

DISCUSSION

Infections caused by yeast are becoming increasingly common in recent years due to substantial increase in number of patients with suppressed immunity as a result of HIV infection, advances in cancer chemotherapy and immunosuppressive therapy for organ transplantation. Not only *C. albicans*, other yeast species have emerged as a significant problem in healthcare.[6-8] Non albicans candida infections have been reported in neonatal infections, nosocomial bloodstream infections, co-infection in pulmonary tuberculosis patients, prosthetic devices and indwelling catheter associated infections.[7, 9-11] Therefore, the identification and speciation of clinical yeast isolates have turned out to be a major concern for both clinicians as well as Microbiologists. Validation of results of biochemical and antifungal susceptibility tests require reference strains for quality control which should be maintained properly without compromising its viability and classic properties. Moreover, clinical isolates need to be preserved for research purpose. Castellani introduced a method of preservation of fungal isolates in water at room temperature in 1939.[1] Although this is easy and economical procedure, it does not ensure the stability of fungal cells.[2] To overcome this limitation other methods have been proposed *viz.* preservation in soil or on slants overlaid with mineral oil, dehydrated gelatin drops, periodic subculture on SDA slopes, lyophilisation and cryopreservation either in liquid nitrogen or at a very low temperature.[2, 12-15] Although lyophilisation and cryo-preservation are the best method for long term preservation of culture collections, most laboratories in resource limited settings practice serial subculture on agar slopes.[3] However, it involves handling of large number of glassware and cultures which are not only space occupying, but also may result in labeling errors.[4] SDA plate method allowed simultaneous short term preservation of 20 isolates with no substantial difference purity, viability and morphological and biochemical results compared to the conventional method. Even though all stock cultures had reduction in number of viable yeast cells over time, there was no significant difference between the conventional and modified method. The proportion of viable cells in most isolates was sufficient for maintaining it in subculture within 3 months. Only three *C. tropicalis*, one *C. albicans* and one *C. glabrata* strain from stock culture on SDA plate and two *C. tropicalis* and two *C. albicans* strains from SDA slope cultures respectively failed to grow on subculture at the end of 3rd month. These strains had significant reduction in the colony count during 2nd month compared to the mean value in both techniques. While inoculating the plate, care was taken to ensure a separation of 5-7 mm between two adjacent strains to avoid overlapping growth. Thick agar medium and sealed preparation prevented quick drying of yeast colonies. Substitution of one petri plate for 20 test tubes in this modified method not only reduced the volume of culture media, number of glassware, storage space for stock cultures, but also resulted in substantial decrease in expenditure. As per our estimate, this modified technique will reduce the cost of maintenance of 20 yeast strains by conventional method from 3.95 USD (260 INR) to 1.35 USD (89 INR). The limitation of this study is the smaller number of isolates

examined. Therefore, these observations should be confirmed by further studies.

CONCLUSION

Maintenance of stock cultures multiple yeast strains on agar plate is a simple and cost-effective technique which also precludes complexities of handling several glassware and cultures. It may be beneficial in resource limited setups and may be considered for short term preservation of yeast culture collections.

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