Original Article

Microbiology

Comparison of Different Media for the Pigment Production of *Cryptococcus neoformans*

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ABSTRACT

Background: Melanin production by their phenoloxidase activity is a distinctive property of the *Cryptococcus neoformans* isolates. An agar medium which contains a precursor of melanin is used to test the pigment production by *C.neoformans.*

Purpose: This study aimed to compare the pigment production of *C.neoformans* on various media.

Materials and Methods: Twenty strains of *C.neoformans* which were obtained from various clinical samples were inoculated on different media and observed for the rate of growth and pigment production.

Results: Sunflower seed agar was the best medium, with the mean day of growth and the pigment production being 1.25 and 2.8 respectively. The least suitable medium for the observation of pigments in our study was mustard seed agar.

Conclusion: Sunflower seed agar is a simple and inexpensive tool for the presumptive identification of *C.neoformans* in clinical microbiology laboratories.

Key Words: *Cryptococcus neoformans,* melanin production, phenoloxidase, sunflower seed agar, mustard seed agar

INTRODUCTION

Cryptococcosis is an acute, subacute or chronic fungal disease which is caused by encapsulated basidiomycetous yeasts which belong to the genus, *Cryptococcus* [1]. This yeast is a particularly fascinating fungal pathogen, because it crosses the entire spectrum of the host immunity. For instance, it produces infections in apparently immunocompetent patients without any known underlying disease, but on the other hand, commonly the yeast invades a severely immunosuppressed host as a result of HIV infection, organ transplantation or malignancy or due to treatment with high doses of corticosteroids [2].

Cryptococcosis is one of the Acquired Immuno deficiency Syndrome (AIDS) defining illnesses [3].

The incidence of cryptococcosis has risen dramatically over the past 20 years. The Human Immunodeficiency Virus (HIV) epidemic and other forms of immunosuppression are the common factors which explain this increase. *Cryptococcus neoformans var. neoformans* (*C.neoformans*) is the species which has been predominantly reported from immuno-compromised patients [4].

C.neoformans has several well-characterized virulence phenotypes. The three classical phenotypes which were under genetic control have been: (1) capsule production (2) melanin formation; and (3) the ability of a yeast strain to grow at 37°C [5]. Melanin production by their phenoloxidase activity is a distinctive property of the *C.neoformans* isolates. The ability to produce these melanin pigments is one of the most used criteriae for the identification of *C.neoformans* from clinical and environmental isolates and for the evaluation of *Cryptyococcus* virulence [6,7]. It has been suggested that a primary mechanism for melanin's importance is its capacity to act as an antioxidant, but there are other possible mechanisms by which the yeast might use melanin for protection from the host, including cell wall integrity and charge, interference with antifungal susceptibility, abrogating antibody-mediated phagocytosis, and protection from extreme temperatures [8].

Melanin production is usually tested in a proper agar medium which contains a precursor of melanin. For this purpose, agar media which contain L-dopa [9], caffeic acid [10], bird seed [11,12], sunflower extracts [13], tobacco [14,15], henna [16] and mustard seed [17] have been reported to be used so far. This study was undertaken to compare the pigment production of *C.neoformans* on various media.

MATERIALS AND METHODS

This study was carried out in the Department of Microbiology of this medical college. A total of 20 *C. neoformans isolates* which were obtained from various clinical samples, which were received in the Mycology section were included in the study. The definitive identification of *C. neoformans* was done on the basis of: [18]

- Growth at 37^0 C.
- Hydrolysis of Christensen's urea agar.
- Inositol and nitrate assimilation.
- Production of brown pigment.

The pigment production of *C.neoformams* was observed on the following media:

1. Niger seed agar.
2. Sunflower seed agar.
3. Tobacco agar.
4. Mustard seed agar.
5. Henna agar.

All the media were prepared in the media section of the laboratory as per the techniques which are described by other workers [11, 13, 14, 16, 17].

The media were inoculated with *C .neoformans* and incubated at 37°C for a period of 2 weeks. The plates were observed daily for growth and pigment production. *Candida albicans* was used as a negative control.

RESULTS

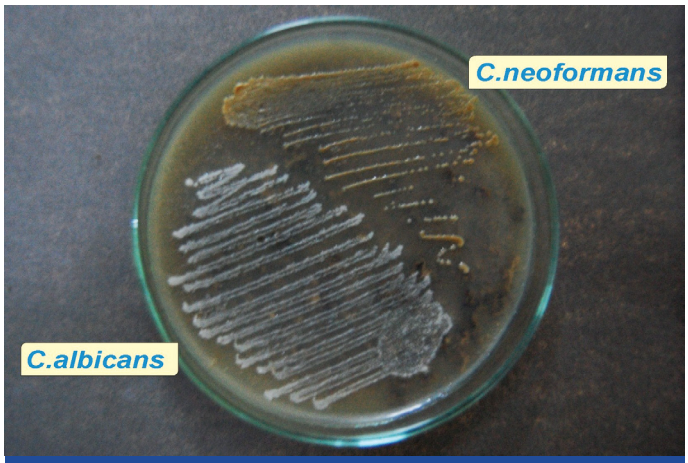
G/P	Sunflower seed agar	Mustard seed agar	Henna agar	Niger seed agar	Tobacco agar
1/2	11	–	–	–	–
1/3	04	–	–	–	–
2/3	05	–	15	14	15
3/4	–	17	03	01	05
4/5	–	03	01	04	–
5/6	–	–	01	01	–

[Table/Fig-1]: Number wise distribution of *C.neoformans* in different media with respect to growth (G) and pigment (P) production.
G = Day that growth was first observed
P = Day that *C.neoformans* produced brown pigment.

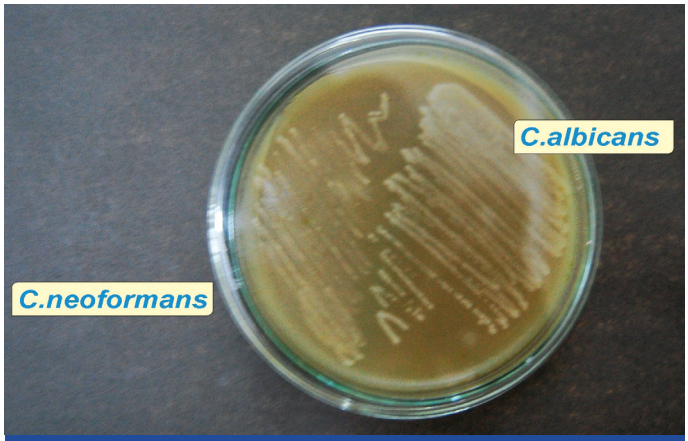
15 isolates of *C.neoformans* showed growth on the first day on sunflower seed agar, whereas 11 isolates showed pigment production on the second day.

Media	Day of growth	Day of pigment
Sunflower seed agar	1.25	2.8
Mustard seed agar	2.6	3.95
Henna agar	2.45	3.3
Niger seed agar	2.6	3.6
Tobacco seed agar	2.25	3.25

[Table/Fig-2]: Mean day of growth and pigment production of *C.neoformans* on various media



[Table/Fig-3]: Figure showing brown pigment of *C.neoformans* on Sunflower seed agar



[Table/Fig-4]: Figure showing brown pigment of *C.neoformans* on Mustard seed agar

DISCUSSION

The need for the rapid and accurate identification of *C.neoformans* in the clinical laboratories has in recent years, led to the development of many identification tests which are based on the phenoloxidase enzyme activity of the organisms. The concept of using different media for the identification of *C.neoformans* is not new, but problems like elevated costs, complex media preparation, the time which is required for the growth and pigment production and ill-defined interpretations are frequently encountered.

Sunflower seed agar, which is prepared from pulverised sunflower seed, is the best medium for the early growth and pigment production of *C.neoformans.* In our study, most of the isolates produced a brown pigment within 48 hours. Khan et al have also reported the production of brown colonies of *C.neoformans* on this medium [19]. Since sunflower seed agar is known to impart a brown pigmentation to the *C.neoformans* colonies, it has been utilized for the isolation and presumptive identification of *C.neoformans* from saprophytic and clinical sources [20]. It is easy to prepare and inexpensive and the contents are readily available in the market. Sunflower is one of the major crops which are cultivated in Maharashtra.

Tobacco agar is the next suitable medium for the appreciation of the brown pigment of *C.neoformans.* Tobacco, which is used in this medium, is also widely available over the counter in India. A maximum number of isolates showed pigments on this media within 72 hours. Tendolkar et al also reported the same finding, where all the isolates produced a brown pigment within 48-72 hours of their incubation [14]. Khan et al reported the appearance of dark brown pigmented colonies of *C.neoformans* on this medium after 72 hours of incubation [15]. In our study, most of the strains of *C.neoformans* produced a brown pigment on henna agar within 72 hours of incubation. Nandhakumar et al have reported that all isolates of *C.neoformans* produced a brown pigment on this medium at 24 hours post inoculation [16]. In this study, the rate of growth and pigment production of *C.neoformans* was late or poor on mustard seed agar, which was in contrast to the observations of Nandhakumar et al, who reported the brown colour effect of *C. neoformans* at 48 hours post inoculation [17]. Since the concept of using mustard seed agar for the appreciation of the pigments of this yeast is new, further evaluation of this medium by using more strains of *C.neoformans* is required to confirm its utility for the diagnosis of cryptococcosis.

It can be concluded from this study, that of the various media which were used for the pigment production of *C.neoformans,* sunflower seed agar was the most valuable tool for the selective isolation and presumptive identification of *C.neoformans.*

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