

Can Pal Sunflower Seed Medium be Considered as a Simple Inexpensive Tool for the Study of *Candida* Species?

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Fungi, which include moulds, yeasts and dimorphic fungi are eukaryotic, heterophilic and versatile group of living organisms, have the potential to cause infections in humans and animals [1]. There are around 5 million species of fungi that are widespread in the nature, of which about 600 are found to be associated with several clinical disorders of in humans and animals [2]. Fungi are isolated from a wide variety of sources, such as the soil, avian excreta, air, water, sewage, bat guano, vegetables, fruits, woods, grains etc. [1,3,4]. There are evidences to believe that humans and animals have an equal opportunity to acquire the zoopathogenic fungi from the immediate environment. Fungal infections, such as aspergillosis, blastomycosis, candidiasis, chromoblastomycosis, coccidioidomycosis, cryptococcosis, fusariosis, geotrichosis, maduromycosis, phaeohiphomycosis, rhodotoruliosis, trichosporonosis, and zygomycosis cause morbidity and mortality among the susceptible hosts. These mycoses are reported to occur in both sexes, in all age groups, in all seasons, in rural and urban settings, in all seasons, and in developing as well as developed nations of the world [1,4,5]. Mycotic diseases, which can occur in sporadic and epidemic form, remain a hidden threat to the immunocompromised and immunocompetent persons throughout the world. A number of factors, such as diabetes mellitus, tuberculosis, HIV/AIDS, cancer, COVID-19, neutropenia, haematological malignancies, chronic kidney disease, besides organ transplantation, prolonged use of antibiotics, corticosteroids, cytotoxic drugs etc. are known to predispose the subjects to fungal infections by decreasing the body immune response [1,6,7].

The genus *Candida* that contains about 200 species. Some of the species of *Candida* include *C. albicans*, *C. guilliermondii*, *C. kefyr*, *C. krusei*, *C. lipolytica*, *C. lusitanae*, *C. parapsilosis*, *C. rugosa*, *C. stellatoidea*, *C. tropicalis*, *C. utilis* and *C. viswanathii* [1,5]. Among these species, *Candida albicans* is the most frequently implicated in the etiology of human and animal candidiasis [1]. Recently, *Candida auris* has emerged as a highly virulent species of *Candida* that carries 30 to 40% mortality [7]. *Candida* is a yeast that occurs as a commensal in humans and animals. The author is credited to report for the first time the isolation of *C. tropicalis* from the lung empyema fluid of an immunocompromised patient [8]. *Candida* species can invade the skin, nail, ear, eye, and several internal organs. Human infections caused by *Candida* include stomatitis, esophagitis, vulvovaginitis, diaper dermatitis, balanitis, endophthalmitis, arthritis, osteomyelitis, otitis, keratitis, endocarditis, pneumonia, and meningitis [1,5]. In addition, *Candida* species are an important cause of nosocomial infections, which can be life-threatening in the patients admitted to the hospitals [5]. In animals, mastitis, endometritis, abortion, pyothorax, dermatitis, gastritis, and vulvovaginitis have been described [1,5].

The isolation of microorganism in pure form from clinical specimens of the patient is imperative to establish an unequivocal diagnosis of a disease. Different types of media, such as blood agar, nutrient agar, brain heart infusion agar, MacConkey agar, eosin methylene agar, brilliant green agar, Sabouraud dextrose agar, mycosal agar Lowenstein Jensen medium, Fletcher semisolid medium etc. are employed for the isolation of microbes from diverse types of clinical specimens in the public health and microbiology laboratories of the world [1,2]. The early and correct diagnosis and prompt treatment is highly pertinent to mitigate the sufferings of the patients [1].

Sabouraud medium is widely used in the mycology laboratory for the isolation of fungi. The growth of *Cryptococcus neoformans* on Sabouraud medium cannot be easily differentiated from other yeasts like *Candida*, *Trichosporon* etc. Hence, sunflower (*Helianthus annuus*) seed was utilized by Pal in 1980 to develop a selective medium for *Cryptococcus neoformans*, which cause a life-threatening infection in humans as well as in animals [6,7]. The medium named as Pal sunflower seed agar (Pal medium) contains 45g of pulverized sunflower seed, 20g agar power, 100 mg chloramphenicol and 1000 ml distilled water [1,9]. *Cryptococcus neoformans* imparts brown coloured colonies on Pal medium (Figure 1) that aids in early recognition of this pathogenic yeast, and thereby, helps the physician to institute the therapy to save the life of the patients. The efficacy of Pal medium for the rapid isolation of *Cr. neoformans* from different types of clinical samples have been investigated earlier [3,6,10,11].

The known strains of *C. auris*, *C. dubliniensis*, *C. glabrata*, *guilliermondii*, *C. kefyr*, *C. parapsilosis*, *C. tropicalis*, *C. utilis* and *Teunomyces (Candida) krusei* were inoculated on Pal medium to see whether this medium can support the growth of *Candida* species. Interestingly, all the inoculated species of *Candida* grew well on Pal medium by producing whitish/cream colonies (Figure 1). *Candida* species failed to produce brown pigmented colonies on Pal medium. It is important to mention that Pal medium is cheaper than Sabouraud agar.



Figure 1: *Candida* species inoculated at the periphery of Pal medium plate showed whitish cream colonies whereas *Cryptococcus neoformans* growth in the centre exhibited brown colour. Note that none of the species of *Candida* yielded brown pigment growth on

Pal medium.

Source: [6].

It is hoped that scientists and researchers working in the microbiology and public health laboratories of the world will find Pal medium an excellent easily available, simple and less expensive tool for the study of *Candida albicans* and also other species of *Candida* including *Candida auris*, a very recently emerging highly virulent pathogen.

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