Mating of Cryptococcus neoformans var. grubii on Eucalyptus camaldulensis Woody Debris

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Abstract

Cryptococcus neoformans (San Felice Vuillemin 1895) Vuillemin 1901, is a globally distributed human fungal pathogen that causes life-threatening meningoencephalitis in immunocompromised patients. Microscopically, most clinical isolates appear as spherical, budding, encapsulated yeast cells in both tissue and culture [1-3]. The most common and serious clinical manifestation of infection is meningoencephalitis, which occurs with any of the serotypes. Cryptococcosis is acquired by inhalation of airborne cells of C. neoformans var. grubii from the environment, but the source and nature of the infectious propagules have not been resolved [3].

C. neoformans is classified into three serotypes based on capsular agglutination reactions: serotype A (C. neoformans var. grubii), serotype D (C. neoformans var. neoformans), and AD hybids. C. neoformans is haploid basidiomycetous yeast with a bipolar mating system. This species exists in two mating types (MATa and MATα) and AD hybrids. C. neoformans var. grubii (serotype A) and C. neoformans var. neoformans (serotype D). C. neoformans is an opportunistic fungal pathogen with a defined sexual cycle involving fusion of haploid MATa and MATα cells [4]. Serotypes A, D, and AD are isolated worldwide from avian excreta, soil, and vegetative debris [3].

In recent years, a natural association has been recognized between C. neoformans var. gattii (serotype B) and flowering eucalyptus trees, such as the red river gum tree (Eucalyptus camaldulensis). Up to date, all of the isolates recovered from Eucalyptus trees have been serotype B. If the isolation of C. neoformans var. neoformans from natural environments reflects only colonization by enrichment, then the true ecology of serotypes A, D, and C remains to be discovered [5,6].

E. camaldulensis (Dehn.) is the most scanned tree type and natural source for C. neoformans colonization. In this research, existence of C. neoformans was scanned in samples taken from E. camaldulensis trees. This study took place in Gökova region on E. camaldulensis trees to study the ability of C. neoformans to produce basidiospor in E. camaldulensis trees. Isolated C. neoformans was examined to determine if mating takes place in natural environment in Turkey.

Keywords: Cryptococcus neoformans; Eucalyptus camaldulensis; Wood debris broth

Introduction

Cryptococcus neoformans (San Felice, 1895) Vuillemin 1901, is a globally distributed human fungal pathogen that causes life-threatening meningoencephalitis in immunocompromised patients. Microscopically, most clinical isolates appear as spherical, budding, encapsulated yeast cells in both tissue and culture [1-3]. The most common and serious clinical manifestation of infection is meningoencephalitis, which occurs with any of the serotypes. Cryptococcosis is acquired by inhalation of airborne cells of C. neoformans from the environment, but the source and nature of the infectious propagules have not been resolved [3].

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E. camaldulensis (Dehn.) is the most scanned tree type and natural source for C. neoformans colonization. In this research, existence of C. neoformans was scanned in samples taken from E. camaldulensis with cultural technique. This study took place in Gökova region on E. camaldulensis trees to study the ability of C. neoformans to produce basidiospor in E. camaldulensis trees. Isolated C. neoformans was examined to determine if mating takes place in natural environment in Turkey.

Material and Methods

Localization

This research was done in the region (Gökova bay, on the Akyaka-Akçapınar road within coordinates: 37° 03’ 14 North, 28° 21’ 33 East and 37° 01’ 37 North, 28° 21’ 32 East) where there are intensive amount of eucalyptus trees and where several other studies were done with C. neoformans isolation. Trees on this study were enumerated and GPS data for each tree was recorded. Samples were collected by swab method as recommended in previous studies [7,8]. According to this method, sterile swabs (prepared with 50-60 cm long bamboo sticks) were mixed with SF in test tubes. Samples were collected from various regions of the cavities of the trees with swab. Cotton part of the swab was sunk into 2 ml steril 0.9% NaCl and was brought to lab with room temperature within the same day [7]. Wood debris was collected from young and old trees, was enumerated separately and was put into plastic bags. Each wood debris sample was taken to be 20-25 gram and was brought to lab after field study.

Strains and media

Plates with Staib, V8, PDA, wood debris were prepared. Pure C. neoformans was brought to lab after field study.
neofomans (Aa) ATCC 208821 (10 μl) and C. neoformans (Aa) IUM 96-2828 (10 μl) strains were sown into PDA plates to revive. In order to prepare wood debris plates, all wood debris, leaves were left to dry and then were grinded in the mill. Wood debris plate (300 cc), consists of 10 gr powder wood debris, 2 gr agar, 100 ml sterile distilled water, 0.1 gr chloramphenicol. Samples collected from working area to lab were sown into plates. Spreading with swab method was used for sowing process [9].

Results

Colonization of cryptococci was observed after 15-20 days of processing samples (from Akyaka region) in lab. During our study, colonization was observed in 11 trees out of 32 eucalyptus trees (36.6%). Broths that we obtained colonization are: Stab and V8. Trees that we observed colonization are: 1, 6, 14, A1, A2, A4, A5, A10, A12, A13, A14 (tree codes).

Pure C. neoformans (Aa) ATCC 208821 (10 μl) and C. neoformans (Aa) IUM 96-2828 (10 μl) strains were mixed and inoculated in E. camaldulensis wood debris broth. The mating (sexual reproduction) capability of C. neoformans was investigated and conjugation tube was observed in 59.3% of these broths (Figure 1). Mating capability of C. neoformans increases the risk of life-threatening meningoencephalitis in immunocompromised patients.

Laetiporus sulphureus (Bull.) Murrill fungus was discovered on all of E. camaldulensis where C. neoformans was isolated (Figure 2).

Discussion

C. neoformans var. grubii isolation related with eucalyptus flora (made in our country) was made as a single origin in Gökova region where 1175 trees were scanned [10]. The region has the minimum pH interval (6.4-7.0). In order to keep yeast alive outside, the environment where 1175 trees were scanned [10]. The region has the minimum pH (made in our country) was made as a single origin in Gökova region (Figure 2). Mating-type-specific and nonspecific PAK kinases play shared and divergent roles in Cryptococcus neoformans. Eukaryot Cell 1: 257-272. That means mating will be made in natural environment in our country and risk factors exist.

The effect of nutritional and physicochemical properties of tree cativies to the lifes of C. neoformans origins isolated from this region is yet not clear. Studying the effect of ecological and chemical combinations to the life cycle of C. neoformans and evaluating the struggle of the yeast to survive will help to clarify the pathogenesis of infectious that is caused by C. neoformans on humans [13]. That is why; we think that it is important to keep track of colonization areas detected in our country long term and/or periodically.

References