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

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Abstract

Cryptococcus neoformans (San Felice) Vuillemin is an encapsulated yeast of the class Basidiomycetes which produces life-threatening fungal infections on especially immunosuppressed individuals. Although flora of *Eucalyptus camaldulensis* (Dehn.) (Which is considered for natural niche for *C. neoformans*) exists in various locations in Turkey, the isolation of the yeast is lower than expected.

In this study, swabbing technique was used for environmental screening of *C. neoformans* in Gökova-Akçapınar region where *C. neoformans* has been isolated in the area in 2011. All of the *E. camaldulensis* wood debris in this region was involved. *C. neoformans* colonization was discovered in 11 of 32 trees in Staib and V8 broths. (36,6%).

Pure *C. neoformans* (Aα) 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. Mating capability of *C. neoformans* increases the risk of life-threatening meningoencephalitis in immunocompromised patients.

Moreover, *Laetiporus sulphureus* (Bull.) Murrill fungous was discovered on all of *E. camaldulensis where C. neoformans was isolated.*

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].

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 hybrids. *C. neoformans* is haploid basidiomycetous yeast with a bipolar mating system. This species exists in two mating types (MATa and MATα) and two varieties, *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 MATα and MATa 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 avian 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* 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.*

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neoformans (Aα) ATCC 208821 (10 µl) and *C. neoformans* (Aa) IUM 96-2828 (10 µl) strains were sow 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 sow into plates. Spreading with swab method was used for sowing process [9].

Results

Colonization of cryptococcals 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: Staib and V8. Trees that we observed colonization are: 1, 6, 14, A1, A2, A4, A5, A10, A12, A13, A14 (tree codes).

Pure *C. neoformans* (Aα) 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 fungous 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 should have wood debris to provide nutritional support needed for its physiology. Humidity and heat of the environment is also effective [8,9]. This shows that Akyaka region, where this study takes place, is suitable for colonization of *C. neoformans* var. *grubii* thanks to its environmental factors.

Sexual reproduction of *C. neoformans* causes yeast to spread around the world and this creates a risky environment for immunocompromised patients [11]. Asexual and sexual reproduction phases of fungus are determined according to different environmental conditions. Sexual reproduction is chosen especially in absence of nitrogen [12]. C. *Neoformans* was determined in asexual form in Akyaka region in our study. With our study, mating was made in medium with *E. cameldulensis* wood debris. That means mating will be made in natural environment in our country and risk factors exist.

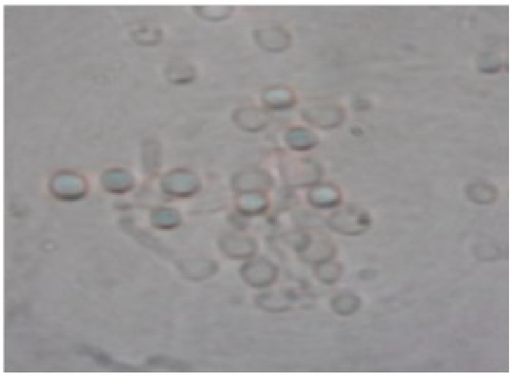

Figure 1: Showing conjugation tubes in *E. camaldulensis wood debris* (tree evaluated as positive).

Figure 2: *L. sulphureus* fungus that resides on the body part of *E. camaldulensis.*

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.

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