Annals of Microbiology, **56 (2)** 97-99 (2006)

Environmental study of *Cryptococcus neoformans* in and around Adana, Turkey

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Received 23 September 2005 / Accepted 16 March 2006

Abstract - Fungal diseases affecting humans generally originate from the environment. Due to the rising of in human fungal infections in recent years, it is now extremely significant to know more about ecology in order to control fungal pathogens. The aim of this study was to evaluate the presence of *Cryptococcus neoformans* in its well-known ecological niches in eight different locations, in Çukurova region, Turkey. For this purpose, for a total of 1835, 1508 vegetable material from tree of the genus *Eucalyptus* trees, 119 pigeon droppings and 208 soil samples were examined for the presence of yeast in a nigerseed medium. *Cryptococcus* spp. was not recovered from any of these samples. We believe that in our region there are elements affecting the life cycle of *Cryptococcus neoformans,* such as alkaline pH and high carbon ratio of the soil.

Key words: *Cryptococcus neoformans,* ecology, *Eucalyptus* spp., pigeon droppings, soil.

INTRODUCTION

The basidiomycetous yeast *Cryptococcus neoformans* is an important pathogen that causes meningitis and pneumoniae in both immunocomprimised and immunocompetent patients. This encapsulated fungus exists in three varieties: *C. neoformans* var. *grubii* (serotype A) and *C. neoformans* var. *neoformans* (serotype D), both with worldwide distribution, and *C. neoformans* var. *gattii* (serotypes B and C), which is limited to tropical and subtropical regions (Kwon-Chung and Bennett, 1984; Franzot *et al.*, 1999).

Cryptococcus neoformans var. *neoformans* is associated with decomposing avian nests; besides droppings, especially those of pigeons, are believed to constitute a major environmental reservoir (Emmons, 1955; Kwon-Chung and Bennett, 1992). A significant progress in understanding of cryptococcal ecology happened in 1990 with the discovery by Ellis and Pfeiffer (1990a) of a specific association between *C. neoformans* var. *gattii* and *Eucalyptus camaldulensis*. This yeast was isolated from debris (wood, bark, leaves, flowers) collected under the canopies of *E. camaldulensis* growing in Australia (Ellis and Pfeiffer, 1990a, 1990b) and this finding was confirmed in many parts of the world (Pfeiffer and Ellis, 1991; Chakrabarti *et al.*, 1997; Montenegro and Paula, 2000). However, *C. neoformans* var. *grubii* was associated with excreta of pet birds (Kielstein *et al.*, 2000).

The aim of the study was to determine the ecological implications of *C. neoformans* in its well-known niches i.e., *Eucalyptus* spp., pigeon droppings and soil samples in and around Adana, Turkey.

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MATERIALS AND METHODS

The city of Adana is the fifth biggest province in Turkey with over 1.5 million inhabitants. It is located at latitude 35º N and longitude 37º E by the Mediterranean Sea. The climate is warm (9.5 ºC) and rainy (132.2 kg/m^2/month) in the winter, and hot (27.5 ºC) and dry (1.6 kg/m^2/month) in the summer time. The relative humidity is high (57.9-76.9%) for most of the year. Tarsus-Karabucak is mainly an eucalyptorium (2000 hectares), where the first commercial eucalyptus plantation, between the provinces of Adana and Mersin was created (Fig. 1).

Environmental sampling. In this study, *Eucalyptus* samples were collected during the blooming period of the late spring of 2005 in and around Adana. The origin and usage in industry of the *Eucalyptus* spp. in this area was discussed in a previous report (Ergin *et al*., 2004a). The samples were a mixture of vegetable debris of *Eucalyptus* including flowers. Most of the trees (> 95%) classified in the study were *E. camaldulensis* Dehn. In addition dry pigeon droppings as well as soil samples collected under *Eucalyptus* trees were analysed. These region-specific samples are presented in Table 1.

Procedure. All the materials were collected in sterile 100 ml containers and brought to the laboratory. About 2-5 g of each sample were suspended in 20-50 ml (1:10, w/v) in sterile physiological saline. The suspensions were allowed to stand for 30 min. Next, 100 µl of supernatant was streaked onto nigerseed medium (Ergin *et al*., 2004a) with 0.1% glucose, containing 0.1% biphenyl (B 6890, Sigma) and 0.1% chloramphenicol (23275, Fluka). The plates were incubated in air at 30 ºC for up to 10 days (Ergin *et al*., 2004a).

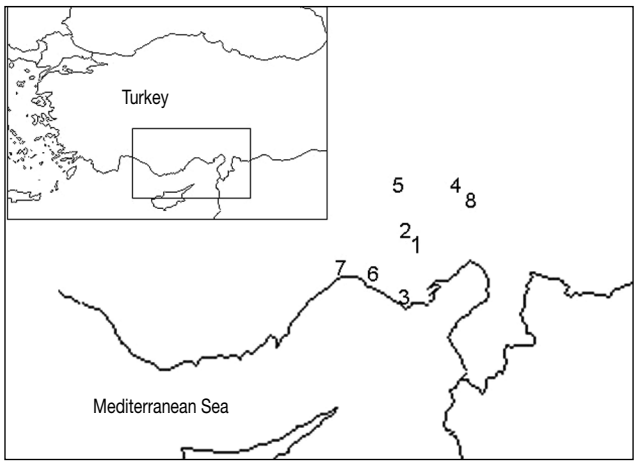

FIG. 1 – Sampling areas. 1: Çukurova University Campus, 2: Adana Equastrian Club, 3: Karataş town, 4: Kozan town, 5: Karaisalı town, 6: Karabucak-Tarsus eucalyptorium, 7: Mersin city center, 8: Kadirli town.

TABLE 1 – Kind and number of samples from different areas of Adana and Mersin regions

Sample	Number
Eucalyptus	
Çukurova University Campus	228
Adana Equastrian Club	125
Karataş (Adana)	294
Kozan (Adana)	275
Karaisalı (Adana)	53
Tarsus-Karabucak eucalyptorium (Mersin)	412
Kadirli (Osmaniye)	121
Subtotal	1508
Piegon droppings	
Park 1 (Atatürk Park, Adana)	62
Park 2 (Çukurova University Campus Park)	40
Mosque (Mersin city center)	17
Subtotal	119
Soil	
Çukurova University Campus	60
Adana Equastrian Club	47
Tarsus-Karabucak eucalyptorium (Mersin)	61
Mersin city center	40
Subtotal	208
Total	1835

Organism. The performance of the nigerseed medium was monitored by the inoculation of *C. neoformans* ATCC 90112.

RESULTS AND DISCUSSION

Cryptococcus spp. was not detected in any of the 1835 samples investigated, including 1508 *Eucalyptus* spp., 119 pigeon droppings and 208 soil samples. Data reported in literature showed that the frequency of *C. neoformans* isolations from pigeon droppings ranges from between 6.0% and 87.5%, all over the world (Lazéra *et al.*, 1993; Castañón-Olivares and López-Martinez, 1994). In studies on *C. neoformans* recovered from pigeon droppings in Turkey, the isolation rates were reported to be, 8.8-14.3% for Izmir (Tümbay, 1977; Sivrel and Tümbay, 1993; Derici and Tümbay, 2003), 3.2-13.9% for Bursa (Karaman *et al.*, 1980; Yılmaz *et al.*, 1989), 1.0% for Istanbul (Aygün, 1998) and 18.9% for Kayseri (Koç and Durkut, 2001). Yıldıran *et al.* (1998), isolated 29 (4.6%) *C. neoformans* strains out of 634 pigeon droppings collected throughout Turkey. Almost all isolates (96.6%) were recovered from roof and dovecote samples. Interestingly, isolation rates for Adana and Mersin were reported as 33.3% (3/9) and 10.0% (1/10), respectively. More recently, Derici and Tümbay (2003), isolated eight (8.8%) *C. neoformans* strains from 90 pigeon droppings. They reported that five (62.5%) of these to be serotype D, and three (37.5%) serotype A. Some other studies reported the isolation of non-*neoformans Cryptococcus* from pigeon droppings, while *C. neoformans* was not recovered in none of them (Kielstein *et al.*, 2000; Pal *et al.*, 1990; Hamasha *et al.*, 2004). However, in our study, no *Cryptococcus* spp. was isolated from pigeon droppings. This might be due to the sampling areas i.e., park and mosques, but not roofs and dovecotes, as well as alkaline soil environment in our region (Ergin *et al*., 2004a).

Regarding *C. neoformans* var. *gattii,* its environmental association with decaying wood in tropical and subtropical regions is well documented (Ellis and Pfeiffer, 1990a, 1990b; Pfeiffer and Ellis, 1991; Chakrabarti *et al.*, 1997; Montenegro and Paula, 2000). More recently, an outbreak of *C. neoformans* var. *gattii* infection was reported in the temperate climate of Vancouver Island, BC, Canada. The yeast was isolated from human, animal, air and soil samples, as well as from multiple native tree groups, including both coniferous and deciduous trees. Limited or none isolation was obtained from imported exotic trees such as *Eucalyptus* (Bartlett, 2005). In addition, *C. neoformans* var. *gattii* was not isolated from eucalypt material in some areas with endemic infection, i.e., Central Africa (Swinne *et al.*, 1994) and Papua New Guinea (Laurenson *et al.*, 1997) despite widespread environmental sampling. Randhawa *et al.* (2001) collected 702 samples of diverse plant materials in India, and isolated only four *C. neoformans* var. *neoformans* strains and obtained no isolation of *C. neoformans* var. *gattii*.

The soil pH of the Tarsus-Karabucak eucalyptus forest in eastern Mediterranean region is between 7.8-8.0, while humidity and carbon rates are, 80-90% and 2.5%, respectively. While, beyond the western border of Taurus Mountains, the soil pH either neutral or acidic (pH 6.4-7.0) with humidity and carbon rates of, 35-90% and 1.5%, respectively. In our previous study, we isolated *C. neoformans* var. *grubii* (serotype A) from honeybee colonies in Tarsus-Karabucak eucalyptorium, but did not isolate any yeast where this flora is not present (Ergin *et al*., 2004b). Recently, we isolated only one strain of *C. neoformans* var. *grubii* in the western Mediterranean region, namely in Turkey, from 1175 *Eucalyptus* samples including debris and flowers, which had been collected from both western and eastern Mediterranean regions (Ergin *et al*., 2004a). The isolation in that study was made in the Taurus Mountains at the juncture of the Mediterranean and the Aegean regions. However, Hamasha *et al.* (2004) in Jordan investigated 500 samples of the mixed soil debris, including tree materials, collected under the *Eucalyptus* tree and failed to detect *C. neoformans*. We believe

that the alkaline soil environment in this region, as an extension to the Taurus Mountains, may have suppressed the life cycle of *C. neoformans.*

This study indicates that none of the three varieties of *C. neoformans* (var. *neoformans*, var. *gattii*, and var. *grubii*) was found in the ecological niches in the Çukurova region, Turkey. This might also explain the lower incidence of human cryptococcal infections in this region. In the last decade, we had only two cases, one with AIDS and the other with systemic lupus erythematosus and also both of them were due to *C. neoformans* var. *grubii* (serotype A, Ilkit M., unpublished data). Thus, in our region, alkaline soil environment (pH 7.8-8.0) with high carbon rates (2.5%), might be responsible for the absence of *Cryptococcus* spp. in any sample.

As a conclusion, we suggest that during the investigation of *C. neoformans* in environmental samples, we should also consider other ecological niches such as plant flora. Further studies are needed to understand the ecological niches of *C. neoformans* over time. Discussing and comparing the data obtained from other studies would greatly help to substantiate the topic of the ecological niches of this yeast fungus.

Acknowledgement

This study was supported by the Research Fund of Çukurova University (Project No: TF2003BAP16). Authors are grateful to Associate Prof Dr Cagrı Ergin in the Department of Microbiology, Faculty of Medicine, University of Pamukkale, Denizli, for general revision of the study.

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