Isolation of *Cryptococcus neoformans* from a chestnut tree (*Castanea sativa*), Denizli, Turkey

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OBJECTIVES

Cryptococcus neoformans is a basidiomycetous encapsulated yeast that can cause life-threatening infections in immunosuppressed humans and animals. *C. neoformans* infections are considered to be acquired via inhalation of aerosolized particles from the environment. Avian guano, decaying tree hollows, and soil are known environmental niches. In recent years, different trees, such as *Eucalyptus camaldulensis*, *Tamarix hispida*, *Platanus orientalis*, and *Punica granatum*, have been reported to have *C. neoformans* colonies in different woody environmental regions of Anatolia, Turkey. In this study, a new *C. neoformans* colonization niche is presented.

MATERIALS AND METHODS

In this study, the colonization screening of *C. neoformans* on chestnut trees (*Castanea* spp.) from the Aydın-Ödemiş-Denizli geographical area is presented. This area consists of mountainous terrain between the fertile plain formed by two major rivers. From this area, 214 chestnut trees (*Castanea* spp.) that had deep fissures or trunk hollows (Fig. 1) were screened during the mid-summer, 2017 (Table 1). A swabbing technique was used, and all samples were cultured on Staib agar medium containing 0.5% biphenyl and 0.4% chloramphenicol. ITS 1-4 primers were used for strain PCR tests as follows ;

ITS1 – TCCGTAGGTGAACCTGCGG

ITS4 – TCCTCCGCTTATTGATATGC

For mating, PCR were tested with reference strain (JEC 20 (VNIV-Da), JEC 21 (VNIV- Da), KN99Aa and KN99Aα) primers as follows;

STE20_Aa_Forward – CTAACCTACTACACCTCACGGCA (457 bp)

STE20_Aa_Reverse – CGCACTGCAAAATAGATAAGTCTG

STE20_Aα_Forward – GGCTGCAATCACAGCACCTTAC (330bp)

STE20_Aα_Reverse – CTTTCATGACATCACTCCCCTAT

STE20_Da_Forward – CACATCTCAGATGCCATTTTACCA (728bp)

STE20_Da_Reverse – AGCTCTAAGTCATATGGGTTATAT

STE20_Dα_Forward – CTTAATTCACAGCACCAGCCTA (592bp)

STE20_Dα_Reverse – GGTCATCACAGTCAGTCACCAC

RESULTS

Candidate yeasts were tested for the presence of *Cryptococcus* by conventional methods and canavanine-glycine-bromothymol agar reactions. Only one (0.47%) strain of *C. neoformans* was isolated from all 214 screened trees (Figs. 2 and 3). This strain was confirmed by ITS 1-4 sequencing. We determined the mating type and serotypes by PCR analysis of the *STE20* genes using *STE20* (Aa), *STE20* (Aα), *STE20* (Da), and *STE20* (Dα) primers. The serotype A *MATα* mating type was observed (Fig. 4).

CONCLUSION

Here, the first *C. neoformans* isolate from a chestnut tree (*Castanea sativa*) is presented. Further studies of distribution of human pathogenic *Cryptococcus* in our country will be helpful in determining risk areas that threaten mammalian health.

References for observed *C. neoformans* niches in Turkey;

(*) Ergin Ç et al., Mycopathologia 2004;158:43–47 (*) Mikrobiyol Bul 2010;44:79–85; (*) Turk Mikrobiyol Cem Derg 2014;44:158–162, (*) Gökçen H, Ergin Ç. Pam Tıp Derg 2014;7:109–112, (*) Cogliati M et al., FEMS Yeast Res 2016;16:fow045, (*) Environ Microbiol 2017;19:4318–4325.

Fig 1. All deep fissures or trunk hollows (arrow) of chestnut trees (*Castanea* spp.) were sampled.

Fig 2. With Staib agar, only one *Castanea sativa* was found as colonized by *Cryptococcus neoformans* (arrow).

Table 1. Sampled regions of *Castanea* spp. in the study area.

Region		Sample (n)	Positive (n)
I (Aydın)	Hamamköy	74	-
	Menteşeler		-
	Cumayanı		-
II (Aydın)	Apaklar	54	-
	Ovacık		-
	Yukarıörencik		-
	Aşağıörencik		-
III (Denizli)	Alandız	36	-
IV (Denizli)	İsrafil Deresi	50	1
Total		214	1

Fig 3. Sampled area and positive (*) point.

Fig 4. PCR reactions: JEC20 (Da); JEC21 (Dα); KN99a (Aa); KN99α (Aα); Test strain (1); Negative control (N); Marker (M).