Malaysian Journal of Microbiology, Vol 7(4) 2011, pp. 233-235

SHORT COMMUNICATION

Can smoking cause melanization of *Cryptococcus neoformans in vivo*?

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Received 30 March 2011; received in revised form 3 August 2011; accepted 1 September 2011

ABSTRACT

Earlier studies have reported on the ability of *Cryptococcus neoformans* to synthesize melanin from tobacco extracts / nicotine incorporated in to the medium. However a study on the utilization of components in tobacco smoke by *C. neoformans* for melanin production was unreported. The present study reports on ability of *C. neoformans* for melanization using tobacco smoke and therefore substantiate the possible link between smoking and pathogenecity in clinical cryptococcal infections as reported by several researchers.

Keywords: Cryptococcosis, Smoking, Cryptococcus neoformans, melanin, tobacco

INTRODUCTION

Cryptococcus neoformans is emerging as one among the 'Champion parasites' in causing life threatening human diseases (Gokulshankar *et al.*, 2009). Melanin production confers pathogenicity and selective advantage of survival to *C. neoformans* (Williamson, 1997; Casadevall *et al.*, 2000; van Duin *et al.*, 2002). Pigment production in *C. neoformans* is dependent upon presence of exogenous phenolic substrates (a precursor of melanin) unlike some other fungi which can make pigments endogenously (Chaskes and Tyndall, 1975). It is reported by earlier studies that *C. neoformans* can utilize tobacco extracts (Tendolkar *et al.*, 2003) or nicotine (Khan *et al.*, 2004) in media to produce brown (melanin-like) pigments.

Khan (2006) raised an interesting discussion on whether a connection exists between Smoking, Melanization, and Cryptococcosis? He further hypothesized that Cryptococcosis being a pulmonary and meningeal disease, the absorption of tobacco ingredients directly into the lungs and subsequently into bloodstream during smoking could provide additional substrates for melanin biosynthesis (Khan, 2006) and further experimentally proved that incorporation of nicotine in the medium enables *C. neoformans* to produce brown pigment *in vitro.*

Tobacco used in cigarettes contains a large number of chemicals that include catechol, hydroquinone, and nicotine (Pryor *et al.*, 1998). It is hypothesized as some of the chemicals could possibly serve as precursors for melanin synthesis *in vivo* (Polacheck *et al.*, 1982). It would be intriguing to understand whether it is possible for *C. neoformans* to use the components in the cigarette smoke directly to produce pigment *in vitro* without directly incorporating the tobacco extracts / nicotine in to the medium.

MATERIALS AND METHODS

Tobacco was collected from the cigarettes (brand not mentioned to avoid any commercial implication) and smoke was produced by heating it using an experimental design (Figure 1). The smoke generated from the tobacco content of the cigarette was allowed to infuse in to the conical flasks containing Defined Minimal Medium (DMM) (15 mM glucose, 29.4 mM KH2PO4, 10 mM MgSO4, 13 mM glycine and 3.0 M vitaminB1, pH 5.5) inoculated with *C. neoformans*. To prevent the entry of soot generated by heating tobacco entering DMM, a cigarette filter was used in the inlet tube. The smoke generation was done for duration of 15 min in the periodicity of 2 times a day up to 5-7 days of incubation. The quantity of tobacco smoked each time is equivalent to the weight of 5 g.

Four environmental isolates of *C. neoformans* and two isolates of *C. neoformans* of clinical origin i.e. IHEM no. 11489 and IHEM no. 3553 (obtained earlier from BCCM／IHEM Culture collection, Scientific Institute of Public health, Bruxelles) were used for the study (Table 1).

RESULTS AND DISCUSSION

All the six isolates produced pigment on DMM changing the colour of the medium to brown / black (Table 1). The medium in the control setup (without inoculation of *C. neoformans*) did not change color which is indicative of the fact the colour change is to be attributed only to the

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 ISSN (print): 1823-8262, ISSN (online): 2231-7538

pigment production by the isolates and not because of the smoke itself.

Earlier studies (Boelaert and Blasi, 1999; Hajjeh *et al.*, 1999) have associated the risk of smoking to Cryptococcosis. Nosanchuk and co-workers (Nosanchuk *et al.*, 2000) reported *in vivo* synthesis of melanin by *C. neoformans* in human brain tissue. *C. neoformans* is considered to metabolize catecholamines to melanin and this ability of *C. neoformans* has been suggested as a possible explanation for its ability to invade and live in neural tissue (Khan, 2006). Certain regions of the brain including the basal ganglia are already known to be affluent in catecholamines and invariably cigarette smoking may further enhance levels of the melanin precursor compounds in these regions (Khan, 2006).

It is a well known fact that nicotine, besides being a precursor for melanin synthesis, also has affinity to melanin containing tissues of human. King *et al.* (2009) have postulated that nicotine-bound melanin provides a reservoir that allows for slow egress of relatively small concentrations of unmetabolized nicotine to migrate from skin and other melanin5 containing tissues through the bloodstream to brain nicotine receptors (King *et al.*, 2009). Therefore smoking might exert its influence on *in vivo* melanization of *C. neoformans* (Khan, 2006) and therefore its pathogenesis in cases of cryptococcal mengingitis.

In view of the present findings, it is evident that *C. neoformans* is capable of utilizing the components present in the smoke when diffused in to its medium (can it be extrapolated to CSF *in vivo* conditions?) to produce pigment and further substantiates Khan's hypothesis (Khan, 2006) of the possible correlation between smoking and melanization of *C. neoformans in vivo*. This is a clear cut siren for smokers whose risk of susceptibility to cryptococcal infections is invariably high.

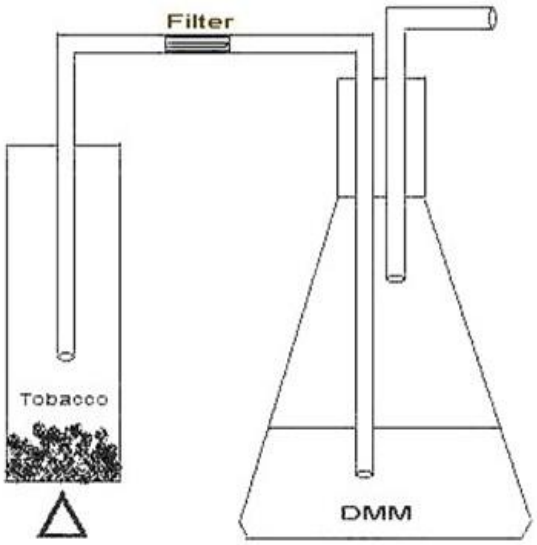

Figure 1: Setup to infuse nicotine into culture medium.

ACKNOWLEDGEMENT

We thank the Head, Department of Microbiology, The New College, Chennai, India and Prof. Danielle Swinne, BCCM ⁄ IHEM Culture Collection, Scientific Institute of Public Health, Bruxelles, Belgium for providing the cultures for the study.

Table 1: Melanization of C*. neoformans* isolates in DMM with infusion of Cigarette smoke

Source	Origin of isolate	Description	Pigment production
Chennai, India	Environment - Pigeon droppings	*Cryptococcus neoformans* var *neoformans*, MAT a, serotype D	+
Chennai, India	Environment - Pigeon droppings	*Cryptococcus neoformans* var *neoformans*, MAT alpha, serotype A	+
Chennai, India	Environment - Fowl droppings	*Cryptococcus neoformans* var *neoformans*, MAT alpha, serotype A	+
Chennai, India	Environment - Crow droppings	*Cryptococcus neoformans* var *neoformans*, MAT a, serotype D	+
BCCM/IHEM Culture collection, Scientific Institute of Public health, Bruxelles	Clinical	IHEM No. 11489 *Cryptococcus neoformans* var. *gattii*, serotype B	+
BCCM/IHEM Culture collection, Scientific Institute of Public health, Bruxelles	Clinical	IHEM No. 3553 *Cryptococcus neoformans* var. *grubii*, serotype A	+

ISSN (print): 1823-8262, ISSN (online): 2231-7538

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 ISSN (print): 1823-8262, ISSN (online): 2231-7538