A new henna-based medium for the differentiation of *Cryptococcus neoformans*

Cryptococcosis due to *Cryptococcus neoformans* is a leading cause of morbidity and mortality among AIDS patients (Mitchell & Perfect, 1995). Melanin production by phenol oxidase activity is a distinctive and characteristic property of *Cryptococcus neoformans* isolates. The ability to produce melanin pigment is one of the widely used criteria for the identification of *Cryptococcus neoformans* and for the evaluation of virulence (Kwon-Chung & Rhodes, 1986; Polak, 1990). Melanin production tested in a medium containing a precursor of melanin, such as dihydroxyphenylalanine, caffeic acid, bird seed or sunflower seed has been reported (Chaskes & Tyndall, 1978; Denning *et al.*, 1990; Hopfer & Blank, 1975; Khan *et al.*, 2004), and we reported the use of a mustard-seed-based medium for the same purpose (Nandhakumar *et al.*, 2006). We now report the brown colour effect (BCE) of *Cryptococcus neoformans* in a medium containing leaf extract of henna.

Henna (*Lawsonia inermis*, syn. *L. alba*) is a flowering plant native to Africa, southern Asia, the Middle East and Australasia. It is not native to most of Europe except Spain. Preparation of henna agar was similar to that of mustard agar (Nandhakumar *et al.*, 2006). Freshly plucked henna leaves were washed and dried in the shade (3–5 days). An aqueous extract was prepared by adding 10 g dried henna leaves to 1 litre distilled water, followed by boiling for 10 min. The decoction was then cooled and filtered through gauze and the volume was readjusted to 1 l. The pH was adjusted to 6.0, and 20 g agar-agar was added before the mixture was autoclaved at 121 °C for 15 min. The medium was allowed to cool to 45–55 °C and dispensed into sterile 90 mm diameter Petri dishes (approx. 25 ml agar per plate).

Three isolates of *Cryptococcus neoformans* var. *gatti*, two isolates of *Cryptococcus neoformans* var. *neoformans* and two isolates of *Candida albicans* were tested.

Two reference strains, *Cryptococcus neoformans* var. *grubii* (H-99) and *Cryptococcus neoformans* var. *neoformans* (B-3501), kindly provided by J. Kwon Chung (National Institute of Health, Bethesda, MD, USA), were included. All the test isolates were initially grown on Sabouraud's dextrose agar at 37 °C for 24–48 h. The plates were incubated at 30 °C on the laboratory bench.

At 24 h post-inoculation, all seven isolates of *Cryptococcus neoformans* could be easily identified by the BCE on henna agar and differentiated from the white colonies of *Candida albicans*. The plates were held for up to 7 days to check for any variations in the colony colour. Strains were also seeded on Pal's agar (Khan *et al.*, 2004) and were observed to produce the BCE.

The concept of using differential media for isolating specific fungal pathogens is not new, but problems encountered with differential media used for identification of *Cryptococcus neoformans* have included an elevated cost and a complex medium preparation procedure. Henna is inexpensive and easily available in many tropical and subtropical regions. Commercial henna powder is available in all countries including Europe and might be used to make the infusion in regions where the plant is not cultivated, although the authors would not like to suggest that commercial henna powder would necessarily give similar results.

The henna agar is simple to prepare, contains only henna and agar and does not require any expensive additives.

This is the first report on the BCE of *Cryptococcus neoformans* on henna agar. Although we are unable to explain the exact component of henna that is responsible for this phenomenon, further analysis of this new medium would enable elucidation of the exact mechanism.

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Chaskes, S. & Tyndall, R. L. (1978). Pigment production of *Cryptococcus neoformans* and *Cryptococcus* species from aminophenols and diaminobenzenes. *J Clin Microbiol* 7, 146–152.

Denning, D. W., Stevens, D. A. & Hamilton, J. R. (1990). Comparison of *Guizotia abyssinica* seed extract (birdseed) agar with conventional media for selective identification of *Cryptococcus neoformans* in patients with acquired immunodeficiency syndrome. *J Clin Microbiol* 28, 2565–2567.

Hopfer, R. L. & Blank, F. (1975). Caffeic acid-containing medium for identification of *Cryptococcus neoformans*. *J Clin Microbiol* 2, 115–120.

Khan, Z. U., Ahmad, S., Mokaddas, E. & Chandy, R. (2004). Simplified sunflower seed agar for differentiation of *Candida dubliniensis* from *Candida albicans*. *Clin Microbiol Infect* 10, 590–592.

Kwon-Chung, K. J. & Rhodes, J. C. (1986). Encapsulation and melanin formation as indicators of virulence in *Cryptococcus neoformans*. *Infect Immun* 51, 218–223.

Mitchell, T. G. & Perfect, J. R. (1995). Cryptococcosis in the era of AIDS – 100 years after the discovery of *Cryptococcus neoformans*. *Clin Microbiol Rev* 8, 515–548.

Nandhakumar, B., Girish Kumar, C. P., Prabu, D. & Menon, T. (2006). Mustard seed agar – a new medium for differentiation of *Cryptococcus neoformans*. *J Clin Microbiol* 44, 674.

Polak, A. (1990). Melanin as a virulence factor in pathogenic fungi. *Mycoses* 33, 215–224.