

## Short communication

**Cryptococcus neoformans var. gattii can be separated from var. neoformans by its ability to assimilate D-tryptophan**

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An additional test to differentiate the varieties of *Cryptococcus neoformans* is described. It is based on the assimilation of D-tryptophan by the variety *gattii*.

**Introduction**

*Cryptococcus neoformans* is an encapsulated yeast which preferentially causes infections in immunocompromised patients. It exists in two varieties: *C. neoformans* var. *neoformans* and *C. neoformans* var. *gattii*.

Some biochemical differences have been reported between the two varieties, including assimilation of dicarboxylic acids [1] and creatinine metabolism [2]. The canavanine-glycine-bromothymol blue (CGB) medium [3] is effective in separating them.

Using the auxanographic method, Dufait *et al.* [4] have demonstrated that *C. neoformans* var. *gattii* can readily be differentiated from *C. neoformans* var. *neoformans* by its ability to assimilate D-proline as a source of nitrogen.

We have extended this study to a larger number of isolates and tested several additional D-amino acid compounds on carbon base agar. (This work was partly presented at the 2nd International Conference on *Cryptococcus* and Cryptococcosis, Milano, Italy, 19-23 September 1993).

**Materials and methods**

The study was performed on 245 isolates of *C. neoformans*: 79 clinical isolates of var. *gattii* and 110 clinical as well as 56 environmental var. *neoformans* isolates.

*C. neoformans* was identified by use of standard biochemical methods [5] and the D-proline assimilation test was the first criteria used to differentiate the two varieties [4].

The following twelve amino acids have been tested: D-alanine, D-(-)aspartic acid, D-glutamic acid, D-histidine

hydrochloride monohydrate, D-isoleucine, D-leucine, D-lysine hydrochloride, D-(-)ornithine hydrochloride, D-phenylalanine, D-serine, D-(-)tryptophan and D-tyrosine. All amino acids were obtained as powders from Janssen Chimica (Beerse, Belgium).

Paper discs impregnated with 20% (w:v) solutions in sterile distilled water of each D-amino acid compound were placed on the surface of plates containing Yeast Carbon Base, Difco (auxanographic method). The plates were incubated at 25-26 °C for 3-5 days.

As a reference method, serotyping was performed with adsorbed polyclonal antibody raised in rabbits by immunizing with whole heat-killed cryptococci.

**Results**

Of the 12 amino acids examined, only D-tryptophan was of value for the discrimination between the two varieties. All isolates of the var. *gattii* (serotypes B and C)

**Table 1** Serotyping and assimilation of D-proline and D-tryptophan for 245 *C. neoformans* isolates

Serotype	D-proline*	D-tryptophan*
A	0/89	0/89
D	0/60	0/60
AD	0/17	0/17
B	53/53	53/53
C	25/26†	26/26

\*Positive assimilation/number isolates tested.

†One isolate from a Rwandese patient (RV 70586 serotype C) did not assimilate D-proline.

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assimilated D-tryptophan, whereas one of 79 isolates failed to assimilate D-proline (Table 1). This isolate (RV 70586) was recovered from a Rwandese patient and was identified as serotype C.

Positive assimilation of D-tryptophan was not only apparent from enhanced growth around the disc, but also by a wide diffusion of a brown pigment in the medium. This pigment could be considered one of the metabolites of D-tryptophan catabolism (for instance, oxidation of aniline-like products). Attempts have to be made to extract and identify this compound.

None of the 166 isolates of the var. *neoformans* (serotypes A, D and AD) assimilated either D-tryptophan or D-proline.

### Discussion and conclusion

From our results, it appears that D-tryptophan assimilation by *C. neoformans* var. *gattii* constitutes an additional easy and reliable test to differentiate the two varieties of *C. neoformans*. This D-amino acid is not only considerably less expensive than D-proline but also provides higher sensitivity (100%) compared with the reference method.

To our knowledge, RV 70586 is the first isolate of the variety *gattii* which does not assimilate D-proline.

### Acknowledgement

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### References

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