IDENTIFICATION OF CRYPTOCOCCUS NEOFORMANS IN A ROUTINE CLINICAL LABORATORY

by

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INTRODUCTION

A firm diagnosis of cryptococcosis depends upon the isolation and identification on culture of the yeast-like fungus, Cryptococcus neoformans. The poor prognosis of many of these cases warrants expeditious handling of the laboratory specimen. The cost and toxicity of the treatment seriously penalize inaccurate diagnosis.

The present study was undertaken to evaluate which taxonomic features of C. neoformans permit the most rapid, practical and reliable differentiation of this species from the nonpathogenic species of Cryptococcus. A new differential medium for the identification of C. neoformans was included in the evaluation.

MATERIALS AND METHODS

Fifty-one isolates of Cryptococcus were used in this study. Thirty-six were isolated in this laboratory from clinical specimens. Eight were obtained from the American Type Culture Collection, Rockville, Maryland, and seven were from the Department of Microbiology and Medical Technology, University of Arizona, Tucson, Arizona (provided through the kindness of Dr. Adelaide Evenson Riker).

For all tests performed the fungi were grown on 1% neopeptone 2% glucose agar (NG agar) for 48 hours at 26°C. To demonstrate growth at 37°C a loopful of inoculum was streaked onto NG agar and incubated for 48 hours. The temperature of the incubator fluctuated ±0.5°C. To test for urease activity, one loopful of inoculum was streaked onto slants containing Christensen’s urea agar and
incubated at 26°C for 48 hours. The method for the production of extracellular starch was that of MAGER & ASCHNER (1947). A suspension of yeast was made in 0.85% NaCl and two parallel streaks were made onto the agar plate, each streak containing one loopful of yeast. The plates were incubated at 26°C. The test for starch was made at the end of one week and if negative it was repeated in two weeks. Use of undiluted Lugol's iodine in the starch test, as has been advocated, was too strong, as the denseness of the solution seemed to obscure the readings. A 1:10 dilution of Lugol's in water was made and the streaks were flooded. If the streaks did not turn blue, a portion of the streak was removed and the agar beneath was tested. If either the streak or the agar underneath turned blue, the reaction was considered positive.

The auxanographic method that was first introduced by BEYERINCK (1889) and later used in a taxonomic study by LODDER & KREGER-VAN RIJ (1952) was utilized in the assimilation studies of potassium nitrate and the various carbon sources. The vitamin solution used was 5 mg of thiamine hydrochloride (Upjohn Co., Kalamazoo, Michigan) per liter of molten medium cooled to about 70–80°C. The medium was poured into petri plates containing 1 ml of a suspension of yeasts in sterile water. The contents were mixed thoroughly and 1–3 mg of the test substances were placed on the surface of the solidified medium. The two most important factors in testing for the assimilation of potassium nitrate and the various carbon sources were thorough mixing of the fungi with the medium in the petri plates and testing not more than three carbon substances per plate. While it was possible to make readings in 24–48 hours, further incubation of 5–7 days proved to give clearer zones.

Creatinine assimilation studies (creatinine obtained from Fisher Scientific Co., Fairlawn, N. J.) were made according to BENHAM'S (1955) plate-smear method. Results with this method were difficult to interpret because many isolates gave growth responses intermediate between no growth and obvious good growth. STAIB (1963a) also noted quantitative differences in response. Those isolates which gave doubtful growth or no growth were retested by the auxanographic method of BEYERINCK (1889) as used by STAIB (1963a).

Medium containing a boiled extract of *Guizotia abyssinica* seed (obtained from William G. Scarlett and Company, Baltimore, Maryland) was evaluated for its use in routine diagnosis. The medium was prepared as described previously (SHIELDS & AJELLO, 1966; STAIB, 1963b) with one difference. The amount of chloramphenicol (50 mg/l) was not sufficient to inhibit bacteria in clinical specimens which were being tested concurrently and therefore was increased to 400 mg/l. A suspension in 0.85% saline was made of each isolate and one loopful was streaked onto a plate containing the above medium. Reactions were recorded at the end of one and two weeks of incubation at 26°C.