TAXONOMY OF CRYPTOCOCCUS NEOFORMANS AND ITS VARIETY UNIGUTTULATUS

by

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The yeast Cryptococcus neoformans has been the subject of studies from various points of view. Since it is known to be pathogenic for man it is of particular importance to have it well-defined. Taxonomic data are given by Lodder and Kreger-van Rij (1952). The species is characterized as follows:

"Cells are round or almost round, 3.5—8 μ in diameter. They are surrounded by a capsule. Under appropriate conditions "starch" is formed in the capsule or in the medium. The appearance of the streak culture is mucous. No pseudomycelium formation. No ascospores. Fermentation absent. Assimilation of glucose, galactose, saccharose and maltose. Lactose and nitrate cannot be assimilated. Splitting of arbutin: slightly positive."

A variety uniguttulatus to the species was recognized for those strains in which the formation of a capsule is very reduced, almost to nought. The appearance of the streak culture on malt agar is not mucous. The type strain of the variety was originally described by Wolfram and Zach (1934) as Eutorulopsis uniguttulata. This strain, as well as a second one classified in the variety by Lodder and Kreger-van Rij, has been isolated from human diseased nails.

Cr. neoformans and its variety uniguttulatus may be distinguished from most other species in the genus Cryptococcus by the assimilation reactions with galactose, saccharose, maltose, lactose and nitrate. From the species Cr. luteolus which has the same assimilation pattern, it differs in the shape of the cells. In Cr. neoformans the cells are round to short-oval, in Cr. luteolus they are oval or long-oval to elongate, (3.5—6) × (6—11.5) μ. The original strain of the latter species does not grow at 37°C.
In a publication on the genus *Cryptococcus*, Benham (1955) mentions the variety *inocuous* as a non-virulent form of the species *Cr. neoformans*. The properties given are: no growth at 37°C.; not pathogenic for mice; assimilation of glucose, saccharose and maltose, and of nitrate. A strain of this variety from Benham’s collection received by the courtesy of Dr. M. Silva appeared to be able to assimilate glucose, galactose, saccharose, maltose and lactose, and could therefore be identified with *Cr. albidos*. The assimilation of galactose and lactose is often weak in this species.

Van Uden, Braço Forte and do Carmo Sousa (1959) isolated a strain of *Cr. neoformans* from the stomach of a horse and studied it together with 22 other strains of this species. They found as the maximum temperature for growth for all strains 39—40°C. Glucose, galactose, saccharose, maltose, D-xylose, mannitol, sorbitol and inositol could be assimilated. Raffinose, L-arabinose, ethanol and soluble starch were assimilated by most strains. Glycerol and inulin were weakly or not assimilated. Lactose and nitrate could not be utilized.

In publications of medical origin the ability to grow at 37°C. is generally mentioned as an important characteristic of *Cr. neoformans*. Out of the 26 strains of this species recorded by Lodder and Kreger-van Rij (1952) 25 grow at this temperature. The strain which makes the exception (CBS. 963) was isolated from the air. The two strains included in the variety do not grow at 37°C.

In the last few years we have investigated strains of *Cr. neoformans* which were not, or only slightly mucous, and which grew at 37°C. Moreover, from one source of isolation more and less mucous strains were obtained all growing at 37°C. The question arose whether these non-mucous forms could be identified with the type strain of the variety *uniguttulatus* which does not grow at 37°C., in other words, whether a reduced capsule was in this case a suitable characteristic for separating the variety from the species. To find an answer to this question capsule formation, growth at 37°C. and the assimilation of a greater number of carbon compounds were studied in strains of the species and the variety.

**Material and methods.**

33 strains were studied. All of them corresponded with the standard