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THE SPECIES DIPODASCOPSIS UNINUCLEATA (BIGGS) BATRA & MILLNER

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(With three Text-figures)

Morphological and physiological properties of Dipodascopsis uninucleata (Biggs) Batra & Millner are described. Two varieties, var. uninucleata and var. wickerhamii nov. var. are recognized.

Introduction

Biggs (1937) gave the following description of Dipodascus uninucleatus:

"Colony circular pulvinate; edge wavy, surface glistening, gyrosely wrinkled; consistency butyrous; color opaque; coloration of the medium none. Hyphae branched, septate, constricted at the cross walls, 2.4–2.6 μ in diameter in young cultures, in old cultures very irregular. Asexual reproduction none. Cells consistently uninucleate. Asci elongate, multisporous, diameter 7–8 μ tapering to 4–5 μ at the tip, length extremely variable, in young cultures 90–180 μ . Spores minute ellipsoid 0.5–1.0×2.0–2.8 μ , liberated from the tip of the ascus by gradual extrusion".

The single strain of this species studied by Biggs had been isolated from a dead pupa of *Drosophila melanogaster*.

Dipodascus uninucleatus corresponds to the first species of the genus, D. albidus, in the formation of the typical elongate multispored ascus, but there are also differences between them: D. uninucleatus forms no arthrospores and the ascospores are very small; D. albidus produces arthrospores and much larger ascospores.

Batra (1959), in a study of the genus *Dipodascus*, described two strains of *D. uninucleatus*, the original one and a strain NRRL-Y-2181, and records Wickerham's finding that the second strain differs from the first one in the inability to assimilate sucrose, raffinose and inulin, and in the ability to assimilate D-arabinose and L-rhamnose.

Kreger-van Rij & Veenhuis (1974) examined the ultrastructure of *Dipodascus* species and found that the septa in *D. uninucleatus* have a simple narrow pore, whereas those of *D. aggregatus* have plasmodesmata. The spore wall in the latter species is thin at first and expands considerably later on, which is not the case in *D. uninucleatus*. The authors pointed to another important difference between *D. uninucleatus* on the one

hand and *D. albidus* and *D. aggregatus* on the other, namely the method of ascus formation. In the last two species gametangia are formed from the lateral wall of the hypha on two adjacent cells. The tips of the gametangia fuse and from the zygote a large cylindrical ascus arises "standing with two feet" on the hypha. In *D. uninucleatus* gametangia are formed at both sides of the septum between adjacent cells, and after fusion, the ascus "hangs" between the gametangiogamic cells. Apart from these differences, in *D. uninucleatus* Kreger-van Rij & Veenhuis observed a capsule on the cells and a starch reaction of the mycelium.

Batra & Millner (Batra, in the press) transferred *D. uninucleatus* to a new genus, *Dipodascopsis*, described as follows: Thin mycelium without conidia; asci formed after gametangiogamy. The gametangia arise on adjacent cells. The ascus is conical and multispored; the spores are reniform or ellipsoidal. No fermentation; nitrate is not assimilated.

In the present paper the results of the examination of three strains of *Dipodascopsis uninucleata* using the methods of "The Yeasts" (Lodder, 1970) are described. The species is subdivided into its type variety and a new variety *wickerhamii*, named after Dr. L. J. Wickerham.

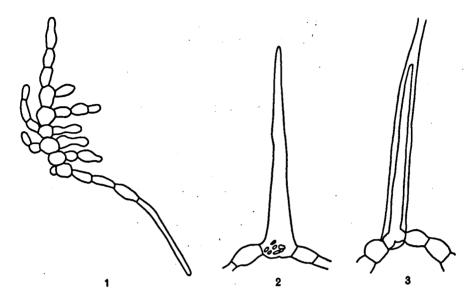
DIPODASCOPSIS UNINUCLEATA (Biggs) Batra & Millner var. UNINUCLEATA—Figs. 1-3

GROWTH IN MALT EXTRACT: After 5 days at 25 °C, a thin loose sediment is formed. After one month at room temperature, a sediment and a dull pellicle are present.

GROWTH ON MALT AGAR: Branched mycelium, often with short cells and constrictions at the septa (Fig. 1). The cells are capsulated. Old cultures give a starch reaction with iodine solution, the wall of the hyphae staining blue. Arthrospores are not formed, but the mycelium may break up between two gametangia. Side branches arise near a septum or in the middle of hyphal cells. After one month at room temperature, the culture is light brown, dull, raised, butyrous and delicately wrinkled.

Formation of the ascus: In two adjacent cells in a hypha cross walls are formed resulting in two new small cells, the gametangia, at both sides of the septum between the original cells. After fusion of the gametangia, a long tapered aerial ascus develops with numerous small reniform or ellipsoidal spores (Fig. 2). The spores do not stain with iodine solution. They are liberated from the tip of the ascus and initially remain clustered. New gametangia may be formed on the first gametangiogamic cells from the same site, and thus within the first ascus (Fig. 3). After fusion, a second ascus develops within the first one, often protruding through the apical pore. A third and a fourth ascus may follow. Occasionally, only one gametangium develops or the two gametangia do not fuse and grow out as septate hyphae inside the former ascus. Perforation of the ascus wall from the inside by the tip of such a hypha has been observed. The spores germinate by swelling to a sphere and then produce a tube. This may happen when the spores are still inside the ascus. Asci are formed on malt agar, glucose-yeast extract-peptone agar and, very profusely, on arbutin agar. Biggs (1937) found that fusion of gametangia from different hyphae may take place and she observed the development of asci without previous fusion of cells.

Fermentation: Negative.



Figs. 1-3. Dipodascopsis uninucleata var. uninucleata. — 1. Mycelium on malt agar. — 2. A long tapered ascus between two gametangiogamic cells. Only a few of the numerous ascospores are drawn in the ascus. — 3. A second ascus has developed inside the first one.

Assimilation of Carbon Compounds:

Glucose	+	D-Ribose	
Galactose	<u>.</u>	L-Rhamnose	
L-Sorbose	+ '	Ethanol	+
Sucrose	<u> </u>	Glycerol	+ (very weak) or —
Maltose	_	Erythritol	-
Cellobiose	<u>-</u>	Ribitol	+
Trehalose	+	Galactitol	
Lactose	'	D-Mannitol	_
Melibiose	+	D-Glucitol	*.
Raffinose	<u> </u>	α-Methyl-D-glucoside	+ .
Melezitose	<u> </u>	Salicin	<u>'</u>
Inulin	1	DL-Lactic acid	
Soluble starch	+ or —	Succinic acid	
D-Xylose	1 0	Citric acid	
L-Arabinose	<u> </u>	Inositol	_
D-Arabinose	+ (weak)	111031101	Т
_			
SPLITTING O	E ADRITTIN' Negative		

SPLITTING OF ARBUTIN: Negative.

Assimilation of potassium nitrate: Negative.

GROWTH IN VITAMIN-FREE MEDIUM: Negative.

GROWTH ON 50% (W/W) GLUCOSE-YEAST EXTRACT AGAR: Negative. GROWTH AT 37°C: Positive.

Cultures examined. — Two strains of this variety have been studied: the type strain described by Biggs (CBS 190.37), and a second strain received from Dr. C. P. Kurtzman with the number NRRL-Y-1268 and indicated as "Mrak 75-Burkholder".

DIPODASCOPSIS UNINUCLEATA var. wickerhamii Kreger-van Rij, var. nov.

Haec varietas a varietate uninucleata differt: L-Rhamnosum assimilatur, at non sucrosum, raffinosum et inulinum.

This variety is similar to var. uninucleata with the exception of the assimilation of the following carbon compounds:

Sucrose	:		Inulin	_
Raffinose		<u> </u>	L-Rhamnose	+

One strain of this variety has been studied and this is the type strain. It was received from Dr. C. P. Kurtzman with the number NRRL-Y-2181, and it had been isolated from *Drosophila* by Dr. H. J. Phaff. This strain has been deposited in the collection of the Centraalbureau voor Schimmelcultures at Baarn; dried material of it is preserved in the Rijksherbarium at Leiden (L).

DISCUSSION

Separation of *D. uninucleatus* from the genus *Dipodascus* and its inclusion in a new genus *Dipodascopsis* seem amply justified. The more important features distinguishing *Dipodascopsis uninucleata* from species of *Dipodascus* are: the absence of arthrospores, the ultrastructure of the septum and the lateral wall, the method of gametangium formation, and, in consequence, the position of the ascus, the formation of a second, third or fourth ascus within the first one, and the shape, size and ultrastructure of the ascospores. Perhaps of less importance are the presence of a capsule on the cells and the starch reaction of the mycelium.

Development of a second ascus within the first one has never before been observed in *Dipodascopsis*. It resembles the proliferation of asci in *Ascoidea* (Brefeld, 1891), but in this genus gametangiogamy is absent.

Examination of the strains of *Dipodascopsis uninucleata* with the methods used for yeasts reveals physiological differences among them. Therefore, two varieties are recognized differing in the assimilation of four carbon compounds. The auxanographic test proved to be suitable for showing these differences.

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