

Some Cytological Studies on Mucorales

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Introduction

Earlier studies on the nuclear behaviour in Mucorales have been mostly restricted to the observations on resting nuclei (Moreau, 1913; Baird, 1924; Callen, 1940; Cutter, 1942; Rai, 1951; Robinson, 1957; and Saksena and Sarbhoy, 1962). This was primarily due to small size of the nuclei and lack of proper techniques. With the recent development of time and techniques now it is possible to study the nuclear behaviour in great detail. The present paper deals with the nuclear behaviour of the developmental stages of spores in five species of Mucorales.

Materials and Methods

Absidia orchidis (Vuillemin) Hagem, *A. ornata* Sarbhoy, *Actinomyces elegans* (Eidam) Benjamin & Hesseltine, *Chaetocladium hesseltinei* Mehrotra et Sarbhoy and *Cunninghamella bertholletiae* Stadel were isolated from the soils of Gangetic plains. Two different techniques were used for the nuclear studies. In the first case the colonies were grown in one percent peptone solution. Two days later the aerial mycelium along with sporangia and sporangiospores were fixed in four different fixatives, viz., modified Flemming's weak solution (Saksena, 1936), Flemming's fluid (strong), Raper's and Formal-acetic-alcohol (FAA). Fixed tissues were washed in running water for twenty-four hours. These tissues were stained with 0.5 percent haematoxylin (Edmund Gurr) or saturated solution of Gentian violet. The staining technique was the same as used by Saksena & Sarbhoy (1962).

In the second case the squashes with Acetorcein were prepared as described by Sharma & Mukherjee (1955). The slides were fixed and stained according to the method of Riddell (1950). All the slides were examined at a magnification of $\times 1,000$.

Nuclei were observed at various stages in different parts of the material fixed with modified Flemming's weak solution and Raper's Fixative. In the resting stage these nuclei were seen surrounded by a nuclear membrane. Each nucleus had a nucleolus. The nuclear behaviour depended on different stages of fungal development.

Sporangiospores

Dormant sporangiospores of all the species studied were mostly multinucleate and rarely uni- or bi-nucleate. The number of nuclei present in each sporangiospore ranged mostly from 4 to 10 only (Fig. 1, A).

Spore Germination

The spores started germinating in all the species after 6 to 12 hours. They were studied first, prior to germination also. Before germination spores swelled by absorbing moisture but the number of the nuclei remained the same as in the dormant stage. On germination, one or two germ tubes were formed and at that time the nuclei in them or in the spores were slightly elongated (Fig. 1, A & B). These elongated nuclei were in the process of division. Finally all the divided nuclei migrated into the germ tubes, forming a typical coenocytic hypha (Fig. 1, B & C). Later divisions of the nuclei were mainly by constriction (Fig. 1, E). Faint spindles with highly chromatic masses of the central body lying in the equatorial region were seen in *Absidia orchidis* only.

Sporangium

In the process of sporangium development mycelial tips swell and the cytoplasm developed many vacuoles in the sporangium. The nuclei in the swollen tips divide very quickly forming a multinucleate sporangium (Fig. 1). The number of nuclei per sporangium greatly varied but mostly they were thirty to forty.

Discussion

There are two types of nuclear divisions occurring in Mucorales (Cutter, 1942, a). He stated that in the rapidly growing hyphae mitosis took place. Generally the formations of the faint spindles with highly chromatic masses of the central body lying in the equatorial region were seen. These chromatic mass separated into two nearly equal halves which moved towards the poles of the spindle. After a time, when these masses reached the poles, the spindle disappeared, a clear region was formed around them and soon after, the two daughter nuclei were seen each with a nuclear membrane. The author only observed structures with faint spindles in the case of *Absidia orchidis*. There is also some evidence that similar structures occur in *Cunninghamella echinulata* (Raizada, 1957). The dormant spores were multinucleate as reported by Cutter for different species of Mucorales studied by him. The present results are in contradiction with Cutter in the presence of uni-, bi- and multinucleate types of spores. Earlier workers could find only multinucleate types (Callen, 1940;

Cutter, 1942 and 1942 a). These uni- and binucleatae types were as viable as the multinucleate ones.

The best results were obtained in Raper's fixative and modified Flemming's weak solution. Dividing nuclei were not observed in the squash preparations.

Constrictions of nuclei during amitosis were seen in mature hyphae in the present studies. Cutter (1942), Robinow (1957) and Bakerspigel (1960) also reported similar results in the case of fungi studied by them.

Summary

Nuclear behaviour of five different species of the order Mucorales was studied in detail.

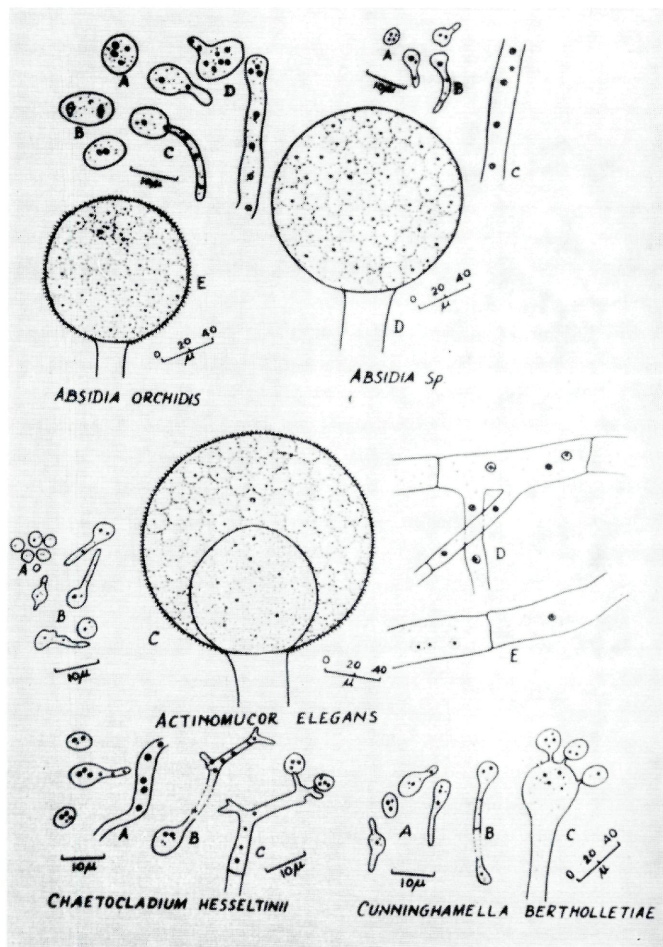
The uni-, bi- and multinucleate spores were found in all the species. Dividing nuclei in *Absidia orchidis* formed faint spindles, in other species only a slight elongation of the nuclei was observed. Nuclei in mature hyphae divided by constrictions.

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Plate 1. Showing the various stages of nuclear division in the following five species

- (i) *Absidia orchidis*
- (ii) *Absidia* sp. (*Absidia ornata* Sarbhoy)
- (iii) *Actinomucor elegans*
- (iv) *Chaetocladium hesseltinei* and
- (v) *Cunninghamella bertholletiae*

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