Bot. Bull. Academia Sinica **13**: 82-91 (1972)

NUCLEAR BEHAVIOR OF CULTIVATED MUSHROOM

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Abstract

Cytological techniques were used to study the nuclear behaviors in the basidium, basidiospore and mycelium of *Agaricus bisporus*. The basidia, 5.0-7.0 ×11.5-22.0 μ, are club-shaped and contains two nuclei. As the basidium develops, the two nuclei fuse to form a larger diploid nucleus which proceeds to meiosis. In the first meiotic prophase, nine bivalent chromosomes were observed. In the metaphase, all chromosomes arranged on the equatorial plate and the spindles were either parallel or un-parallel to the long axis of the basidium. Both first and second meiosis take place in the basidium resulting in the formation of tetrad nuclei.

The size of basidiospores ranged between 3.6-8.3 × 5.0-15.2 μ, Number of nuclei in a basidium can either be one, two, three, four, five, six seven, or eight. During spore maturation the original nucleus or nuclei may divided further by mitosis, and the nuclear division of the basidiospores is unsynchronous.

Each vegetative cell contains 4 to 25 nuclei, most often between 6 to 10. The size of the nucleus ronges from 0.9 to 1.4 μ, The nuclei of a hyphal cell do not divide simultaneously. Though the typical metaphase plate and centrioles were not observed during the division of hyphal nuclei, the spindle and dot-like chromosomes were visible. The clamp connection was not observed, but the fusion of the mycelia occurred frequently, there was a high frequency of nucler movement between the fusion hyphae.

Introduction

Since 1954, mushroom culture has become a booming industry in Taiwan (Luh, 1967). However, the production per unit area is still far from comparable to those of United States and Japan (Hu and Song, 1967). Whereas the cause for the inferior per unit production is not known, it is suggested that genetic defect of the spawn might be a contributing factor. In order to elucidate the problem, it is felt necessary to clarify the cytological as well as the genetic features of the mushroom cultivated locally.

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Cytogenetic studies on *Agaricus bisporus* have been done by several researchers. Evans (1956), Lin and Wu (1968) studied the nuclear behavior both in somatic cells and basidium. Kligman (1943), Sass (1936) and Jiři (1965) especially concentrated on the meiotic process and the behaviour of the haploid nuclei in basidia and basidiospores. Meiotic behaviors and chromosome number of the fungus, however, are not clear. We report here the nuclear phenomena in the basidia, basidiospores and hyphal cells.

Materials and Methods

The cultures of *Agaricus bisporus* (#101) used in this investigation was supplied by the Mushroom Laboratory of National Taiwan University, maintained in malt yeast extract agar containning malt extract 15 gram, yeast extract 3 gram, and 20 gram agar in a liter of distilled water. Spawn was then prepared and planted in well prepared compost in the Mushroom Laboratory of the University.

In order to study basidia and basidiospores, young gills were severed from fruiting-bodies whose pileus was convex in shape and the veil just separated from the stipe. The gills were squashed on slides and stained with HCl-Giemsa (Aist, 1965; Lin and Wu, 1968). After fixed in Carnoy's fluid (Absolute ethyl alcohol: chloroform: glacial acetic acid, 6:3:1) or Farmer's fluid (Absolute ethyl alcohol: glacial acetic acid, 3:1) for 30 minutes, the slides were rehydrated in 95% ethyl alcohol for 20 minutes followed by 75% ethyl alcohol for 30 minutes. After rinsed with distilled water for several times, the squashed gills were hydrolyzed with 1 N HCl at 60°C for 10 minutes. The preparation was rinsed with water again and stained with freshly prepared 5% HCl-Giemsa solution for 40 minutes. After repeated rinses with water, the excessive water drops on slides were removed with a piece of filter paper, then the specimens were mounted with Canada balsm for examination under phase contrast microscope. The Giemsa solution was prepared with one ml Giemsa stock solution (Kanto Chemical Co. Inc.) and 10 ml 0.1 M phosphate buffer (pH 7), the solution was filtered through filter paper (Toyo No. 5) before use.

For cytological study of vegetative mycelium, six pieces of cover slips were laid on a circular filter paper which in turn was placed in a petri-dish. The petri-dish with entire content was sterilized and a mycelial disc (3 mm in diameter) inoculated on each cover slip and some sterile water was added to maintain moisture. Several days after inoculation, the vegetative mycelia were stained with HCl-Giemsa technique as previously described.

All preparations were examined with a phase contrast equipment (Olympus PA) using positive contrast objectives (PLL 20×, 40×, 100×) and a green or

blue filters. Measurements were made with a Filar micrometer eyespiece (OSM) inserted in Olympus microscope, and photographs were taken with black and white negative Kodak Panatomic-X.

Results

Nuclear behavior in basidium

The club-shaped basidium, 3.7–7.0×11.5–22.0 μ, generally has two sterigmata on it's terminal (Fig. 1). A young basidium contains two oval or ellipsoidal nuclei of 0.9 to 1.4 μ in diameter (Fig. 2). As it develops, the two haploid nuclei gradually fused resulted in a single large diploid nucleus (Fig. 3, 4, 5, 6). During the initial stage of fusion, a dumbbell-shaped nucleus was observed. There was a nucleolus in the center of the diploid nucleus (Fig. 7). The subhymenium cells have two nuclei (Fig. 2, 8).

In early meiotic prophase, the chromosomes appeared as long slender threads with many bead-like structures coiled each other (Fig. 9). In zygotene stage, the homologous chromosomes appeared attracted to each other (Fig. 10, 11). The paired chromosomes then progressively shortened in the pachytene stage (Fig. 12, 13). At the diplotene stage, each chromosome became bivalent and splits lengthwise into two halves, the separation was distinctly visible except at the regions of chiasmata (Fig. 14). The chromosomes became more compact and thicker bodies (Fig. 15, 16, 17) at diakinesis. The chromosome number appeared to be nine (Fig. 12, 13, 14).

In metaphase, all bivalent chromosomes arranged on the equatorial plate was similar to that of *Coprinus macrorhizus* Rea. f. *microsporus* Hongo (Kimura and Takemaru, 1955b) (Fig. 18), and the direction of nuclear division was observed parallel to the long axis of the basidium. The two univalent components separated at anaphase (Fig. 19, 20). Consequently, two daughter nuclei were formed and the first meiotic division was completed (Fig. 21, 22).

After the first meiotic division, the two daughter nuclei immediately began the second meiotic division, with the tetrad nuclei thus produced in a basidium (Fig. 23, 24, 25, 26). During the second nuclear division, the spindles were usually obliquely placed in the basidium, and always parallel to each other (Fig. 24). Actual migration of nuclei through the sterigma into the basidiospore was not observed.

Nuclear behavior in basidiospore

Size of the oval or ellipsoidal basidiospore ranged between 3.6–8.3×5.0–15.2 μ with thick cell wall and without germination pore. Besides one (Fig. 27, 28, 29), two (Fig. 30, 31, 32), three (Fig. 33, 34, 35) and four nuclei (Fig. 36, 37, 38) in a basidiospore, five (Fig. 39), six (Fig. 40), seven (Fig. 41) and eight

nuclei basidiospores (Fig. 42) were also found precisely in the present investigation. The frequency of nuclear distribution in the basidiospore, as shown in Table 1, indicated that the basidiospores with two nuclei were predominant.

Table 1. *Frequency of nuclear distribution in the basidiospore of Agaricus bisporus.*

No. of nucles	1	2	3	4	5	6	7	8	Total
No. of spore	148	360	72	159	14	8	3	4	768
Frequency(%)	19.27	46.86	9.38	20.70	1.82	1.04	0.39	0.52	100

Nuclear behavior in mycelium

The hyphal cells were 2.0–7.5×24.5–156.0 μ and multinucleated. Number of nuclei per hyphal cell was extremely variable. Generally it ranged from four to twenty-five, but most often between six to ten (Fig. 43, 44). However, as many as thirty nuclei were observed in the young hyphal cells (Fig. 45) and in the older cells the nuclei may reduced to one, two or no nucleus at all. The distribution of nuclei in hyphal cells was random and showed no tendency of pairing. Sometimes, several nuclei laid closely together (Fig. 45).

The size of nucleus measured between 0.9 to 1.4 μ in diameter. Division of the nuclei in a cell was not synchronous (Fig. 49) and the direction of mitotic apparati were either parallel or perpendicular to the long axis of the hyphae. At the beginning of nuclear division, chromosomes contracted gradually, resulting in nine distinct chromosomes (Fig. 46, 47). Typical metaphase plate was not seen during the nuclear division of hyphal cells. At the anaphase, the two clusters of daughter chromosomes pulled apart, and separated into two rounded daughter nuclei at the telophase (Fig. 48, 49).

Although clamp connection was not observed, the fusion of the mycelia occurred frequently. Nuclear exchange between hypha took place via hyphal anastomosis (Fig. 50). There was a high frequency of nuclear movement between the fused hypha, and the moving nuclei appeared in slender shape.

Discussion

Since the fructification of the fungus is genetically regulated (Pelham, 1967), it is important to clarify the cytological feature of the cultivated mushroom, *Agaricus bisporus* to serve as a foundamental information for the genetics and breeding of the mushroom.

Sass (1929) grouped the two-spored Hymenomycetes in two categories on the basis of the nuclear cycle: (1) Haploid forms, in which there is no karyogamy. The vegetative and hymenial cells are uniformly uninucleated

and only one nuclear division occurred in the basidium. Each spore received one nucleus and nuclear division might occur in the spore; (2) Diploid forms, in which karyogamy occurred and was followed by meiosis, producing four nuclei in the basidium. The cultivated mushroom reported in this paper belongs to the latter as far as the nuclear behavior was concerned.

The young basidium of *Agaricus bisporus* is binucleated in the primary stage. After the fusion of these two nuclei, meiosis follows and gives rise to four daughter nuclei. All this can be confirmed in the electron microscopic observation (Thielke, 1969). This is similar to the nuclear behavior in the basidia of other higher fungi (Chang and Chu, 1969; Huffman, 1968; Kimura and Takemaru, 1955a, 1955b; McClaren, 1967). During meiosis, thread-like chromosomes are also observed. This is reminiscent to the observations for *Coprinus atramentarius* (McClaren, 1967), *Coprinus lagopus* and *Coprinus comatus* Lu and Raju, 1970).

Controversial statements on the number of chromosomes indicate the difficulty in working with this particular fungus, the cultivated mushroom. Colson (1935) found that the haploid chromosome was nine in both two-spored cultivated and fourspored wild mushroom of *Agaricus.* Kligman (1943) and Jiři (1965) also found the chromosome number of cultivated mushroom to be nine. However, Evans (1959), and Lin and Wu (1968) found the haploid chromosome number to be 12, and according to Sass (1928) and Sarazin (1938) it is 4. In the present investigation, nine bivalent chromosomes were observed at the late meioticprophase and metaphase as well as during the somatic nuclear division. The discrepancies on the chromosome count may be due to the different techniques such as squash method and paraffin section method or different isolates used for cytological studies (Evans, 1956).

Generally speaking, each spore of the four-spored form Basidiomycete receives one nucleus which divides once in the spore. In the two-spored forms, there is considerable evidence to indicate that a spore may receive two nuclei from the basidium (Jiři, 1965). In the case of *Naucoria semiorbicularis* f. *bispora* and *Galera tenera* f. *bispora,* several nuclear divisions occur in the basidiospores thus the mature spores contains eight nuclei before germination (Sals, 1929). Table 1 indicates that basidiospores of the cultivated mushroom are usually binucleated, occasionally uninucleated and tetranucleated. Spores with more than four nuclei are also observed. The uninucleated, parts of the binucleated and tetranucleated basidiospores may be due to the migration of the previously divided nuclei in basidium. Because, besides two-spored form, one, three, four and more than four basidiospores were formed to be borne on a basidium (Song *et al.*, 1972). Parts of the binucleated and tetranucleated spores and the occurrnece of five, six, seven, and eight nucleated spores may

suggest mitotic division of the one, two or four nuclei which enter the basidiospores (Fritsche, 1965; Sass, 1929; Wakayama, 1930), whereas three, five, and seven nuclei indicate the un-synchronous somatic division of the nuclei in basidiospore.

Because the nuclei divide independently, a single hyphal cell contains a different number of the nuclei (Evans, 1959; Fritsche, 1965). Kligman (1943) described that the nuclear number of hyphal cell of the cultivated mushroom ranging ordinarily from 7 to 20, sometimes as many as 25 to 28 in long cells. He also stated that the nuclei in each cell were disposed at random and showed no observable tendency of pairing. Division of the nuclei in a cell did not occur simultaneously. Our results for the mushroom are similar to Kligman's observations.

There are two opposite opinions in the somatic division of the fungi. Bakerspigel (1959), Dowding and Weijer (1962), Robinow and Caten (1969) and Saksena (1961) held that the somatic nuclei undergo non-classical mitotic division. The process of non-classical mitosis is simple, the nucleus increases in size and elongate, constrict at the midregion, finally, two daughter nuclei are formed. On the other hand, Brushaber *et al.* (1967), Hartmen (1964) and Kno-Davies (1967) observed that the nuclear division in the fungal mycelium undergo classical mitotic division. Furthermore, Chang and Ling (1970) and Kowalski (1966) substantiated that both the classic mitosis and non-classic mitosis were found in the vegetative hyphae. Though centrioles and typical metaphase plate were not seen during the mycelial mitosis, spindles and nine chromosomes were observed in this investigation. The authors therefore suggest that the mitosis of the vegetative hyphae is similar to that in the higher plants.

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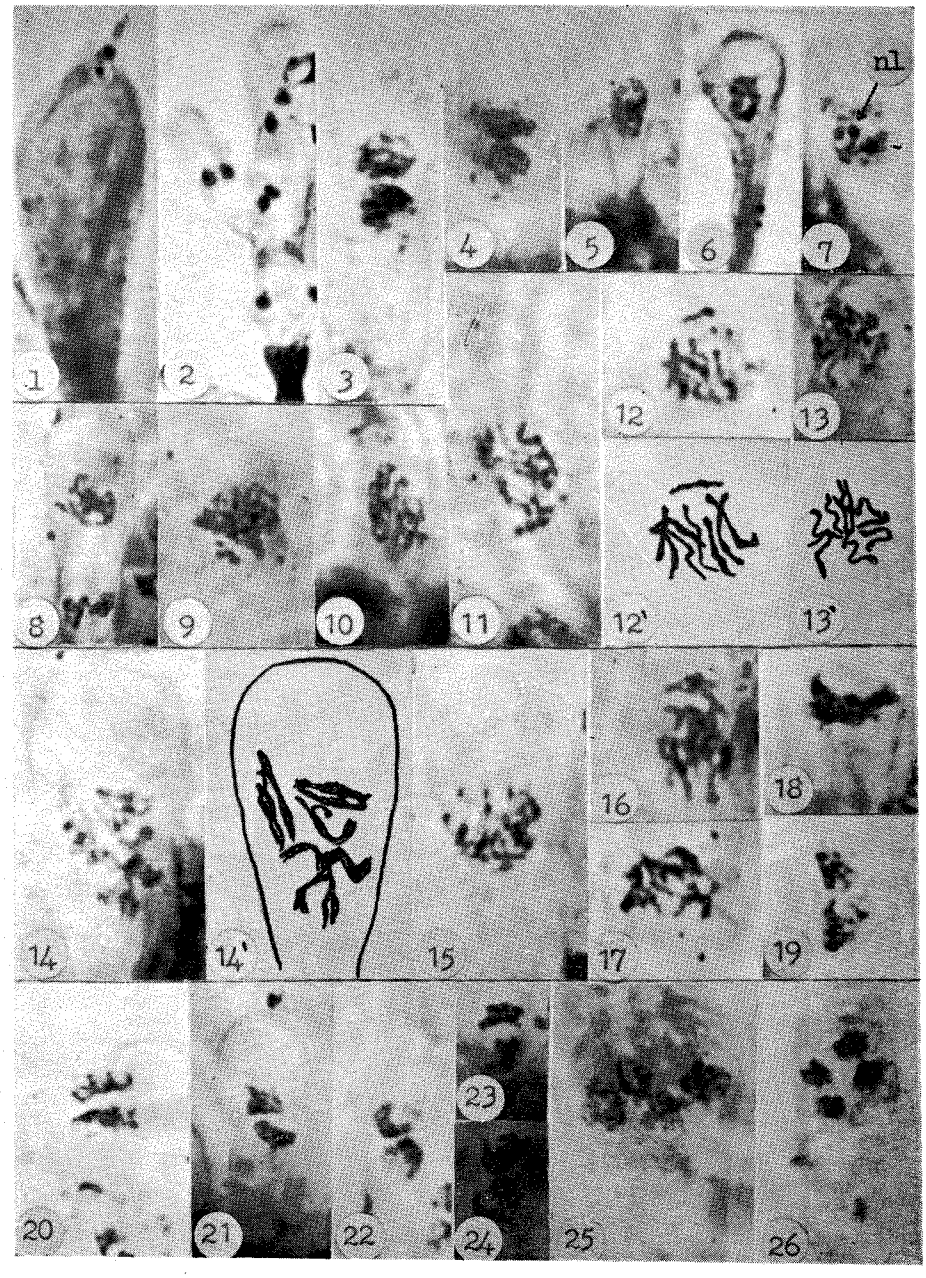

Fig. 1-26. Nuclear behavior in basidium of cultivated mushroom, *A. bisporus*. Fig. 1. Basidium of *A. bisporus* with two sterigmata. ×3200. Fig. 2. Two haploid nuclei in young basidium. ×1650. Fig. 3. Basidium with prefusion nuclei. ×2400. Fig. 4. Early fusion in the basidium, the nuclei appear as dumbbell-shaped. ×2400. Fig. 5, 6. Diploid nucleus. ×200. Fig. 7. Diploid nucleus with nucleolus (nl). ×2800. Fig. 8. Subhymenium cell with two nuclei and the basidium with a nucleus. ×2400. Fig. 9. Chromosomes with bead-like structures in early prophase 1. ×2800. Fig. 10, 11. Zygotene of prophase 1. ×2800, ×3500. Fig. 12, 13. Pachytene in prophase 1, showing nine chromosomes. ×3500. Fig. 12', 13'. Reconstruction of chromosomes from Fig. 12, 13. Fig. 14. Diplotene in prophase 1 with chiasmata. ×3500. Fig. 15, 16, 17. Diakinesis of prophase 1. ×3500. Fig. 18. Metaphase 1. Note the chromosomes arranged on the metaphase plate. ×3500. Fig. 19, 20. Anaphase 1, two univalent components began to separate. ×2800. Fig. 21, 22. Completion of first meiotic division. ×2650. Fig. 23. Metaphase 2. ×2200. Fig. 24. Anaphase 2. ×2200. Fig. 25. Telophase 2. ×3200. Fig. 26. Basidium with tetrad nuclei. ×3000.

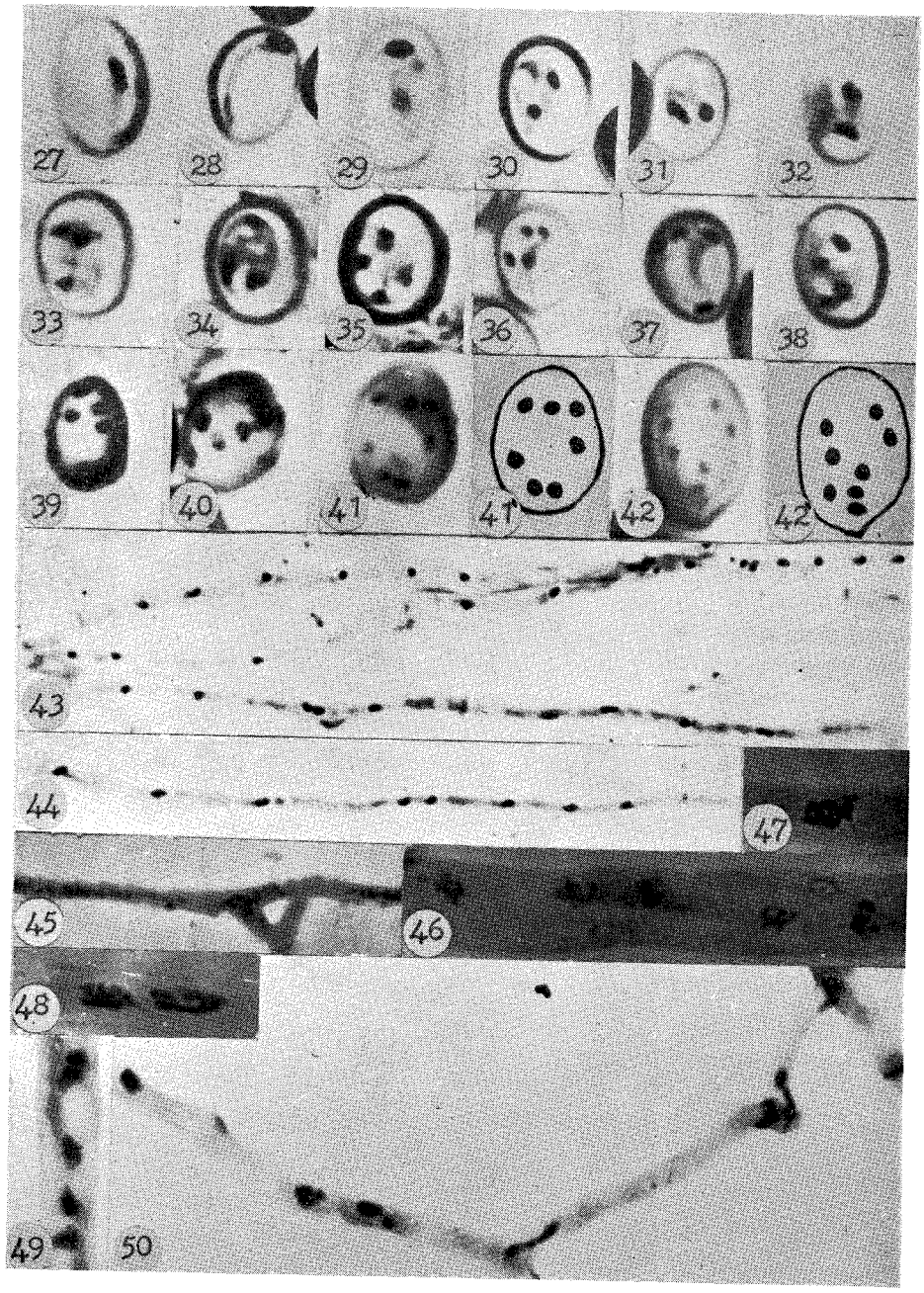

Fig. 27-50. Nuclear behavior in basidiospore and mycelium of cultivated mushroom, *A. bisporus*. Fig. 27, 28, 29. Uninucleate basidiospores. ×2400. Fig. 30, 31, 32. Bincleate basidiospore. ×2400. Fig. 33, 34, 35. Basidiospore with 3 nuclei. ×2400. Fig. 36, 37, 38. Basidiospore with 4 nuclei. ×2400. Fig. 39. Basidiospore with 5 nuclei. ×2400. Fig. 40. Basidiospore with 6 nuclei. ×2400. Fig. 41. Basidiospore with 7 nuclei. ×2500. Fig. 41′. Reconstruction of Fig. 41. Fig. 42. Basidiospore with 8 nuclei. ×2500. Fig. 42′. Reconstruction of Fig. 42. Fig. 43, 44. Multinucleate hyphal cell. ×450. Fig. 45. Young hyphal cell with about 30 nuclei. Note several nuclei lie close together. ×450. Fig. 46. Prophase of mitosis in hyphal cell. The chromosomal materials form a reticulum. ×2400. Fig. 47. Somatic mitosis with 9 chromosomes. ×2400. Fig. 48. Anaphase of mitosis, two clusters of daughter chromosomes are pulling apart. ×2400. Fig. 49. Telophase of mitosis. The nuclear division is not synchronous. ×1800. Fig. 50. Hyphal anastomosis. Note the moving nuclei elongate into slender shape. ×1200.

洋菇細胞核行為的研究

侯信雄 吳龍溪

洋菇擔子柄（Basidium）呈棍棒狀，大小爲 3.0～7.0×11.5～22.0 μ，一般於先端形成2枝小柄。吉氏染液（HCl-Giemsa solution）染色後可見初期含有2核，隨擔子柄之發育而結合變大，繼行減數分裂（Meiosis）。分裂過程中可見9對染色體。中期染色體排列在赤道板上，而後逐漸向兩極移動，形成2個子核並再分裂一次，因此成熟擔子柄內含有4核。核分裂中可見紡錘絲。分裂方向不定。

擔孢子圓形或橢圓形，直徑在 3.6～8.3×5.0～15.2 μ 之間。核數變異很大，除2核及4核外、並有具 1, 3, 5, 6, 7 以及8核的擔孢子。可知細胞核在擔孢子成熟過程中仍有分裂現象。3, 5, 7 核的存在，證明擔孢子內核分裂並非同時進行。

營養菌絲每一細胞 4～25 核，但以 6～10 核爲常，無配對的現象。細胞核直徑 0.9～1.4 μ。同一細胞內核分裂不同時進行，分裂方向亦不定。分裂過程中雖未發現典型中心體及赤道板，但初期及中期均可見染色體。後期形成兩染色體團，向兩極分開形成子核。無扣子體（Clamp connection），但菌絲間經常有結合現象（Anastomosis）。結合菌絲細胞核移動頻繁，並有核交換的現象。移動中的細胞核呈細長形。