Ultrastructure of conidia and conidium ontogeny of *Pestalotiopsis palmarum*

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Taxonomy of Coelomycetous fungi is still a matter of controversy and is hampered by different mode of conidium ontogeny. The definition of annellidic and phialidic conidium ontogeny was proposed by Hughes (7). It has been modified by Kendrick (9), Hamill (4, 5) and Carroll and Carroll (1). But a little attention has been paid on ultrastructural studies in genera of Coelomycetes by Sutton and Sandhu (12), Jones (8), Purohit and Chawla (11). Therefore, keeping this aspect in mind ultrastructural studies were performed to reveal the annellidic mode of conidiogenesis in the fungus *P. palmarum*. Leaves of *Phoenix sylvestris* infected with *Pestalotiopsis palmarum*, were collected from Mount Abu. The fungus was isolated and cultured on potato dextrose agar medium (PDA) at 28±2°C. The colonies of *P. palmarum* developed after 2-3 days and sporulated within 7 days. Acervuli and conidia from sporulating culture of *P. palmarum* were first fixed over night in 2.5% glutaraldehyde and washed two times in 1% phosphate buffer (pH-7.2) for 1 hr and dehydrated in an acetone series of 50, 70, 80, 90 and 95% for ~ hr, each followed by a final change in 100% copper sulphate. The samples were critical point dried in liquid CO₂ and mounted on specimen stubs and coated with gold. The material was examined under Philips SEM-513 B and LEO 4355 VP Stereoscan microscope.

For the TEM studies, the sporulating culture of *P. palmarum* was pre fixed in 2.5% glutaraldehyde in 0.1M phosphate buffer for 2 h. and was again washed in 0.1 M phosphate buffer for 1hr. These samples were dehydrated in 30, 50, 70, 80 and 90% acetone series for ½ hr each, followed by two changes in dry acetone and finally cleaned with toluene. Samples were immersed first in a mixture of araldite and toluene at room temperature and finally in araldite mixture at 50°C for 2 hr. The specimens were placed over night in the block of araldite mixture at 60°C in a polymerization oven. Semithin sections of samples were cut (1μ) and stained with 1% toluidene blue in 0.1% borax and observed under light microscope for selecting the proper region. Subsequently ultra thin sections of the samples were cut with a glass knife on an ultratome, mounted on copper grids and post stained with saturated solution of 50% uranyl acetate and lead citrate for 10 minutes each. The specimen-mounted grids were dried and viewed under Philips C M 10 TEM.

The primary conidium arises as a protrusion of the apex of conidiogenous cells and develops holoblastically. The wall of conidiogenous cells is continuous with the conidial initial. The developing primary conidium initial elongates and shows thickening of vertical walls. The primary conidium secedes as a result of schizolytic ruputuring of the outer conidial wall layer adjacent to the septum. The septal pore is plugged by Woronin body (Fig. 1). Thus the primary conidium development is clearly holoblastic (Fig. 1). The septum at the base of conidium is double layered and each layer performed separate function during conidial maturation and proliferation of conidiogenous cell. Formation of successive conidia from conidiogenous cell is enteroblastic (Fig. 2). The outer annellide wall layer usually ruputuring at the base of first formed conidium adjacent to the double layered septum leaving an annellation.
Subsequently, the annellide undergoes percurrent proliferation after maturation of each enteroblastic conidium leaving a series of the annellations at annellide apex. During formation of successive enteroblastic conidia, the central septal pore at the base of each conidium, which seceded from the annellide is sealed by a Woronin body, similar to holoblastic conidium.

Electron micrograph of longitudinal section through the conidium shows three median thick walled coloured cells between two thin walled hyaline cells. (Fig. 4) The cytoplasm of each median cell has a nucleus, numerous mitochondria, and large number of small vacuoles. Each conidium has distinct wall showing an outer melanized electron dense zone and inner hyaline electron transparent zone (Fig. 4). The apical and basal hyaline cells show cytolysis and are devoid of cytoplasm contents. The basal appendage develops endogenously as an extension of the inner hyaline zone of basal hyaline cell while apical appendages develop during conidium initiation from extension of the inner hyaline zone of apical hyaline cell (Fig. 3). The apical appendages branch at later stage. Scanning electron microscopic studies show that conidia are smooth surfaced with wrinkled lower most median coloured cells (Fig. 5).

Figs.1-5. (1) Transmission electron micrograph of conidiogenous cell with holoblastic conidium (x 6300); (2) Transmission electron micrograph of conidiogenous cell with enteroblastic conidium (x 4600); (3) Transmission electron micrograph of upper appendages (x 7300); (4) Transmission electron micrograph of conidium (x 2400); (5) Scanning electron micrograph of Single Conidium of *P. palmarum* (x 5000)

AA = Apical Appendages; AC = Apical Cell; UCC = Upper Colour Cell; MCC = Middle Colour Cell; LCC = Lower Colour Cell; BC = Basal Cell; BA = Basal Appendage; HC = Holoblastic Conidium; EC = Enteroblastic Conidium; A = Annellation; WB = Woronin Body; CC = Conidiogenous Cell; S = Septum
Cole and Samson (2) and Cole (3) proposed, on the basis of ultrastructural studies of some hyphomycetes, the formation of first conidium in annellidic and phialidic mode of conidiogenesis is holoblastic. Present ultrastructural studies of conidiogenesis in *Pestalotopsis palmarum* also revealed that the first conidium from annellide is formed holoblastically. Hammill (4) noted that in annellidic fungi the only primary conidium is holoblastically produced and successive conidia are developed by blowing out an inner wall layer of the conidiogenous cell i.e. enteroblastic. Similar development has been seen in present study. Cole and Samson (2) opined that outer annellide wall layer usually ruptures at the base of the first conidium adjacent to double layered septum leaving an annellation. In the present fungus the double layer septum and annellation has been seen. Morgan-Jones et al (10) reported that in annellidic development the percurrent proliferation of the conidiogenous cell must involve the half septum left behind after secession of previous conidium. In present fungus the percurrent proliferation or annellation has been seen. Carrol and Carrol (1), Cole and Samson (2) and Hammill (6) reported that presence of pore and Woronin bodies in delimiting septa are additional characteristic features of annellidic conidiogenesis. The septal pore at the base of conidium in *P. palmarum* with Woronin body was also observed showing an important feature of annellidic development. The number of conidia developed through each successively formed annellation is also considered as a characteristic feature of proliferating annellides. Purohit and Chawla (11) reported that the single layer conidium wall is differentiated into an outer melanized electron dense and inner hyaline electron transperant zone with basal hyaline wrinkled cell. Similar result was found during present studies. Therefore, on the basis of the present studies it can be concluded that both the hyphomycetes and the coelomycetes show similar pattern of annellidic conidiogenesis.

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REFERENCES


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