

## Chlamydospore Development in Soil by *Protomyces thirumalacharii*

*Protomyces thirumalacharii* Pavgi, the incitant of purple leaf spot of *Sesbania grandiflora* Pers., is of common occurrence in Varanasi<sup>1</sup>. The chlamydospores developed abundantly in the mesophyllar interspaces are liberated over the soil after rotting and disintegration of the infected leaflets. They germinate by forming (endo)spores in the extruded vesicles and the (endo)spores germinate to form a vegetative mycelium under favorable conditions of moisture and moderately high temperature during the rainy season (July–August). The growth on potato dextrose agar (PDA) medium is snow-white when young, turning creamy yellow to greyish brown on aging. Formation of chlamydospores was observed on PDA (with 0.1% yeast extract) and host extract cultures incubated at 26–28°C for 35–40 days. Development of chlamydospores in artificial cultures indicated possibility of their formation in the field soil.

Mycelial mats from 15-day-plated colonies on PDA, washed in sterile distilled water and spun into fragments were uniformly mixed in partially sterilized soil (autoclaved at 15 lb/sq. for 30 min), dispensed in lots of approx. 100 g into 500-ml Erlenmeyer flasks and aseptically moistened with 10–15 ml sterile distilled water; the flasks were incubated at temperatures ranging between 20 and 35°C. The cultures were sampled at regular intervals. Mycelial growth removed by sifting the soil after 15 days appeared viable at all the temperatures and was profuse at 25°C. The protoplasmic contents from the neighboring cells accumulated in most of the apical cells, leaving the former vacuolate. The terminally borne, thin-walled, hyaline and globular chlamydospore initials were observed after a month in the soil cultures incubated at 25–28°C. The spore walls thickened, became deep chocolate brown developing characteristic ornamentations and the spores matured in about 45 days. No chlamydospores developed in cultures incubated at 20 and 35°C. The chlamydospores were induced to germinate on slide mounts as suggested by THIRUMALACHAR et al.<sup>3,4</sup> after a steep in acidulated distilled water<sup>4</sup>. The development and germination of chlamydospores on PDA and in soil cultures were normal and similar to those from the infected leaf tissues. Viable chlamydospores were recovered from the infested field soil, which were likely to have been added through the leaf debris and development in the soil.

*P. thirumalacharii* is a foliar fungus parasite and was scarcely considered as a facultative soil inhabitant. The

observations present evidence of its ability to survive and hibernate in the soil humus through the chlamydospores developed in vitro. The vegetative mycelium as well plays a vital role in its survival and carry-over in the soil. Earlier studies here indicated ability of the mycelium to resist desiccation and exposure to high temperature up to 70°C. The hyphae undergo marked morphogenic changes during the period of desiccation. The oversummering chlamydospores embedded in the host tissues and those formed in the soil germinate equally well and subsequently form the vegetative mycelium in the soil. The facultative saprobic nature of the pathogen suggests its perpetuation in the soil during the host-free period and in the absence of any fresh inoculum added during the season. This ability adds another dimension to its mode of carry-over, increasing the concentration of primary soil-borne inoculum. Consequently, the disease, once started, gradually spreads with greater intensity every year. A similar trend of in vitro chlamydospore development is exhibited by *Protomyces macrosporus* Unger. Dispensation of the host by this species of *Protomyces* (both the genera belonging to the family of Protomycetaceae) is unique and not observed in any other species so far.

**Zusammenfassung.** Chlamydosporen (Dauersporen) des parasitischen Pilzes *Protomyces thirumalacharii* (Protomycetaceae, Exoascales) können sich sowohl in vitro als auch im Boden entwickeln. Dauersporenentwicklung findet in der Regel in dieser Familie nur auf der Wirtspflanze statt.

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<sup>1</sup> M. S. PAVGI, *Experientia* 21, 281 (1965).

<sup>2</sup> M. J. THIRUMALACHAR and M. S. PAVGI, *Indian Phytopath.* 3, 177 (1950).

<sup>3</sup> M. J. THIRUMALACHAR and M. J. NARASIMHAN, *Mycologia* 45, 461 (1953).

<sup>4</sup> A. N. MUKHOPADHYAY and M. S. PAVGI, *Experientia* 20, 619 (1964).

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## Formation of Gigantic Mitochondria in Hypoxic Isolated Perfused Rat Hearts

Gigantic and fused mitochondria have been reported previously in the rat diaphragm treated with plasmocid<sup>1</sup>, in the myocardium of dogs with aortic stenosis<sup>2</sup>, and in the myocardium of hypoxia dog without persantin<sup>3</sup>. The present communication reports that gigantic mitochondria are observed in the hypoxic isolated perfused rat hearts.

Hearts from healthy adult rats were isolated and perfused with Krebs-Henseleit bicarbonate medium equilibrated with 95% O<sub>2</sub>, 5% CO<sub>2</sub> with or without added 8 mM glucose. Hypoxia was induced by changing the gas mixture to 95% N<sub>2</sub>, 5% CO<sub>2</sub>. Isometric tension development and heart rate were monitored on a Sanborn recorder. At the end of each experiment, small pieces of ventricular tissue (1 mm cubes) were fixed in 1% osmium tetroxide (buffered with White's saline at pH 7) for 1 h. After dehydration, pieces of tissue were embedded in

epon 812. Sections were stained with lead citrate and examined in an RCA-3G electron microscope.

The isolated rat heart was made hypoxic by changing the perfusion medium from an oxygenated substrate-free medium to one devoid of oxygen after 15 min. Electron micrographs of the longitudinal sections of the right ventricle taken 3 min after hypoxia showed that the majority of the mitochondria had maintained their sizes. From a population of 352 mitochondria, 11% showed an increase in size, and 6.5% showed fusion. Occasionally

<sup>1</sup> C. N. SUN and D. BOWDEN, *Cytologia*, in press.

<sup>2</sup> A. WOLLENBERGER and W. SCHULZE, *J. biophys. biochem. Cytol.* 10, 285 (1961).

<sup>3</sup> B. B. LOZADA and R. P. LAGUENS, *Cardiologia*, suppl. 49, 33 (1966).