Chlamydospore Development in Soil by *Protomycopsis thirumalacharii*

Protomycopsis thirumalacharii Pavgi, the incitant of purple leaf spot of *Sesbania grandiflora* Pers., is of common occurrence in Varanasi[1]. 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 Erlenmayer 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.[3,4] after a steep in acidulated distilled water[4]. 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 *Protomycopsis* (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 *Protomycopsis 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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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[1], in the myocardium of dogs with aortic stenosis[2], and in the myocardium of hypoxia dog without persantin[3]. 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_2, 5% CO_2 with or without added 8 m*M* glucose. Hypoxia was induced by changing the gas mixture to 95% N_2, 5% CO_2. 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

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some mitochondria were found to be arranged in a disorderly manner in the heart muscle. A marked alteration of mitochondria in the heart was observed 7 min after hypoxia. From a population of 780 mitochondria, 30% had greatly increased in size and were swollen, 4% of them were small bodies or degenerated mitochondria, and 25% had fused together. 1% of the sample was huge mitochondria with floating cristae or mitochondria forming an elongated giant structure of about 6 to 7 sarcomere in length (Figure 1). Mitochondria in the early stages of fusion formed a 'giant structure' of different shapes as illustrated in Figure 2 and 3. Narrow channels or bridges were seen along the border of the adjoining mitochondria; the interior parts of the organelles had already been fused in some instances. This phenomenon is probably due to the inherent property of mitochondrial structural protein to reform hydrophobic surfaces after rupture of the mitochondrial membrane. Some of the mitochondria contained clear matrices as well as disrupted cristae. The formation of vacuoles in the myoplasm was occasionally observed.

Fig. 1, 2 and 3. Electron micrographs were taken from right ventricles of isolated rat heart 7 min after hypoxia illustrating fusion of mitochondria. × 26,000.

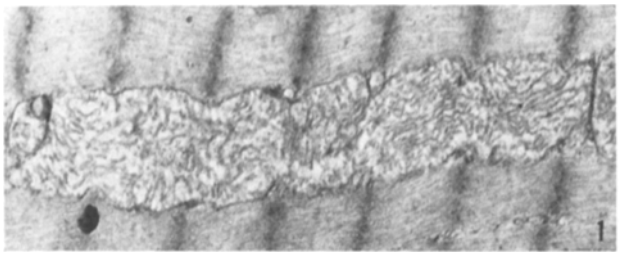

Fig. 1. A rod shaped mitochondrion ran parallel to the course of the myofibrils through 6 or 7 sarcomeres.

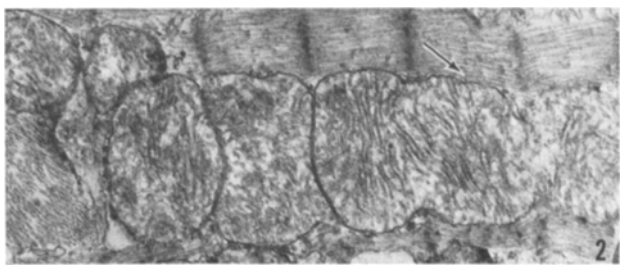

Fig. 2. A mitochondrion ran through 3 sarcomeres (arrow).

Fig. 3. Mitochondria were in different stages of fusion to form giant structures of different shapes.

Zusammenfassung. Herzen gesunder, erwachsener Ratten wurden mit Krebs-Henseleit-Bikarbonatlösung durchströmt (95% O_2:5% CO_2). Bei künstlich herbeigeführter Hypoxie (95% N_2:5% CO_2) erschienen die Herzmitochondrien innerhalb von 7 min bedeutend vergrössert und zu 25% miteinander verschmolzen.

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Observation on the Metamorphosis of Larvae of *Ascidia malaca* in Sea Water Devoid of $SO_4^=$ Ions

Previous investigations carried out on embryos of Ascidiaceae (*Ciona intestinalis* L., *Ascidia malaca* and *Phallusia mamillata*) allowed to develop in sea water devoid of $SO_4^=$ ions led to the development of larvae with marked abnormalities solely affecting the tails which were shorter than normal and exhibited anomalous muscle cells[1]. Subsequent cytochemical and autoradiographic studies led to the conclusion that the anomalies seen in the muscle cells of the tails were probably the consequence of a disorder of protein synthesis[2-4].

Preliminary studies carried out after metamorphosis had revealed changes of the morphogenesis of the adhesive papillae in *Ascidia malaca*[5]. In the present article, a report is given of the results of further experiments carried out during and after metamorphosis in embryos of *A. malaca*.

The embryos were allowed to develop in ordinary sea water, artificial sea water and $SO_4^=$-free sea water, using the methods previously described[1]; some of the embryos were photographed in vivo. In one experiment, groups of larvae developed in normal sea water or in artificial sea water to which 25 μC of $^{35}SO_4$ was added 10–12 h after hatching; after 30 min, these larvae were washed repeatedly in sea water and were then treated with Nile

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