SHORT COMMUNICATION

The Effect of Supraoptimal Temperatures on the Fine Structure of *Merulius lacrymans* (Jacq.) Fr.

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It has been known for a long time that the wood-rotting fungus *Merulius lacrymans* is very sensitive to heat. The optimum temperature for growth is about 23°, and growth is completely inhibited at 28°. At temperatures above 27° a material is excreted into the medium, which is probably a degradation product of nucleic acids (Langvad & Goksøyr, 1967). Respiration is not immediately affected, since at 37.5° the organism continued to respire with a respiratory quotient of 1 even 2 h after its death (thermal death time at 37.5° being 4 h).

This work was undertaken to study the effects of high temperatures on the fine structure of *Merulius lacrymans*.

Stock cultures of the CARTWRIGHT strain of *Merulius lacrymans* (Jacq) Fr. obtained from Centraalbureau voor Schimmelcultures, Baarn, Holland, were maintained on a malt extract medium containing 2% agar.

For electron-microscope studies colonies were grown on strips of cellophane resting on plates of 2% (w/v) malt extract agar. After two to three days at 23°, plates were incubated at 37.5° for periods ranging from 10 min to 4 h.

The colonies were fixed and embedded as described by Langvad (1971 *a, b*). The sections were examined in a Phillips EM 200 or a Siemens Elmiskop 101 electron microscope.

The results are based on several experiments in which many sections were examined. The electron-micrographs presented are selected as typical portrayals of the phenomena described.

Hyphae of *Merulius lacrymans* grown at 23° showed a normal eukaryotic ultrastructure (Fig. 1*a*). Nuclei often seemed to be very large and elongated. Ribosomes were numerous and uniformly distributed throughout the cytoplasm. Lomasomes and cytoplasmic membrane inclusions were observed in many hyphae.

At 37.5° the first effect of heat was observed after 20 min exposure. This initial effect was not restricted to a single organelle, since mitochondria, nuclei and the cytoplasm were all affected.

Mitochondria seemed to be very sensitive to heat. After 20 min exposure at 37.5° the cristae started to break down and soon disappeared leaving double membrane vesicles (Fig. 1*b*). After 1 h normal mitochondria could no longer be observed.

An early effect of high temperature was also observed on the nuclei; the nuclear membrane broke up, and material from the nucleolus leaked out (Fig. 1*c*). After 40 min exposure to 37.5° only a few compartments showed this damage but after 1 h exposure normal nuclei could no longer be observed.

The cytoplasm assumed a granular appearance when exposed to high temperatures (Fig. 1*d*). Prolonged exposure at 37.5° caused severe cytological damage. After 4 h the hyphae were completely disorganized. All organelles and membrane systems, including the

Fig. 1 (a) Section of a hypha of *Merulius lacrymans* grown at optimum temperature, 23°. N, nucleus; Nu, nucleolus; M, mitochondria; Cm, cytoplasmic membrane inclusion. (b) Different stages of breakdown of mitochondria after 20 min exposure at 37.5°. (c) Disrupted nucleus after 40 min exposure at 37.5°. (d) Hyphae showing beginning of granulation of the cytoplasm after 1 h exposure at 37.5°. (e) Completely disorganized hypha after 4 h exposure at 37.5°.

plasma membrane, were disrupted. At 4 h the hyphae contained only large granules and small pieces of membranes (Fig. 1*d*).

It has earlier been found that *Merulius lacrymans* was still able to respire (at a reduced rate) after 6 h at 37·5°, 2 h after the thermal death time at this temperature (Langvad & Goksøyr, 1967). The membrane fragments present in the compartments (Fig. 1*d*) 4 h after the onset of heat treatment could be fragments of the outermost mitochondrial membrane, and, if so, probably contain enzymes still able to respire.

It has also been found that a material is excreted into the medium when the fungus is exposed to high temperatures, most probably degradation products of nucleic acids. Fig. 1*c* shows that the nuclear membrane is disrupted, and material from the nucleolus leaks out.

Baker & Smith (1970) have studied the fine structure of germinated spores of *Rhizopus stolonifer* and *Monilinia fructicola* heated for 2·5 min at 52°. They report the absence of mitochondria in treated material and the disruption of nuclei.

The granular material in Fig. 1*d* and *e* possibly consists of aggregations of ribosomes. These structures resemble those found in heat treated *Rhizopus stolonifer* spores (Baker & Smith, 1970). These authors also draw the conclusion that they are ribosome aggregations.

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