SHORT COMMUNICATION

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

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(Accepted for publication 7 October 1971)

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 & Goksoyr, 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 (1971a, b). The sections were examined in a Phillips EM 200 or a Siemens Elmscope 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. 1a). 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. 1b). 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. 1c). 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. 1d). 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. 1d).

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. 1d) 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. 1c 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. 1d 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.

This work was in part supported by a grant from Meltzers Høgskolefond, Norway. Most of the work was done at the Astbury Department of Biophysics, University of Leeds. The help and encouragement in the work of Professor R. D. Preston and several members of his staff are gratefully acknowledged. The author wants especially to thank Mr S. Chou, Astbury Department of Biophysics, for his interest in the work, and for many discussions concerning the interpretation of the pictures.

REFERENCES


