1. EXPERIMENTAL INVESTIGATIONS ON THE TELIOSPORES OF USTILAGO MAYDIS DC

A REVIEW

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Abstract

This paper summarizes and gives a comprehensive review of the experimental results on the teliospores of *Ustilago maydis* carried out in our Laboratory by LM and TEM methods. Besides the importance of melanins, this review is about the effects of high temperature, partial degradation and X-ray irradiation of the teliospores.

Key words: Palynology, recent, *Ustilago maydis*, LM, TEM.

Introduction

The most important disease of maize is caused by smut fungi, followed by viruses, and bacteria. Of all smut genera the genus *Ustilago* most species (over 350), of which parasitize a great number of host families. *Ustilago maydis* occurs on Gramineae, especially on *Euchlaena* and *Zea*, worldwide: "Sori in stems, leaves or inflorescence forming pustules or irregular galls of considerable size, at first covered by a thin, grayish-silvery, later brown, smooth membrane which ruptures irregularly to expose the medium to dark brown, powdery spore mass. Spores globose, subglobose, ovoid to sometimes elongated or slightly irregular, 7-11 x 7-13 μm, light olive-brown; wall c. 0.5 μm thick, finely rather densely echinulate. Germination by four-celled promycelium laterally and terminally bearing basidiospores" (VÁNKY, 1985).

Ustilago maydis is well known for the extremely resistant spores, which are easily available, and occur in large masses in the infected plant. The spores retain their ability to germinate for many years. Reports of children who had fallen ill from eating bakery products, made from maize flour infected with spores of *Ustilago maydis* in 1920, in Paris (UBRIZSI, 1965). Interestingly, BUSTOS et al. (2000) and RENDUELES et al. (2000) have shown that the occurrence of the spores of *Ustilago* is moderately frequent in the air. Formers have pointed out that if the relative humidity increases, the frequency changes, for example *Phoma* and *Ustilago* become abundant.

The complex study of the biopolymer systems of the wall demonstrated that the teliospores of *Ustilago maydis* contain melanins. Therefore it is considered worth while exploring the significance of the presence of melanins.

The aim of this contribution is to give a comprehensive review of the results of experiments, which were carried out in the Cell Biological and Evolutionary Micropaleontological Laboratory.

Materials and Methods

The investigated material was collected by Dr. A. PALÁGYI on 22.8.1991. Locality: Ságvári Experimental Research Station of the Cereal Research Institute. The spores were frozen at -20°C after collection.

The experimental investigations were divided into two parts: LM and TEM methods, and included the examination of three effects: 1. High temperature, 2. Partial degradation, 3. X-ray irradiation.

1. *High temperature effect*

The high temperature effect on the spores was investigated in detail by LM method. The temperature: 200°C; length of time from 10 minutes until 300 hours: (10', 1 hr, 5 hrs, 10 hrs, 25 hrs, 50 hrs, 100 hrs, 200 hrs, 300 hrs). The slides for light-microscopical investigations were mounted in glycerine-jelly hydrated at 39.6%. 200 specimens of each sample were investigated.

2. Partial degradation

The temperature of the partial degradation was 30°C. Different organic solvents were used in LM and TEM methods. Diethylamine or merkaptoethanol were used as organic solvents. By LM 20 mg spores + 5 ml dist. water + 2 ml organic solvents, length of time: 30, 60 and 90 days. The slides for light-microscopical investigations were mounted in glycerine-jelly hydrated at 39.6%.

There were two different experiment-series with TEM. In the first series, the used organic solvent was 2-aminoethanol. There were four samples with different solvents: in the first (20 mg teliospores + 1 ml 2-aminoethanol, length of time: 24 hrs), in the second (20 mg teliospores + 1 ml 2-aminoethanol, length of time: 24 hrs, after washing + 10 ml $KMnO_4$ 1%, during 24 hrs), in the third (20 mg teliospores + 1 ml 2-aminoethanol, length of time 24 hrs, after washing + 10 ml $KMnO_4$ 1% during 48 hrs), and in the last one (20 mg teliospores + 1 ml 2-aminoethanol, length of time: 24 hrs, after washing + 10 ml $KMnO_4$ 1%, during 24 hrs, washing again + 2 ml acetic anhydride, length of time: 24 hrs) were used. But at the second series the used organic solvent was diethylamine. There was only one experiment (20 mg teliospores + 5 ml dist. water + 2 ml diethylamine, during 30 days). Partially dissolved spores were prepared for the TEM investigations as follows: Fixation in Millonig buffered OsO_4 1% (aq. dil.) for 1 hour. Washing in Millonig phosphate buffer overnight. Dehydration was performed in an ascending series of ethanol in 15 min. steps including uranyl acetate staining in 70% ethanol. The samples were embedded in Araldite, Durcupan (Fluka) epoxy resin in gelatine capsules and polymerized in 56°C thermostat for 3 days. The ultrathin sections were made on a Porter Blum ultramicrotome in the Electron Microscopical Laboratory of the Institute of Biophysics, at the Biological Research Center, Hungarian Academy of Sciences.

3. X-ray irradiation

The teliospores were irradiated with CuKα X-ray with BRON UM1 instrument at 35 KV, 20 mA. The length of time of the irradiation was, 5, 15, 35, 60 and 300 minutes. The irradiated spores were prepared for LM and TEM investigations. The methods of preparation for LM and TEM investigations were as described above.

The importance of melanins

Melanins are complex black polymers of resonance stabilized cyclic subunits with widely differing chemical composition, and as yet unknown molecular structure. They occur in most groups of living organisms, in the animal kingdom, plants and some fungi. "Melanins are insoluble in boiling water, hot concentrated mineral acids, and organic solvents, although they can be bleached by strong oxidizers like hydrogen peroxide, and some are degraded by treatment with strong alkalis" (BUTLER et al., 2001). In addition to the absorption of visible light, melanin absorbs gamma rays, X-rays, ultraviolet light, and infrared wavelengths, transferring energy deep into its molecular structure. It is remarkable that few researchers insist on limiting the term melanin to describe the alkali-insoluble black pigments synthesised by mammals, often called dopa melanins.

The usual sites of melanin deposition are the external parts of organisms (skin, integument and outer wall of cells), though melanin can occur in the internal organs as well. There are roles of melanins in the adaptive colouration of insects and lower vertebrates, bearing on the formation of humus in soils, in the preservation of geological record and can give data about the volume of cosmic radiation. Moreover, melanins take part in forming resistance to microbial degradation and provide a barrier against water loss under conditions of osmotic stress and also have a function as a photoprotector. Melanogenesis in animals is initiated in response to UV radiation. The protection function is not the exclusive domain of the melanins for there are other pigments which perform the same function, but apparently less efficiently, in animals, plants and fungi. "The high electron acceptor capacity of melanins, the presence of free radicals in them, and their semi-conductor properties may be related to the mechanisms of migration of energy in biological systems" (BLINOV, 1973, cited by PIROZYNSKI, 1975).

Many plant and animal disease are caused by melanized fungi. According to KUO and ALEXANDER (in PIROZYNSKI, 1975) melanized components of fungi in soils are more resistant to microbial degradation than their unmelanized components. There are lots of publications about the spreading of melanized microbes, for example higher numbers are found on rock and leaf surfaces and other exposed locations. The barrier offered by melanin may also provide protection against fungicides and heavy metals. BUTLER et al. (2001) emphasized the work of ZHDANOVA et al. (1994), who indicated that greater numbers of melanized and radiation-tolerant fungi were appearing in contaminated soils around the Chernobyl reactor in the Ukraine. But the heavy melanization is not good either, because heavy deposition of pigment may produce a very brittle wall and less resistance. RAST et al. (1981) established that the plate-like particles were of medium electron density and appeared to contain melanin in the form of granules (isolated from the spore wall of *Aspergillus bisporus*).

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Results

1. LM RESULTS

At first the effect of high temperature on the spores of *Ustilago maydis* was studied by KEDVES and TÓTH (1993) with the LM method. It was observed that the outer part of the spore wall became detached at 200°C. This started after 10 hours and advanced after 100 hours of heating. The writers observed that the outermost wall layers lost spores,

and when mounted in glycerine-jelly, formed interesting patterns which they considered to be very useful in the modelling of biopolymer stuctures of the partially degraded plant cell wall.

KEDVES and GÁSPÁR (1994) observed by LM that the teliospores of *Ustilago maydis* have an extremely resistant wall after dissolving the spores with diethylamine or merkaptoethanol. They supposed that melanin took part forming considerable resistance. KEDVES and GÁSPÁR (1995) did not observe any recognizable alterations during studying the results of light microscopy of X-ray irradiated teliospores of *Ustilago maydis*.

2. TEM RESULTS

KEDVES, PÁRDUTZ and BORBOLA (1998) carried out the TEM of X-ray irradiated teliospores. The relatively low X-ray dose (5 minutes) resulted in considerable alterations to the outer part of the spore wall. There were electron-dense particles in the outer wall, moreover, an electron-dense layer could be seen at the border of the exospore and epispore. The degradation of the organelles of the cytoplasm was advanced after 15 minutes irradiation. The ultrastructure of the epispore was finely lamellar or granular. In particular at the non electron-dense granuli, the sculptural elements of exospore were destroyed. At the strongest irradiation (300 minutes) the protoplasm organelles were disintegrated and epispore and endospore were homogenized and swollen. The experiment revealed molecular units such as chains, cyclic units with pentagonal or hexagonal symmetry within the wall.

KEDVES, PÁRDUTZ and MADARÁSZ (2001) investigated the ultrastructure of partially dissoluted spores with diethylamine over 30 days. This experiment revealed the biopolymer and molecular structure of the outer wall of the teliospores. Details of the linear and for the most part cyclic molecules and highly organized globular units, similar to the Penrose-like biopolymer structures, were published. These results are similar to those of the X-ray irradiation over 300 minutes.

KEDVES and BORBOLA (2002) studied the ultrastructure of the teliospores of *Ustilago maydis* with organic solvents and oxidizing agents. Important alterations were observed at the fine structure of the wall, and in the inner part of the teliospore. The most important were as follows: the endospore swelled against 2-aminoethanol; the degradation process by 2-aminoethanol, $KMnO_4$ and acetic anhydride revealed globular biopolymer units on the outer and inner surface of the exospore. Sometimes the inner part of the endospore seemed to be more electron-dense and three layered.

Discussion and Conclusions

Differences in the composition of the biopolymer systems of the walls of different kinds of spores and pollen grains can be observed by different methods. LM results supported extreme resistance of the teliospores of *Ustilago maydis*. By TEM method, the partial degradation experiments revealed different kinds of biopolymer structures in the wall layers of the teliospores. Globular biopolymer units were observed on the surfaces. RAST et al. (1981) gave an account of similar units. The differences within samples may be the consequence of the different state of maturity of the spores.

It is rather interesting that different TEM experiments were similar or nearly the same results. For instance one of the TEM results (teliospores + 1 ml 2-aminoethanol, 24 hrs. after 10 ml $KMnO_4$ 1%, 24 hrs, and 2 ml acetic anhydride, 24 hrs) were similar

to or more or less identical with the X-ray irradiated teliospores over 15 minutes. In this way, the application of the acetic anhydride as degradation agents may be useful again for the further partial degradation experiments.

The molecular alteration of the biopolymer system is carried out by aromatization process (POTONIÉ and REHNELT, 1971). Thus the study of this kind of biopolymer structure is complicate. The presence of melanins in the wall increases complexity of the biopolymer system of the wall. Results support the assumption of the protective function of melanins against radiation, but the sporopollenin without melanins may be resistant to X-ray radiation.

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