

## **Intracellular bacterial infection in *Agaricus bisporus* (Lange) Sing.**

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K a l b a r c z y k J: *Intracellular bacterial infection in Agaricus bisporus (Lange) Sing.*, Acta Mycol. XXI(2): 261-264, 1985 (1987).

Rod-shaped Gram-bacteria were observed in preparations made from the sporocarp of mummy – diseased *Agaricus bisporus* in the electron microscope. In cells of diseased rhizomorphs from several to a few dozen bacteria were found. Cells filled with a large number of bacteria were dead and the cellular wall was degraded. Probable the entrance of bacterie penetration into the mushroom cell was observed. The bacterium, after its isolation, was identified as *Pseudomonas* sp.

### INTRODUCTION

The bacteria are probably the most important factor in mushrooms growth (C u r t o 1972). The role of the bacteria in the production of *Agaricus bisporus* is particularly clear in symptomatic mushrooms. The mummy disease of the mushroom is very dangerous (S t o r e y 1954). The symptoms of the disease resemble viral paralysis but they are the characteristic feature only of this disease. This consists in an excessive development of rhizomorphs and thus the diseased sporocarp is taken from the medium together with a big tuft of spawn, the stem of the mushroom clearly dilated at the base, often spindle-shaped and curved, brown in cross-section. Diseased sporocarps are harder than healthy ones, the cover cracks too soon. The disease spreads very fast. In 1968 S c h i s t e r et al. (1968) showed that bacteria were the cause of the disease. *Pseudomonas* sp. was found to be the only causal organism of the disease. Isolation from the diseased rhizomorphs always showed the presence of this bacterium. When part of the spawn was taken away from the box in which there were mushrooms with clear symptoms of the disease and put into another box with the healthy spawn the

disease occurred in 25-30%. This low infection rate could not be explained. The authors of those studies thought there was an unknown factor controlling over the infection. They suggested it was an intracellular bacterial infection.

The isolated bacterium which caused the mummy disease was determined as *Pseudomonas tolaasi*. There is a great similarity between *Pseudomonas tolaasi* and other colonies of *Pseudomonas* ssp. which may also – in some circumstances – cause the sporocarps turn brown. There are a few tests to identify *Pseudomonas tolaasi* none of them is perfect. Only Wong's studies (1979) made it possible to work out a reliable way to identify *Pseudomonas tolaasi*. Wong detected a characteristic behaviour of *Pseudomonas tolaasi*. Different species of *Pseudomonas* are found in the enriched nutrient agar, *Pseudomonas tolaasi* is always surrounded with a white line separating these bacteria from other *Pseudomonas* ssp. (Bread 1957, Young 1970).

In 1974 van Zaaayan (1969, 1974) using her own method for virus detection, was the first person to detect bacteria in the mushroom cell. Inside the cells of diseased rhizomorphs, she found from 1 to 12 rod-shaped bacteria. After having isolated them, they were determined as *Pseudomonas* sp.

#### MATERIALS AND METHODS

Sporocarps of *Agaricus bisporus* showing mummy symptoms were collected from infected mushroom farms and prepared for electron microscopy. Young sporocarps were used at the time when their cover was not cracked yet. The method of ultrathin sections taken from the stem base was used. A sample of a diseased mushroom was first fixed in 4% glutaric aldehyde, in 0,1 M cacodylan buffer at pH = 7,3 for 3 hours at 4°C. Then it was washed in 0,1 M cacodylan buffer at pH = 7,3 and postfixed in 2% OsO<sub>4</sub> in cacodylan buffer. Following dehydration in ethyl alcohol it was embedded in Sparr Low Viscosity resin. The preparation was cut with a Reichert ultramicrotome and examined in a BS-500 Tesla electron microscope.

#### RESULTS

From a few to a few dozen bacteria were found in infected sporocarps of *Agaricus bisporus* inside the cells of diseased rhizomorphs. Cells of the mushroom filled with a large number of bacteria were dead. Cytoplasm of the mushroom cells with a small number of bacteria shrank surrounding them. When a cell was completely filled with bacteria the wall of the mushroom cell was damaged. In the mushroom cell infected with the bacteria undetermined structures were always found at or close to the place where the mushroom cell wall was vividly damaged (Plate I). If there was only a small number of bacteria in the mushroom cell they

Plate I

*A* - The mushroom cell with the bacteria in it, on both sides of the cell undetermined structures situated near the damaged cell wall,  $\times 17\ 300$ ; *B* - The same image more magnified with a clear damage of the cell wall,  $\times 20\ 340$ ; *C* - A cell filled with the bacteria, a healthy cell next to it,  $\times 20\ 000$ ; *D* - A few bacteria situated inside the cell concentrated close to one of the mushroom cell walls,  $\times 36\ 000$

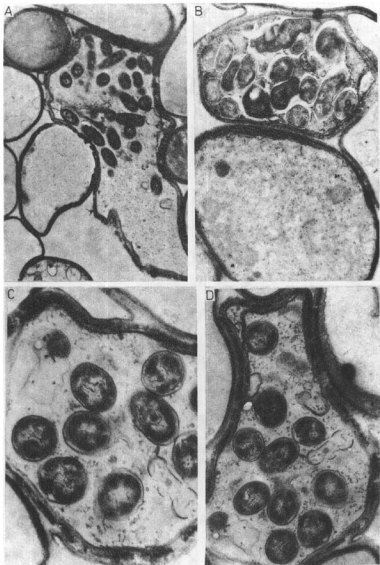
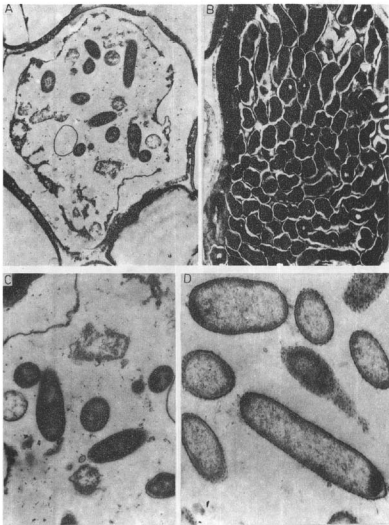


Plate II

*A* - Vivid rod-shaped bacteria, among them undetermined structures, shrinking cytoplasm also visible,  $\times 12\ 000$ ; *B* - A mushroom cell filled completely with bacteria,  $\times 7\ 000$ ; *C* - Cytoplasm of the mushroom cell shrinks surrounding the bacteria,  $\times 32\ 000$ ; *D* - Bacteria isolated from the infected mushrooms,  $\times 4\ 800$



were always located near one of the cell walls. This distribution of the bacteria with the vivid damage of the cell wall seem to suggest the entrance of bacteria penetration. The way the damage in the cell wall was caused needs to be explained. Those cells are also of interest in which other undetermined structures were found (Plate II), probably necrotic bacteria with indestructible DNA.

In preparations from the sporocarps without the pathological symptoms no bacteria were found in the mushroom cells. The isolated bacteria from diseased sporocarps were cultured on enriched nutrient agar and a few strains of bacteria were obtained. All Gram- rods were analysed. According to S c h i s l e r (1968) and B e r g e y's characteristics they were determined as *Pseudomonas* sp. Physiological influence of the isolated bacteria is being analysed at present.

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