Intracellular Occurrence of Bacteria in Mummy-Diseased Mushrooms

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ABSTRACT

Rod-shaped bacteria were observed in ultrathin sections of stipe cells of *Agaricus bisporus* sporophores affected by mummy disease. From one up to twelve bacteria per intact mushroom cell were found in only a few cells per section. No bacteria were detected in cells of healthy mushroom sporocarps.

The constant association and intracellular nature of bacteria with sporocarp tissue of cultivated mushrooms affected by mummy disease are confirmed.

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Additional key words: Pseudomonas sp., electron microscopy.

The mummy disease of the cultivated mushroom, *Agaricus bisporus* (Lange) Sing., was first described by Tucker and Routien (7) in 1942 and by Kligman and Penny (2) in 1943. Complete crop failure may result when the disease is present.

Several workers (3, 4, 6) investigated the disease without detecting the causal organism. In 1968, Schisler et al. (5) reported the presence of a bacterium in constant association with diseased rhizomorph and sporophore tissue. The bacterium was identified as *Pseudomonas* sp. Infection experiments with pure cultures of the *Pseudomonas* sp., isolated from mummy-diseased mushrooms resulted in symptomatic mushrooms on 30-35% of the trays planted with infested spawn. The failure of Schisler et al. (5) to achieve consistently high infection rates was attributed to unknown factors interfering with infection, and those authors suggested an intracellular occurrence of the bacterium. It is questionable, however, whether a photograph of a section through an infected sporophore, published by these authors, provides unequivocal evidence of intracellular occurrence of gram-negative rods.

In our opinion, ultrathin section electron microscopy of infected tissue could give decisive information on the presence of rod-shaped bacteria within mushroom cells.

MATERIALS AND METHODS.—Fresh sporocarps showing mummy symptoms were collected from mushroom farms where the disease occurred; healthy mushrooms were obtained from the Mushroom Experimental Station at Horst. Caps and stipes of mostly young, unopened fruiting bodies were separately prepared for electron microscopy, using a modified method previously applied to virus-infected mushroom tissue (1). Tissue blocks were fixed for 1.0 h at 4 C in 5% (v/v) glutaraldehyde, washed and postfixed for 1.0 h at 4 C in 1% (w/v) osmium tetroxide. After washing, the blocks were left overnight in 0.5% aqueous uranyl acetate at 4 C. Following dehydration the tissue was embedded in a 1:3 (v/v) mixture of Epon 812 and Araldite 6005. Sections were poststained in 2% uranyl acetate and in Reynolds' lead citrate, and examined in a Philips EM-300 electron microscope.

RESULTS.—From one to twelve rod-shaped bacteria were found in sections of intact stipe cells of diseased sporophores (Fig. 1-4). The bacteria measured 0.4 to 0.7 by 1.4 to 1.8 μm. The adjacent cells, containing no bacteria, often had a disarranged appearance; unusual membranous structures never observed in healthy tissue were sometimes found near the cross wall (Fig. 4). In Fig. 5 the mushroom cell is filled with bacteria; degraded cell contents and many membranes can be distinguished. Fig. 6 shows a cell with contracted cytoplasm in which the bacteria are noticeable by their high electron density.

In the cytoplasm of mushroom stipe cells, initial stages of bacterial reproduction by fission were frequently observed (Fig. 3, 5, 6). In Fig. 1, reproduction has just been completed as was suggested by the connection between the two bacterial cells visible in a preceding section.

No bacteria were detected in sections of healthy mushroom cells.

As the bacteria observed were always found within the cytoplasm of cells of mummy-diseased mushrooms and never in healthy tissue, they may very well represent the *Pseudomonas* sp. presumed to be the causal organism of the disease (5). Investigations on epidemiology and control of the disease are in progress.

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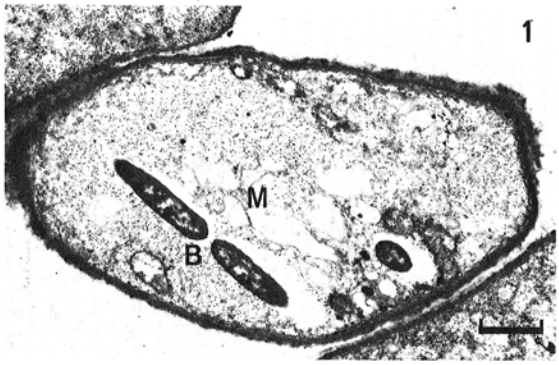
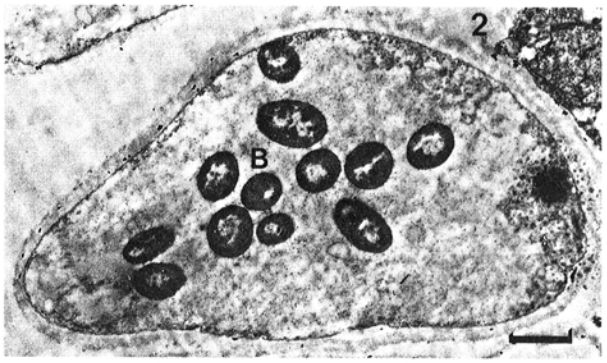
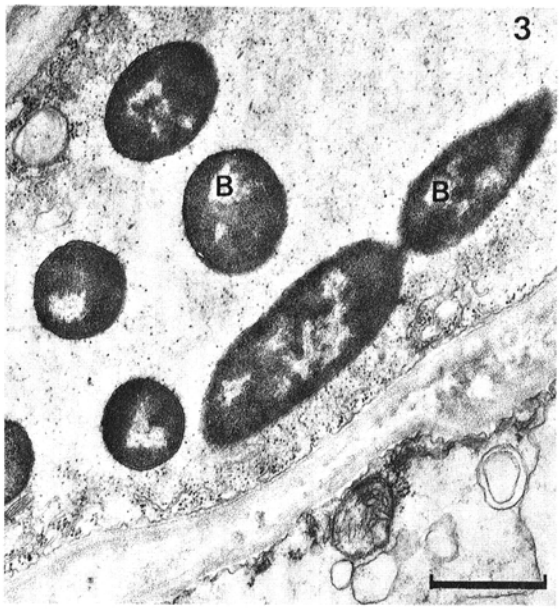
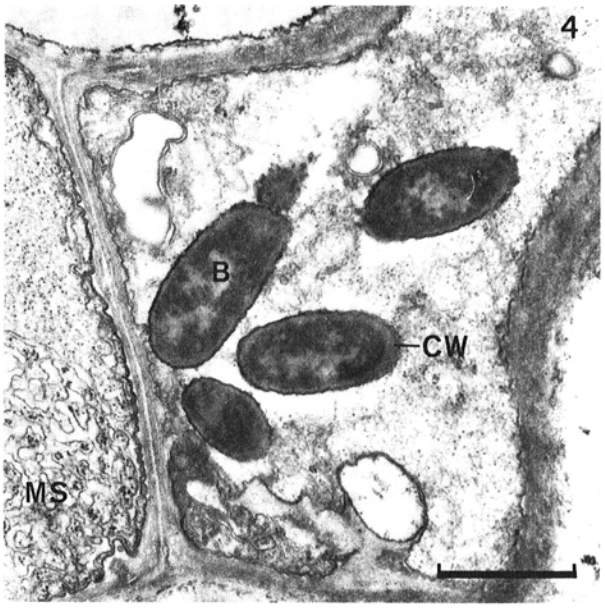
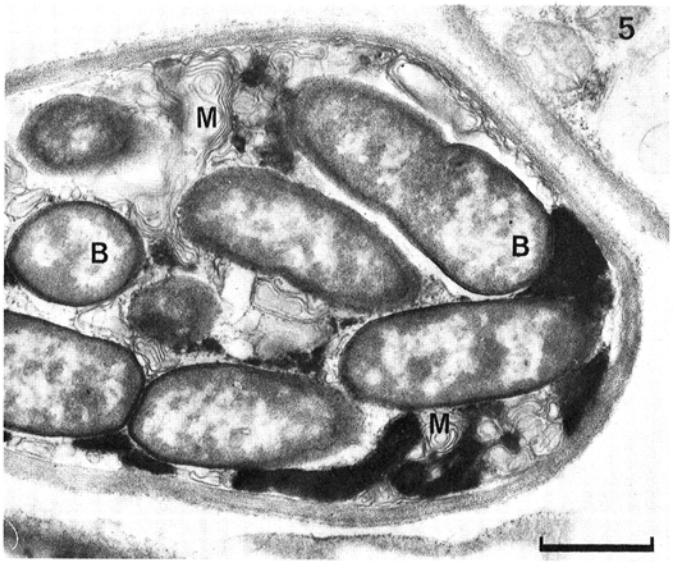
Fig. 1-6. Ultrathin sections of stipe tissue of mummy-diseased cultivated mushroom, *Agaricus bisporus,* showing intracellular bacteria (B) in varying numbers. **1)** Reproduction by fission has just been completed; many membranes (M) are visible. **2)** Fungal cell with 12 bacteria in cross section. **3)** Reproduction by fission. **4)** Bacteria with a pronounced cell wall (CW). The adjacent cell shows unusual membranous structures (MS) near the cross wall. **5)** Mushroom cell filled with bacteria besides degraded cell contents and many membranes (M). **6)** Cell in which the cytoplasm (C) has contracted around the bacteria. Scale bars represent 1.0 μm.

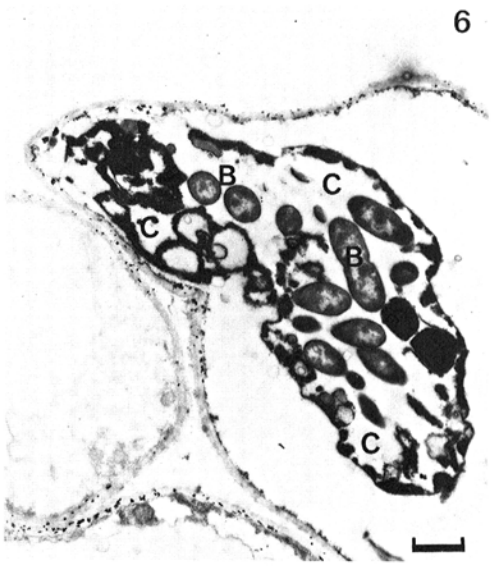

1
M
B


2
B


3
B
B


4
B
CW
MS


5
M
B
B
M


6
B
C
C
B
C