日植病報 55: 676-679 (1989)
Ann. Phytopath. Soc. Japan 55: 676-679 (1989)



# Electron Microscopic Observation of the Root Tumor of Melon Caused by *Streptomyces* sp.

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**Key words:** root tumor, melon, *Streptomyces* sp., electron microscopy.

In 1987, Kobayashi and co-workers[1] proved that "root tumor of melon" was caused by Actinomycetales. The mold-like bacterium was identified as a member of the genus *Streptomyces* of the family *Streptomycetaceae*[2]. The distribution of pathogenic bacteria in the tumor has not been investigated. Therefore, to clarify distribution sites of pathogenic bacteria in the tumor, electron microscopic observation of the tumor tissue was put into practice.

The inoculation and sample preparation were carried out as follows. The pathogenic bacterium (isolate M2138) was cultured in YEME medium[3] (yeast extract 3 g, peptone 5 g, malt extract 3 g, glucose 10 g, sucrose 340 g, $MgCl_2 \cdot 7H_2O$ 1.15 g, distilled water one liter, pH 6.8) at 30 C for 96 hr. The culture was centrifuged at $10,000 \times g$ for 30 min and the supernatant was discarded. The pellet was suspended in distilled water at the concentration of about $10^8$ cells/ml. The bacterial suspension was poured into soil at the basal part of the seedling of melon (cultivar: Kosack, *ca.* 5 cm in height). The soil and pots were autoclaved for 20 min at 120 C before use. The inoculated plants were incubated in the growth chamber at 25 C. After three weeks, the tumors with smooth surface about 2 mm in diameter were collected from the roots and washed with tap water. The tumors were immediately fixed in 2.5% glutaraldehyde at 4 C overnight, washed in 0.1 M phosphate buffer (pH 7.2) for 5 hr and postfixed for 2 hr in 1% osmium tetroxide. The tumors were dehydrated in an ethanol series and embedded in Luveak-812. Ultrathin sectioning was carried out by ordinary techniques using Porter-Blum MT2-B ultramicrotome and sections were stained with uranyl acetate and lead citrate for observation under the electron microscope. The isolate M2138 grown on YEME medium fortified with 1.7% agar was also subjected to ultrathin sectioning by the same procedure as described above for electron microscopic observation.

Bacterial cells were observed only in a few surface cell layers of cortex of the tumor (Plate I-1). The existence of bacterial mass in the tissue suggested that the invaded bacteria multiplied there to produce tumor. The cytoplasmic contents of the host cells adjacent to the bacterial cells were degenerated or almost disappeared. The wall of the host cells invaded by or adjacent to bacterial cells was frequently collapsed (Plate I-2). Since the infected cells showed deformation and irregular increase in number and size, they might be affected by some physiologically active substances produced by the bacteria. The branched and intertwined mycelia were occasionally observed (Plate II-1A). The septum was observed sometimes in elongated mycelia (Plate II-2A). The similar branched mycelia and septum were observed in the clump of M2138 cells grown on the medium (Plate II-1B, 2B). No bacteria were observed in any inner parts of the tumor (Plate II-3). The cells of the inner tumor tissue frequently possessed amyloplasts (AP) and continued irregular proliferation.

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From the results mentioned above it will be presumed that the bacterial cells invaded into host tissue, multiply in a few cell layers of the cortex and secrete physiologically active substances that induce deformation of the host cells or stimulate the cell division or enlargement of inner host cells. To clarify the mechanism of tumor formation in detail, further studies on the host-parasite relationships are required.

The authors are grateful to Prof. S. Wakimoto, Kyushu University, for critical reading of the manuscript, and to Prof. A. Kono, Miyazaki University, for his support.

## 和 文 摘 要

上運天 博・菅 康弘：放線菌に起因するメロンがんしゅ病こぶ組織の電顕観察

メロンがんしゅ病は放線菌によって根にこぶを生ずる新病害であるが，罹病組織中における病原菌の存在部位を明らかにする目的でこぶ組織の電顕観察を行った。その結果，組織内で増殖したと思われる多数の放線菌がこぶ組織の表層，すなわち表皮から2～3層の細胞内に観察された。それより内側のこぶ組織では放線菌は認められなかったが，細胞は不規則な増殖を続けた。細胞内の大部分の菌体は単細胞であったが，なかには菌糸が分枝し，隔壁が認められるものもあった。

(Received June 12, 1989)

### Explanation of plates

**Plate I**

1. Bacterial cells (B) in the cortex of the tumor. The lower left is the direction of the tumor center. Bar: 2 $\mu$m.
2. Collapsed wall of the host cell (CW) adjacent to the bacteria. The lower left is the direction of the tumor center. Bar: 2 $\mu$m.

**Plate II**

1A. Intertwined mycelia in the tumor tissue. Bar: 0.3 $\mu$m.
1B. Branched mycelium of the pathogenic *Streptomyces* isolate M2138 grown on the medium. Bar: 0.3 $\mu$m.
2A. Septum of the mycelium in the tumor tissue. Bar: 0.3 $\mu$m.
2B. Septum of the mycelium of the isolate M2138 grown on the medium. Bar: 0.3 $\mu$m.
3. Inner tissue of the tumor. No bacterial cell was observed. AP: amyloplast. Bar: 2 $\mu$m.

**Plate I**

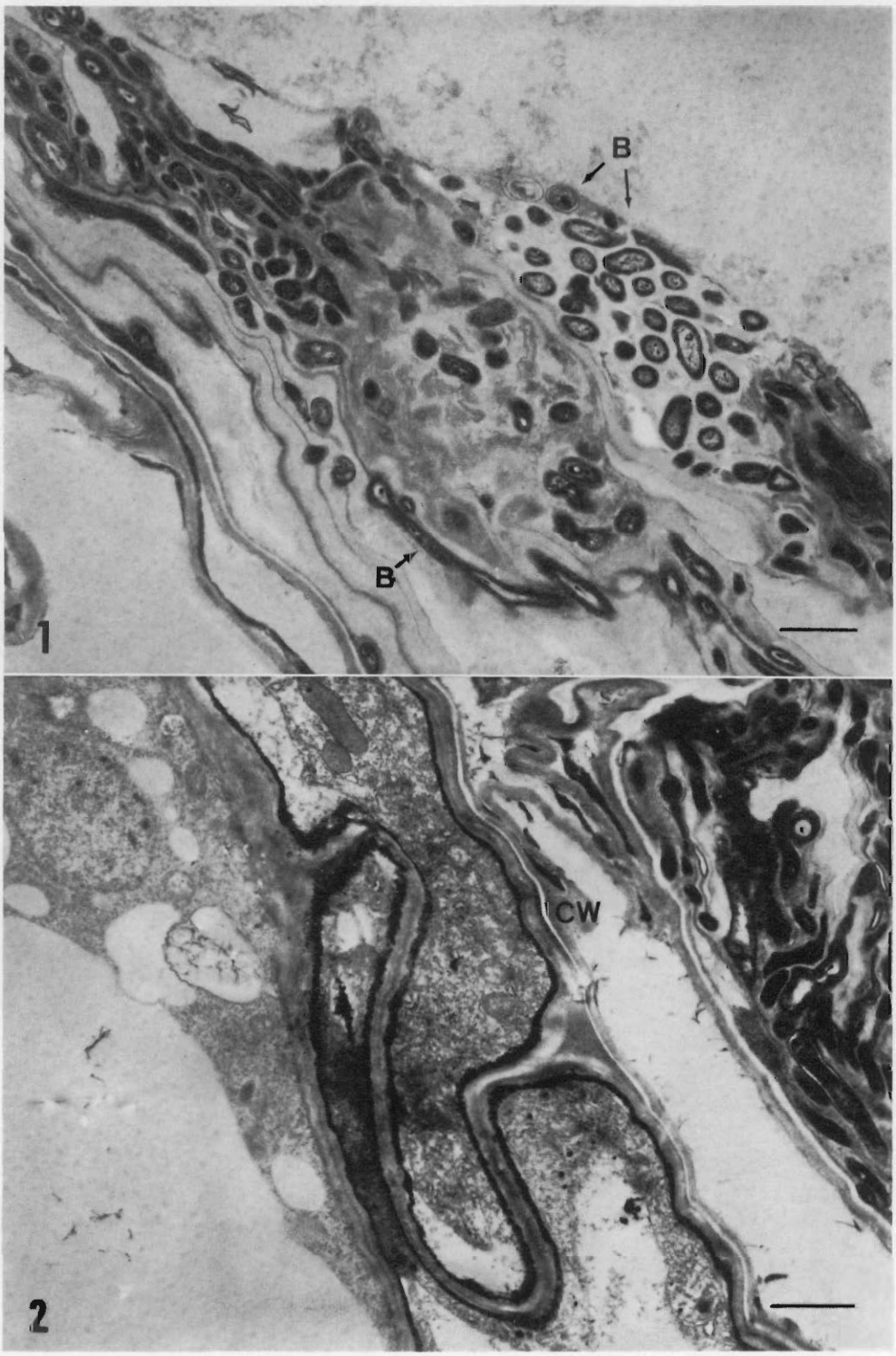

Plate II