Effect of Polyoxin on Fungi VIII Electron Microscopic Observation of Bursting of the Bulbous Germ Tubes of Polyoxin-treated *Alternaria kikuchiana* Tanaka in Pear Leaf Extract*

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石崎 寛**・河野 満**・久能 均** : 糸状菌に対するポリオキシンの影響 VIII ナシ葉煎汁
中におけるポリオキシ処理ナシ黒斑病菌胞子発芽管の破裂に関する電子顕微鏡観察

In the previous papers^{1,2,3)}, we reported effects of polyoxin on spore germ tubes and hyphae of *Alternaria kikuchiana* Tanaka. In another paper⁴⁾, we showed that the bulbous germ tubes induced by polyoxin burst and released their contents in pear leaf and fruit extracts. In the present paper we studied such an effect of pear extracts on the bulbous germ tubes in more detail at the ultrastructural level.

The pear leaf extract was prepared as follows. Fifty grams of fresh Japanese pear leaf (var. Kikusui) was chopped and boiled in 500 ml of deionized water for 30 min. The extract was filtered through a layer of filter paper (Toyo, No. 2). Twenty grams of glucose was added to the filtrate, which was then diluted to a volume of 1000 ml with deionized water. Finally the extract was autoclaved at 1 atm for 30 min.

A polyoxin-sensitive isolate of *A. kikuchiana* Tanaka (Al-3, the stock number of Mie Univ.) was cultured on potato-glucose agar at 28C for two weeks. Spores formed on the mycelial mat were harvested and then floated on 5 ml of the pear leaf extract containing raw material of polyoxin-AL wettable powder (Nihon Noyaku Co. Ltd.) at concentration equivalent to 10 ppm polyoxin B in a Petri dish (3 cm in diameter), and incubated at 28C in an incubator. After 6, 12 and 20 hrs incubation, the spores were collected by centrifugation and embedded in melting agar at 45C. The agar blocks including the spores were cut into small pieces of ca. 1 mm³ and fixed with 4.1% paraformaldehyde plus 5.1% glutaraldehyde, followed by 1% OsO₄. They were dehydrated through an ethanol series and embedded in epoxy resin. Ultrathin sections were stained with uranyl acetate and lead nitrate and observed with a JEOL-7A electron microscope.

As shown in the previous paper⁴⁾, the leaf extract accelerated the appearance of bulbous germ tubes induced by polyoxin. In the specimens fixed after 6 hrs incubation, the wall thickness of bulbous germ tubes appeared homogeneous (Fig. 1). The plasma membrane was closely adpressed to the inner cell wall and appeared

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wavy (Fig. 1). Mitochondria, ribosomes, endoplasmic reticula, and vacuoles were observed in the cytoplasm (Fig. 1). Some of the bulbous germ tubes contained large vacuoles in the cytoplasm (Fig. 1), while others did a number of small vacuoles. Cell walls of the bulbous germ tubes after 12 hr-incubation had the heterogeneous thickness; parts of the cell wall appeared thick in some places (Figs. 2,3). In some of the bulbous germ tubes, the plasma membrane initiated separation from the inner cell wall and also the cytoplasm initiated degeneration. As shown in Fig. 3, individual cytoplasmic organelles became less distinguishable and non-bounded, vacuole-like space occupied the central portion of bulbous germ tubes. After 20 hrs incubation, the plasma membrane separating from the inner cell wall surrounded the degenerating cytoplasm. In Fig. 4, individual cytoplasmic organelles degenerated and vesicular and granular structures remained in plasma membrane-bounded region. The cell wall thickness became heterogeneous; parts of the cell wall appeared thick and other parts extremely thin (Fig. 4). The thin wall portion appeared to extrude (Fig. 4, arrow). Finally, the cell wall split at a thin portion near the distal region of the bulbous germ tube and the contents of the germ tubes began to flow out through the openings (Fig. 5). Cytoplasmic organelles and matrices some of which had degenerated gathered near the plasma membrane, providing high electron density to near-region of the plasma membrane. Some vesicles and splitting plasma membranes remained in the bulbous germ tubes from which their contents had flowed out (Fig. 6). The majority of bulbous germ tubes followed similar steps of bursting as above and about 68% of them burst after 24 hrs incubation.

These ultrastructural observations are consistent with the light microscopic observations reported in the previous paper⁴⁾. As mentioned in the previous paper⁴⁾, such a bursting phenomenon of the bulbous germ tubes was never observed in potato-glucose medium or water containing polyoxin. These results lead us to speculate two possibilities that some constituents of pear might be responsible for bursting of the bulbous germ tubes and that exudates from pear leaves might enhance the effect of polyoxin in the field. Further experiments should be done in order to examine these possibilities.

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Explanation of plate

Abbreviation

cw : cell wall cy : cytoplasm er : endoplasmic reticulum m : mitochondrion
n : nucleus op : cell wall opening pm : plasma membrane sp : spore t : tonoplast
v : vacuole

- Fig. 1. A bulbous germ tube observed in polyoxin-containing pear leaf extract after 6 hr-incubation. Note that the thickness of cell wall is homogenous. $\times 7400$.
Fig. 2. A bulbous germ tube observed after 12 hr-incubation. Note that the thickness of cell wall is not homogenous. High electron density of the cytoplasm is due to the excess stain for giving the cell wall sufficient electron density. $\times 9100$.
Fig. 3. A bulbous germ tube observed after 12-hr incubation. Note that the plasma membrane has separated from the inner cell wall and that the cytoplasm appears degenerating. $\times 8900$.
Fig. 4. A bulbous germ tube observed after 20-hr incubation. The thickness of cell wall is heterogeneous and a thin portion of the cell wall has extruded (arrow). The plasma membrane separating from the cell wall surrounds the degenerating cytoplasm. $\times 15800$.
Fig. 5. A bulbous germ tube releasing its contents through a cell wall opening, observed after 20-hr incubation. $\times 21000$.
Fig. 6. A bulbous germ tube from which its contents have been released, observed after 20-hr incubation. Note that the plasma membrane has split. $\times 15800$.