Effect of Polyoxin on Fungi (X) Microautoradiography of Conidia of *Alternaria kikuchiana* Tanaka*

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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 (X)
Alternaria kikuchiana Tanaka の分生胞子のマイクロオートラジオグラフィー*

Key Words: *Alternaria kikuchiana*, microautoradiography, polyoxin, incorporation of N-acetyl-glucosamine.

Polyoxins have been reported to inhibit chitin synthesis in fungal cell walls and therefore to have an inhibitory effect on fungi which have chitin as a cell wall component¹⁻³⁾. In previous papers⁴⁻⁶⁾ and chemical⁷⁾ analyses, we reported that the effects of polyoxin on hyphae of *Alternaria kikuchiana* Tanaka could be classified into four types, i) induction of a characteristic bulbous structures, ii) induction of intracellular hyphae, iii) acceleration of septation, and iv) acceleration of pigmentation. In addition, the polyoxin treatment rendered cell walls of *A. kikuchiana* hyphae resistant to acid, alkali, and enzymatic degradation⁸⁾. On the basis of the above results, we suggested that polyoxin had several effects on this fungus in addition perhaps to the inhibition of chitin synthesis. Whether N-acetyl-glucosamine is actually incorporated or not into cell walls of germ tubes of *A. kikuchiana* treated with polyoxin was investigated using electron microscope autoradiography in this paper.

A polyoxin-sensitive isolate of *A. kikuchiana* Tanaka (AL-3, the stock number of Mie Univ.) was cultured on potato-glucose-agar medium at 28 C for 2 weeks to allow the conidial formation. Conidia were floated on a drop of distilled water or aqueous solution of polyoxin-AL wettable powder (equivalent to 5 or 50 ppm of polyoxin B) on a glass slide and incubated in a humid petri dish at 28 C. Three hours after the incubation, N-acetyl-glucosamine (acetyl-1-¹⁴C) (New England Nuclear) was added to each of the incubation drops at a final concentration of 50 μ Ci/ml. The conidia were incubated for further 2 hr. All the samples were thoroughly washed in non-radioactive N-acetyl-glucosamine and distilled water prior to fixation for electron microscopy.

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They were fixed with 5% glutaraldehyde (pH 7.4) and post-fixed with 1% OsO_4 . After dehydration through an ethanol series, they were embedded in epoxy resin. Specimens for autoradiography were prepared by the Mizuhira's method⁹⁾.

Cell walls of germ tubes were consisted of two layers: outer, electron-dense and inner, electron-lucent layer. Silver grains were located over the cell wall and cytoplasm of the control germ tube as indicated in Fig. 1. However, the silver grains were too large to determine their location on either inner or outer layer of the cell wall. Figure 2 illustrates a bulbous germ tube induced by 5 ppm of polyoxin. The cell wall of the germ tube was labelled as indicated by the presence of silver grains. Several silver grains were located also in cytoplasm of the germ tube. These results indicate that the exogenous N-acetyl-glucosamine has been incorporated into cell walls of control and polyoxin-treated germ tubes of the present fungus. Manocha and Letourneau¹⁰⁾ reported that when 14 hr old hyphae of *Choanephora cucurbitarum* grown with *Piptocephalis virginiana* were transferred onto a medium containing [^3H] N-acetyl-glucosamine and 380 μM of polyoxin D, autoradiographs showed a remarkable decrease in the number of silver grains and hence label incorporation in the cell wall of haustorium of the latter fungus as compared to the controls without polyoxin D, and they suggested that polyoxin D suppressed the label incorporation of [^3H] N-acetyl-glucosamine in the cell wall of *P. virginiana*. However, the present result indicates only the location of incorporated N-acetyl-glucosamine but whether it is actually assimilated into chitin or not remains unknown. The possibility can not be ruled out that N-acetyl-glucosamine may be assimilated into cell wall constituents other than chitin. Moreover, the present results give no reliable answer on the quantitative difference of incorporated N-acetyl-glucosamine in control and polyoxin-treated germ tubes.

Figure 3 illustrates the distribution of silver grains in an ungerminating conidium treated with 50 ppm of polyoxin. This concentration perfectly inhibits the conidial germination. However, new cell walls were formed in cells of the conidium to separate one cell into two to three compartments. Appearance of the compartmented cells was similar to that of intracellular hyphae induced by 10 ppm of polyoxin as had been shown in a previous paper⁵⁾. The newly-formed cell walls were thinner than the original ones. Such thin cell walls never occurred in the polyoxin-untreated conidia. Silver grains were located preferentially over the newly-formed cell walls, suggesting that the exogenous N-acetyl-glucosamine may be used for new cell wall synthesis accelerated by the polyoxin treatment even in the ungerminating conidia. The newly-formed cell wall in the ungerminating conidium consisted of only one electron-lucent layer. It was different from the two-layered cell walls of germ tubes in Figs. 1 and 2. The presence of silver grains over such newly-formed cell walls suggests that the exogenous N-acetyl-glucosamine might be incorporated also into the electron-lucent inner layer of the germ tube.

The preferential distribution of silver grains in cell walls may be resulted from non-specific binding of N-acetyl-glucosamine to the constituent materials. In order to

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examine this possibility, the germinating conidia treated with labelled N-acetylglucosamine should be fractionated into subcellular components, and the distribution of radioactivity in each fraction should be determined. These data will be published elsewhere.

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Explanation of Plate

Plate I.

Abbreviation in figures

co: conidium cw: cell wall cy: cytoplasm gt: germ tube ncw: newly-formed
cell wall v: vacuole
scale=1 μ m

- Fig. 1. A germ tube emerging from the control conidium. Silver grains were located over the cell wall and cytoplasm of the germ tube.
- Fig. 2. A bulbous germ tube emerging from a conidium treated with 5 ppm of polyoxin. Most silver grains are present over the cell wall of the germ tube.
- Fig. 3. An ungerminated conidium treated with 50 ppm of polyoxin. Silver grains were mostly associated with the newly-formed, thin cell walls.

Plate I