



HOKKAIDO UNIVERSITY

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学位論文内容の要旨

博士の専攻分野名称：博士（農学）

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Confirmation of the presence of jasmonoyl-L-isoleucine in Mosses and
elucidation of biotoxins upon *Marchantia polymorpha* from the infectious fungi

(ジャスモノイル-L-イソロイシンのMossesにおける存在と糸状菌からの
*Marchantia polymorpha*生育阻害物質の単離とその構造決定)

It is known that Jasmonates (JAs) act as signaling molecules for wound responses in common plants such as dicot and monocot. JAs, class of oxylipins derived from α -linolenic acid (α -LAs), are phytohormone which play crucial roles in plant development and defense response against herbivores and necrotrophic pathogens. Jasmonic acid (JA) and its biologically active form jasmonoyl-L-isoleucine (JA-L-Ile) regulate the defense responses to various environmental stresses. In vascular plants, phytohormones perform essential growth and development processes such as germination, vegetative growth, flowering, seed development, senescence, distribution of nutrients, and stress tolerance. It is known that dinor-*iso*-OPDA is the key compound in *Marchantia polymorpha*, although there is little accumulated knowledge about other findings such as the existence of JA-L-Ile in Lycophyte and Bryophyte. Furthermore, no studies have been done on toxins against Lycophyte and Bryophyte produced by pathogens that can invade Lycophyte and Bryophyte.

The purpose of this study was to clarify the presence of JA-L-Ile in Bryophyte and to elucidate the biotoxins against *M. polymorpha* produced by the fungi that can infect *M. polymorpha*.

1. Unveiling the presence of JA and JA-L-Ile in mosses

12-oxo-Phytodienoic acid (OPDA), JA and JA-L-Ile were found in *Selaginella moellendorfii* belonging to Lycophytes. A moss, *Physcomitrella patens* is known as a useful experimental plant, and the existence of JA has been discussed in *P. patens*, but nothing conclusive. *M. polymorpha*, it has been proven that the endogenous level of JA was increase by the stimulation of airborne deuterium labeled methyl jas

monate (MeJA), although no definitive decision had been made regarding the existence of JA-L-Ile in the liverwort. Taxonomically, mosses and liverworts together with hornworts are related plant groups belonging to Bryophytes. There are no definitive decisions determining the existence of JA and JA-L-Ile in the group. This study aims to verify the presence of JA and JA-L-Ile in four different species of commercially available mosses, *Calohypnum plumiforme*, *Lencobryum bowringii*, *Pyrrhobryum dozyanum*, and *Bartramia pomiformis*. The existence of JA-L-Ile was confirmed in three of the four species, *C. plumiforme*, *L. bowringii*, and *P. dozyanum* using the UPLC MS/MS analysis. Meanwhile, JA was not able to be found in any of the four species. In addition, the accumulations of *cis*-OPDA, *iso*-OPDA, dinor-*cis*-OPDA, and dinor-*iso*-OPDA were also found in all four species.

2. Investigating biologically active compounds from fungi that inhibit the growth of *M. polymorpha*.

M. polymorpha is a well-known model plant used for evolutionary molecular-microbe interactions. It was reported that filamentous fungi *Bjerkandera adusta* and *Irpex lacteus* had ability to invade *M. polymorpha*. However, the infection mechanisms remain unknown. Therefore, the study aims to investigate the toxins from cultured *B. adusta* and *I. lacteus* that infected the growth of *M. polymorpha*. In order to obtain the compounds, diffusion assays were performed as a preliminary experiment of growth inhibitory test. Using growth inhibitory activity-guided fractionation, the active compounds methyl vanillin (**1**) and tremutin E (**2**) were isolated from filamentous fungi *B. adusta* and *I. lacteus*, respectively. Verification of the biological activity of both compounds showed that they inhibited the growth of *M. polymorpha*, and this activity was 100 times stronger than that of acetic acid, the main active ingredient of a commercially available *M. polymorpha* control agent. It was hypothesized that treated *M. polymorpha* using isolated compounds might affect the defense hormone in plants. To confirm the hypothesis, analyzing OPDAs were conducted using UPLC-MS/MS. The results reveal that treatment of *M. polymorpha* with **2** isolated from *I. lacteus*, which has a stronger invading ability, significantly reduced the accumulation of dinor-*iso*-OPDA, which is the key compound in the defense response of *M. polymorpha*. Based on the experimental data that it was found that dinor-*iso*-OPDA was important enough to be targeted by the infectious fungi, the finding supported the conventional theory that dinor-*iso*-OPDA is the key compound in the defense reaction of *M. polymorpha*.