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学位論文の要旨

Abstract of Thesis

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学位論文題目 Title of Thesis（学位論文題目が英語の場合は和訳を付記）

Insecticidal compounds produced by *Pochonia suchlasporia* TAMA 87 in solid-state fermentation
(*Pochonia suchlasporia* TAMA 87 株の固体培養により生産される殺虫性物質)

学位論文の要旨 Abstract of Thesis

In Chapter 1, research background and objectives of the study on insecticidal compound(s) produced by *Pochonia suchlasporia* TAMA 87 are described. Over the past century, agrochemicals have helped to improve food production. However, adverse effect of these agrochemicals arouses the searching of new active compounds that are safer for environment and human health. Among the promising sources of natural products, microorganisms have been extensively screened for the production of active compounds. During the screening of microbial metabolites for chitinolytic enzyme inhibitors, solid-state fermentation (SSF) of a fungal strain *P. suchlasporia* TAMA 87 was found to produce a novel compound pochonicine. This compound showed potential inhibition of β-*N*-acetylglucosaminidases (GlcNAcases), which is essential for normal growth of insects. Since pochonicine is a strong GlcNAcase inhibitor, MeOH extract of the SSF culture of *P. suchlasporia* TAMA 87 was examined for its insect growth regulatory activity. A preliminary study revealed that the MeOH extract of the SSF culture showed insecticidal activity against the blowfly, *Lucilia sericata*. Insecticidal assay of the fractions obtained by partitioning the MeOH extract between EtOAc and water indicated that most of the insecticidal activity was recovered in the EtOAc-soluble fraction but not in the pochonicine-containing water-soluble fraction. Hence, the insecticidal activity was attributed to compound(s) other than pochonicine.

In Chapter 2, production of the insecticidal compound(s) of *P. suchlasporia* TAMA 87 by solid-state fermentation is described. Twenty agar discs of *P. suchlasporia* TAMA 87 were inoculated into twenty different Erlenmeyer flasks (200 mL), each containing an autoclaved rolled barley-based solid medium consisting of 9 g of rolled barley, 1 g of peeled oats, 10 mL of water, 20 mg of yeast extract, 10 mg of sodium L-tartrate dihydrate, and 10 mg of KH_2PO_4. Fermentation was performed in a static condition at 22°C for 22 days. The surfaces of its fungal colonies were white with yellow reverse. The colonies would be more yellow in a longer incubation. The fungal culture was extracted by adding 50 mL of MeOH to each flask, shaking the flasks well, and keeping them overnight at room temperature. The mixture was filtered and concentrated *in vacuo* to give an MeOH extract (9750 mg). This amount was enough for purification and structural analysis of the insecticidal compound(s).

In Chapter 3, purification of the insecticidal compound(s) is described. In this study, bioassay-guided purification was employed to isolate the insecticidal compound(s). Insecticidal compound(s) were purified from the MeOH extract obtained from the production step. The MeOH extract (2922 mg) was partitioned between EtOAc and water. The EtOAc-soluble

fraction was concentrated *in vacuo* to yield an EtOAc extract (550 mg), and subsequently purified by silica gel column chromatography using hexane/EtOAc and EtOAc/MeOH to afford nine fractions (F1-F9). F3 (45 mg), which was eluted by the 30:70 (v/v) hexane/EtOAc mixture, was further purified by preparative HPLC to give ET-1 (6.1 mg). ET-1 was obtained as a yellowish amorphous solid, with the retention time (*t*R) value of 28 min, monitored using UV absorbance at 280 nm.

In Chapter 4, chemical structure elucidation of ET-1 is discussed. The structure of ET-1 was determined with a combination of spectroscopic methods, including 1D, 2D NMR, HRESIMS, and UV-Vis analyses. The results showed that the MeOH solution of this compound exhibited UV absorption maxima at 378, 283, and 212 nm, indicating the presence of conjugated double bonds. The ^{1}H NMR spectrum of ET-1 revealed the presence of five methyl, one methylene, four methine, seven olefinic, and two hydroxy protons. The ^{13}C NMR spectrum of ET-1 displayed 23 carbon signals. Comparing spectroscopic data of ET-1 to those of asteltoxin, a trienic α-pyrone-containing mycotoxin, ET-1 was considered to be an analog of the asteltoxin. Along with 2D NMR, the structure of ET-1 was established as a new asteltoxin that differs from asteltoxin in its α-pyrone moiety. The α-pyrone moiety in ET-1 has two methyl and no methoxy substituents. In addition, by considering NOE correlation, the relative stereochemistry of ET-1 was determined to be identical to that of asteltoxin. Comparison of ET-1's optical rotation value with asteltoxin (and its analogs) indicated that the absolute configuration of ET-1 is the same with asteltoxin. Moreover, ET-1 is indicated to be the first member of a new asteltoxin series, which is characterized by 3,5-dimethyl α-pyrone moiety. ET-1 was finally named asteltoxin H.

In Chapter 5, evaluation of the ET-1 as the insect growth inhibitory and insecticidal compound is described. The bioassay was carried out against prepupae of the blowfly, *L. sericata*. By employing agar gel as the matrix for test samples, five doses of the test sample (measured in µg per mg of prepupal body weight) were examined. To perform the insecticidal assay, agar gels were placed in separate covered Petri dishes. After ten prepupae were laid on each agar gel, a filter paper sprayed with 0.26 g of water was placed correctly between Petri dish and its lid to maintain the humidity. Petri dishes were kept in a dark condition at 22±1°C for 30 days. Prepupae death were counted every day. The death of a prepupa was ascertained by the loss of mobility. The dose of the test sample that killed half of the prepupae on the seventh day was determined as its LD_{50}. The results showed that LD_{50} value of ET-1 was found to be 0.94 µg/mg prepupal body weight.

In Chapter 6, analogs of ET-1 is discussed. During the purification of ET-1, at least six compounds with similar UV spectra to that of ET-1 were detected in the SSF culture of *P. suchlasporia* TAMA 87, indicating that this fungus produces analogs of ET-1. One of the analogs of ET-1 was successfully isolated by a similar purification method to that of ET-1, named ET-4. The structural analysis of ET-4 was performed similarly to that for ET-1, indicating that ET-4 has the structure in which the ethyl group at C-3 of ET-1 is replaced with a methyl group. ET-4 was a novel compound with the same α-pyrone moiety with ET-1.

In conclusion, this is the first report for a novel insecticidal compound with a unique α-pyrone moiety that differs from the so far reported astletoxin-type compounds. The obtained compound ET-1 was finally named asteltoxin H. Furthermore, at least six compounds of the analogs of ET-1 were observed in the SSF culture of *P. suchlasporia* TAMA 87. One of them has been isolated and determined its structure, revealing that this compound, named ET-4, is a novel compound possessing the same α-pyrone moiety as ET-1.