Summary of Doctoral Thesis

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Title	Study of New Metabolites Produced by Endophytic Fungi from Merapi Volcano Area in Java Island, Indonesia

Natural products have played a major role in the search for new drugs or drug candidates throughout the course of many years. Because of their enormous structural diversity and complexity, they are a continuing and inspiring source for researchers. Fungi are one important source of pharmacologically active and structurally diverse natural products. This thesis describes investigations of new compounds from endophytic fungi, through fermentation process. The isolation of secondary metabolites from the fermentation products have done utilizing several steps of chromatography. The structures of the isolated compounds were established by extensive spectral analyses of 1D/2D-NMR and HRESITOFMS. The absolute configurations were determined using a combination of the modified Mosher's ester method, X-ray crystallography, experimental and calculated ECD analysis, X-ray crystalline sponge method, and/or comparison with reported data.

Mount Merapi in Java island, Indonesia, is known as the most active and hazardous volcano in the world. Merapi is 25–30 km north of Yogyakarta City and is home to approximately 1.6 million people. One of the most feared aspects of Merapi is the pyroclastic flow eruption type, which consists of revolving clouds of superheated gases. A recent large eruption in 2010 caused many changes to the environment, including plants and microorganisms. It was assumed that during the post-eruption in this area, fungal endophytes were not investigated as metabolite sources. We explored new metabolite sources from the damaged area, which led to the isolation of several fungal strains. Three of them, *Nectria pseudotrichia* 120-1NP, *Fusarium solani* B-18, and *Colletotrichum boninense* AM-12-2, were described in this thesis.

N. pseudotrichia 120-1NP is an endophytic fungus isolated from the stem of *Gliricidia sepium*. Seven new compounds, nectrianolins A–C (**1**–**3**); 6,8-dihydroxy-3,4,7-trimethylisocoumarin (**5**); 8-hydroxy-6-methoxy-3,4,7-trimethylisocoumarin (**6**); nectriaquinone B (**8**); and zythiostromic acid C (**11**), were isolated from the brown rice culture of this strain, along with four known compounds (**4**, **7**, **9**, and **10**). To the best of our knowledge,

this is the first isolation of **7** from a natural source. Compounds **1** and **2** have a rearranged monocyclo-farnesyl skeleton (which is uncommon to sesquiterpene-epoxycyclohexane conjugates) instead of a bicyclo-farnesyl skeleton. Compounds **1–3** were evaluated for their in vitro cytotoxicity against HL60 and HeLa cell lines by the MTT method. Compounds **1–3** exhibited cytotoxic activity against the HL60 cell lines with IC_{50} values of 1.7, 1.5 and 10.1 μM, respectively. Compound **1–3** also exhibited cytotoxicity against the HeLa cell lines with IC_{50} values of 34.7, 16.6 and 52.1 μM, respectively. Additionally, compounds **4–11** were evaluated for their antimicrobial activity against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Candida albicans*, and *Aspergillus clavatus*, phytotoxicity, and in vitro cytotoxicity. Unlike the other compounds, **8** and **9** exhibited antibacterial activity against *S. aureus* and *P. aeruginosa* with MIC values (μg/mL) of >50 and 6.25, and of >50 and 3.125, respectively. The phytotoxicity was tested using lettuce seeds; notably, only **9** induced significant seedling growth inhibition compared to control. Moreover, **7-10** exhibited cytotoxicity against HL60 cells with IC_{50} values (μM) of 11.9, 1.33, 1.93, and 11.6, respectively. The higher cytotoxicity of **8** and **9** compared to that of the related compounds **7** and **10** was attributed to their increased cell membrane permeability due to the presence of the hydroxyl group.

F. solani B-18 is an endophytic fungus isolated from an unidentified forest litter. Four new compounds with γ-methylidene-spirobutanolide core, fusaspirols A–D (**12–15**), were isolated from the brown rice culture of *Fusarium solani* B-18. Oxaspirol analogues have been reported to possess various biological activities, **12–15** and its derivatives did not show cytotoxicity against murine macrophage derived RAW264.7 cells. Thus, they were tested for osteoclastic differentiation activity using the same cells. After four days of the osteoclastic induction, mature osteoclasts of multinucleated-TRAP (tartrate-resistant acid phosphatase)-positive cells were counted. Compounds **12** and **12a** significantly increased the number of mature osteoclasts at the comparable levels to the positive control (kenpaullone) and the negative control (DMSO), suggesting that **12** and **12a** activated a signaling pathway in osteoclastic differentiation. In the scale-up fermentation based on this strain, with the same method, additional four new polyketides, fusopoltides B–E (**20–23**), were isolated along with four known compounds (**16–19**). Fusopoltide B (**20**) is a diastereomer of its co-isolated known compound, fusopoltide A (**19**), featuring a pentaleno[1,2-*c*]pyran ring system. Fusopoltide C (**21**) and fusopoltides D–E (**22–23**) are incorporated the first natural polyketides featured decahydro-pentaleno[1,2-*c*]pyran and pentaleno[1,2-*c*]furan ring systems, respectively.

C. boninense AM-12-2 is an endophytic fungus isolated from the stem of *Acacia mangium*. Seven new compounds, 3-(3-hydroxy-2-(hydroxymethyl)phenyl)propanoic acid (**24**), 2-hydroxymethyl-3-hydroxy-(*E*)-cinnamic acid (**25**), and colletofurans A–E

(**26–30**), were isolated from brown-rice culture of *Colletotrichum boninense* AM-12-2. Colletofurans A–E (**26–30**) are first natural compounds featured an unprecedented 1-octyl-1,3-dihydroisobenzofuran core. Additionally, colletofuran A (**26**) contained a unique 1,6-dioxaspiro[4.4]nonane ring system. Compounds **24–30** were evaluated for their anti-aphicidal activity. Aphids are a serious insect pest in agriculture worldwide. This assay was performed to test the susceptibility of *Aphis gossypii* (Glover) and *Myzus persicae* (Sulzer), against **26–30**. The commercial insecticide milbemectin was tested as the positive control. Compound **26** and **29** exhibited strong aphicidal activities against *A. gossypii* with the LC_{50} (μg/mL) values of 1 and 0.5, respectively. Furthermore, **27**, **29**, and **30** showed moderate activity against *M. persicae* with the LC_{50} (μg/mL) values of 169, 118, and 26, respectively. Notably, **29** exhibited strong aphicidal activity (LC_{50} = 0.5 μg/mL) against *A. gossypii* and weak activity (LC_{50} = 118 μg/mL) against *M. persicae*. In contrast, **30** exhibited moderate aphicidal activity against *M. persicae* (LC_{50} = 26 μg/mL), but inactive against *A. gossypii*. Other compounds displayed either weak or no aphicidal activity (LC_{50} >200 μg/mL) against tested aphids. In general, the data showed dose dependent responses and *M. persicae* was more resistant than *A. gossypii* to the treatments.

This thesis describes the chemical investigation of 30 fungal metabolites, including 22 new compounds and two compounds firstly isolated from fungal cultures. Complex interactions with their host may involve the presence of these fungi in volcanic areas. The interactions possibly related to their chemistry in some respects and have been shown to produce or elicit secondary metabolites that are new, attractive, and biologically active. Since endophytic fungi from Merapi volcano area are unexplored, there is a high likelihood that new metabolites will be discovered. Although certain new compounds exhibited no activity in bioassay, the new chemistry faced difficulties in the determination of the structure that had to be addressed. These results also disclosed that endophytic fungi from Merapi volcano area, harbor an enormous reservoir of new compounds for drug discovery.