To identify the secondary metabolites produced by *Ganoderma boninense* in the interaction with oil palm *in Vitro* by LC-MS

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Abstract

Ganoderma boninense has been recognized as a white rot pathogenic fungus and main reason of Basal stem rot (BSR) in oil palm. Interaction between Ganoderma and oil palm produces some secondary metabolites such as ergosterol, stigmastrol, campestrol and sitostrol. This study was analyzed extracellular metabolomics that were secreted by G. *boninense* in the interaction with oil palm *in Vitro* by Liquid Chromatograph - Mass Spectrometer (LC-MS). Roots of three months old oil palm plantlets were artificially infected with G. *boninense.* Metabolites that were secreted by G. *boninense* in the interaction with oil palm plantlets and spreated in the enviorment compared to metabolites that are released by microbial cells without plantlet. 4-Hydroxy-3-methylbenzoic acid, Palmitic amid, Kojic acid, Ethionamide, Oleamide and Ganoderic acid beta from Infection sample were detected in interaction crude. This study provides information that related compounds secreted in the interaction between Oil Palm and G. *boninense*

-Key words: *Ganoderma boninense*, Oil palm, secondary metabolites, LC-MS

INTORDAUCTION

Secondary metabolites have been described as metabolic productions not necessary for growth and without distinct function within its living (Keller et al., 2005). A probable function for peptaibols as virulence factor such as AM toxin in *Alternaria alternata* (and the maize pathogenic HC toxin from *Cochliobolus carbonum* has been described the toxic amanitines and phalloidines (*Amanita* sp), the immunosuppressive cycloamanides (*Amanita phalloides*) and the nematicidal omphalotins (*Omphalotus olearius*) are Peptides produced by basidiomycetes (Eisfeld, 2009). The secondary metabolites can extract using different solvents or fermentation and elucidation of the active metabolites and their structures can be performed by various methods such as GC-MS (gas chromatography-mass spectrometer), LC-MS (liquid chromatography-mass spectrometer) and tandem mass spectrometry (MS/MS) and NMR (nuclear magnetic resonance) (Tiwari et al., 2015). Maximum investigation of biologically active molecules in Ganoderma species has been accomplished on the extracts of their fruiting bodies, spores and cultured mycelia. The main constituents which were characterized in Ganoderma species include polysaccharides, triterpenes and steroids (Baby et al., 2015). Biologically remarkable components in Ganoderma species consist of Proteins, nucleosides peptides, fatty acids, amino acids, alkaloids and inorganic elements (Li et al., 2013). Extracellular metabolomics is the search of low molecular weight extracellular metabolites that are released by microbial cells into their surroundings, specifically the culture media. The all supplement of these metabolites is almost known to as the exometabolome. It consists all of the metabolites which secreted out of the microbial cell, also those components are discovered in the supernatant (Pinu and Villas-Boas, 2017). This study was

analyzed metabolomics that were secreted by G. *boninense* in the interaction with oil palm *In-Vitro* by LC-MS. **Materials and methods:** *In-vitro* infection test was carried out by infecting the oil palm plantlets with pre-grown G. *boninense* mycelium. The extraction crude was Prepared for the Analysis of metabolites by LC-MS.

MATERIALS AND METHODS

Murashige and Skoog (MS) media was selected to supply nutrients to the oil palm plantlets (Murashige and Skoog, 1962) and autoclaved at 121 °C for 15 minutes. *In-vitro* infection test was carried out by infecting the oil palm plantlets with pre-grown *G. boninense* mycelium (grown in PDB for 14 days). The oil palm plantlet Approximately 100 ml of MS media was measured and poured into a sterilized 250 ml conical flask. Then, several mycelium clumps were added to the flask using a sterilized spatula. All flasks will be harvested after two weeks. The flasks were then incubated at 28 °C with 16 hours of light and 8 hours of dark. The mycelia were separated by 8 layers of muslin filter after two weeks. In the next step, an equal of methanol (CH₃OH)- ethyl acetate (EtoAC) (1:1 v/v) were added into inoculums cultured in an Erlenmeyer flask. The CH₃OH /EtoAC phase was separated from the medium using a separation funnel to deactivate the enzymatic activity and concentrate the extracellular metabolites, the culture supernatant must be freeze-dried under vacuum before chemical derivatization. Immediately after evaporation, the CH₃OH /EtoAC extracts were each diluted in 100 mL of CH₃OH for LC-MS investigation. LC-MS analysis was performed on an Agilent1290 infinity LC system coupled to Agilent 6520 accurate-Mass-Q-TOF mass spectrometer with dual Electrospray ionization (ESI) source using column Agilent Zorbax Eclips XBD-C18, Narrow-Bore (2.1 x 150mm, 3.5-micron, p/n:93-990-902). The mobile phase consisted of 0.1% formic acid in water (solvent A) and 0.1% formic acid in CH₃OH (solvent B). The column was equilibrated for 5 min prior to each analysis and the metabolites were separated 25 min under the following conditions: 0 min (5% B), 5 min (5% B), 20 min (100%B) and 25 min (100% B). Flow rate for column was set at 0.6mL/min, column temperature at 25 °C and the injection volume was 0.5 µL. The MS parameters were as follows: Capillary voltage 4000 V for ion positive polarity and 3500 V for ion negative polarity with a gas temperature of 300 °C. Mass range were performed from 100-1000 m/z for ion positive polarity and 115-1000 m/z for ion negative polarity .LC-MS analysis of the crude extract of Samples indicated the presence of various proposed compounds when the spectrometer was operated in both positive and negative ion modes.

Result and discussion

Metabolites that were secreted by G. *boninense* in the interaction with oil palm plantlets and spreated in the enviorment were detected with in both positive and negative modes (Table 1).

Table 1. G.boninense secondary metabolites were indicated that effected in pathogenesis

No.	Metabolite	Mode of action	Reference
1	4-Hydroxy-3-methylbenzoic acid	The methylation	Krohn et al., 2004
2	Palmitic amid	Antibiotic	Betnia,1995
3	Kojic acid	mycotoxin, synergizing the toxicity of nicotine	Dowd,1998
4	Ethionamide	Antibiotic, it is an inhibitor of inducible nitric-oxide synthase in cells.	Sanchez et al., 2012
5	Oleamide	fatty acid amide hydrolases	Farrell and Merkler, 2009
6	Ganoderic acid beta	Apoptosis induction	You et al., 2015

CONCLUSION

In this study the secondary metabolite secreted was investigated in interaction between oil palm and G.*boninense* using LC-MS *in vitro*. 4-Hydroxy-3-methylbenzoic acid, Palmitic amid, Kojic acid, Ethionamide, Oleamide and Ganoderic acid beta were released in the environment.

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