Analysis of lingzhi (Ganoderma lingzhi) metabolites during developmental stages with demonstrated bioactivity: a metabolomics approach

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Title : Analysis of lingzhi (*Ganoderma lingzhi*) metabolites during developmental stages with demonstrated bioactivity: a metabolomics approach
(生理活性を示した生育段階における霊芝（*Ganoderma lingzhi*）代謝産物のメタボローム解析）

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Thesis Summary

Metabolomics has been applied in many fields including natural product chemistry. The use of metabolomics for informative, predictive, and discriminative purpose has been applied to lingzhi (*Ganoderma lingzhi*).

As initial step prior to establishing the analytical method for analysis of lingzhi during developmental stages, the isolation efforts succeed to isolate a new lanostane-type triterpenoid, lucidumol D. Its structure was elucidated on the basis of extensive 1D- and 2D-NMR studies as well as mass spectrometry. The cytotoxicity of lucidumol D against proliferation of several cancer cells were assayed by using MTT method and the obtained result suggested selective anti-proliferative and cytotoxic effects against MCF-7, HepG2, HeLa, Caco-2, and HCT-116.

The current analysis methods of lingzhi are focusing to analyze triterpenoid due to various bioactivity of its metabolite. Two-dimensional liquid chromatography (2D-LC) for separation of lingzhi components was constructed for increasing peak capacity due to the limitation in unidimensional. We found the combination of cyano (CN) or amide columns as first dimension and C18 for the second dimension gives more potent resolving power than unidimensional LC. The number of detected peaks was increased significantly on 2D-LC whereas 1042 and 1439 peaks detected on RP(CN)× RP(C18) and HILIC(Amide)×RP(C18) 2D-LC system, respectively. To explain the dissimilarity in selectivity, the percent of orthogonality (%O) were 64% and 89% for RP(CN)× RP(C18), and HILIC(Amide)×RP(C18) 2D-LC system respectively.

As for predictive metabolomics approach, we applied it to identify the anti-diabetic compounds in lingzhi using HPLC-based metabolomics approach. The chemical profile of ethanol, hexane, chloroform, ethyl acetate and water fraction which were monitored at 222, 252, and 272 nm was analyzed by HPLC, and the anti-diabetic activity profiles of all fractions were determined by alpha-glucosidase inhibition assay. There were 45 HPLC chromatograms resulted from triplication of fraction. To discriminate the fractions with different anti-diabetic profile, the chromatograms were compared to the data of IC50 from alpha-glucosidase activity of each fraction using metabolomics along with multivariate analysis. The orthogonal projection to patent (OPLS) analysis result suggested that constituent at retention time of 7.7 min of an ethyl acetate fraction has the highest contribution to the activity. To identify the correlated peak, we did semi-preparative isolation and found that this peak has maximum UV absorption at 222 and 258 nm. ESI-MS analysis of its peak shows the molecular ion peaks m/z 569.0 on negative ion mode. MS/MS analysis of its precursor ion shows product ion at m/z 129.0 on negative ion mode. We suggested that this peak contain lucidenic acid Q,

that reported has strong alpha glucosidase inhibitory activity.

Finally, we applied the metabolomics for discrimination of eight developmental stages of lingzhi. Analysis of the changes in metabolites during developmental stages of lingzhi is important to understand the underlying mechanism of its biosynthesis, as well as its bioactivity. In this study, mass spectrometry based untargeted metabolomics was carried out to analyze the alteration of metabolites during developmental stages of lingzhi. Eight developmental stages were categorized on the basis of morphological changes; starting from mycelium stage to post-mature stage. GC/MS and LC/MS analyses along with multivariate analysis of lingzhi developmental stages were performed. Amino acids, organic acids, sugars, polyols, fatty acids, fatty alcohols, and some small polar metabolites were extracted as marker metabolites from GC/MS analysis; while, lanostane-type triterpenoids were annotated from LC/MS analysis of lingzhi. The marker metabolites from untargeted analysis of lingzhi developmental stages were correlated with the alpha-glucosidase inhibitory activity. Two metabolites, 34 and 35, were potentially contribute to the alpha-glucosidase inhibitory activity.