SHORT COMMUNICATION

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GC/Q-TOF-MS-BASED METABOLOMICS: UNVEILING THE TEMPORAL METABOLIC PATHWAYS IN *Ganoderma boninense* USING PATHWAY ANALYSIS TOOLS

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ABSTRACT

Metabolomics research aims to uncover the complex biochemical pathways involved in biological processes, but the interpretation of metabolites, which play diverse roles within biological systems, remains a significant challenge. In this study, we utilised pathway analysis modules, such as Kyoto Encyclopedia of Genes and Genomes (KEGG) Mapper and MetaboAnalyst to facilitate the visualisation and interpretation of Ganoderma boninense *metabolomics data, derived from the gas chromatography/quadrupole-time-of-flight (GC/Q-TOF) mass spectrometry-based experiments. Our analysis revealed a time-dependent classification of 39 identified extracellular metabolites from the methanolic extract of* G. boninense*, where several metabolic pathways, i.e. starch and sucrose metabolism, galactose metabolism, valine, leucine and isoleucine degradation and citrate (TCA) cycles were found significantly enriched in* G. boninense*. The integration of pathway analysis tools enabled enhanced biological interpretation, contributing to a deeper understanding of temporal primary metabolic pathways linked to the* G. boninense *developmental process, energy production and cellular functions over time. These findings underscore the importance of the pathway analysis tools in metabolomics, helping to reveal the biological insights which are hidden within the complex metabolite profiles and thus advancing our understanding of the* G. boninense *developmental process* in vitro.

Keywords: functional analysis, *Ganoderma boninense*, GC/Q-TOF, pathway.

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INTRODUCTION

A comprehensive study of metabolites has become an essential approach that advances the understanding of the complex molecular relationships found in biological systems (Chen et al., 2022). The multifunctional roles of metabolites are being analysed for their consistent change in biological processes that underlie the profiles, as shown in metabolomics experiments (Oh et al., 2023; Qiu et al., 2023). In the context of metabolomics research, pathway analysis links the detected metabolites to known metabolic pathways, thus enhancing our understanding of their roles in various physiological and pathological conditions (Kanehisa & Sato, 2020; Xia & Wishart, 2011). In metabolomics, pathway analysis increasingly relies on advanced methods such as over-representation analysis (ORA) and topology-based approaches to interpret complex datasets. These approaches map the metabolites onto established biological pathways and evaluate their significance under various conditions (Wieder et al., 2021, 2022).

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Understanding the microbial metabolism and evaluating its biological importance to various environmental signals, requires recent advances in measuring extracellular metabolites (Pinu & Villas-Boas, 2017). With the various analytical equipment and high throughput methodologies available, the extracellular metabolite analysis has become a significant technique for monitoring microorganism growth parameters (Liu et al., 2022; Qiao et al., 2020). One of the promising analytical platforms in metabolite analysis is gas chromatography-mass spectrometry (GC-MS), which provides sensitive, reproducible and robust techniques for measuring volatile metabolites. Furthermore, GC-MS has an advantage over other analytical techniques, as it allows for faster and more reliable identification of compounds based on their mass spectra, using highly established and widely used public spectral databases (Fiehn, 2016).

Recent research highlights the importance of pathway analysis in metabolomics for data visualisation, as it enables researchers to interpret complex datasets by mapping metabolites to known biochemical pathways. Pathway tools like MetaboAnalyst and Kyoto Encyclopedia of Genes and Genomes (KEGG) Mapper are commonly used for this purpose, offering statistical workflows and visualisation features that assist in identifying significant metabolic changes under different conditions, including disease states or environmental changes (Kanehisa et al., 2022; Li et al., 2023; Pang et al., 2024). For example, in fungal research, KEGG enrichment analysis has identified 47 significant metabolic pathways which are associated with *Metarhizium anisopliae* sporulation, thus highlighting the role of amino acid metabolism, particularly glutamate, aspartate, serine, glycine, arginine and leucine in the sporulation process (Yang et al., 2023). Using the pathway tools, several metabolisms associated with *G. boninense* growth and development, virulence and pathogenicity have also been identified (Santiago et al., 2024).

This current study focuses on *G. boninense*, a wood-decaying basidiomycete fungus responsible for oil palm basal stem rot (BSR). *G. boninense* is of interest because it is the causal agent of BSR disease in oil palm, particularly in Malaysia and Indonesia. *G. boninense* presents a major threat to the oil palm industry, with projections suggesting it could infect up to 860,610 ha of mature oil palms by 2040, thus posing significant economic risks (Olaniyi & Szulczyk, 2020). Within just six months, an infection can lead to a 43% economic loss in affected plantations (Khoo & Chong, 2023). In terms of combating the immediate threat of *Ganoderma*, breakthroughs have been made in using molecular techniques to diagnose infections at the early stages (Murphy, 2014). For example, GC-MS-based metabolomics has been increasingly applied to study the interaction between oil palm and *G. boninense,* particularly for identifying bioactive compounds and understanding metabolic responses under various conditions (Abdullah et al., 2021; Hailini et al., 2020; Isha et al., 2020; Rozali et al., 2017; Rupaedah et al., 2024).

Despite extensive research on *G. boninense*, most studies have only primarily focused on the metabolites produced by its oil palm host, thus leaving gaps in our understanding of the metabolites generated by the fungus itself. Moreover, interpreting the functions of metabolites, which often have diverse roles within the biological systems, remains a significant challenge. To address this, our study aimed to investigate the metabolic pathways underlying the complex *G. boninense* metabolite profiles, through pathway analysis tools in metabolomics. This approach enhances biological interpretations, thus offering deeper insights into the intricate metabolic profiles of *G. boninense* and contributing to a better understanding of the dynamic metabolic processes of *G. boninense*.

MATERIALS AND METHODS

Preparation of Fungal Culture

Ganoderma boninense isolate PER71 was provided by the Plant Pathology and Biosecurity Unit, Malaysian Palm Oil Board (MPOB), Malaysia. *G. boninense* was cultivated using a liquid culture protocol (Nurazah et al., 2021b; Wahab, 2016). The culture was initially grown on six Difco™ PDA (Becton, Dickinson and Company, USA) plates and incubated at 27 ± 1ºC for eight days, as biological replicates. One plug of 9.55 mm from each of the six individual PDA plates was inoculated into 50 mL of MEB (Becton, Dickinson and Company, USA) in a 175 cm^3 Nunclon™ cell culture flask (ThermoFisher Scientific, USA). Three technical replicates were prepared. A total of six mycelial plugs were grown in the cell culture flask at 27 ± 1°C for six days. After six days, the mycelial plugs were collected on sterile filter paper, washed with sterile distilled water (3x) and placed into 50 mL of basal medium supplemented with 5 g of glucose (carbon source) and 3.9 g of 2-N-morpholinoethanesulphonic acid (MES) (nitrogen source) at pH 5.5, in a 175 cm^3 Nunclon™ cell culture flask (ThermoFisher Scientific, USA). A negative control with only growth medium was prepared and labelled as day 0. The culture fluids containing *G. boninense* extracellular metabolites from each flask were collected at different time points (day 2, 4, 6 and 8) to study the dynamic changes in *G. boninense* metabolite profiles over time (Nurazah et al., 2021b).

Three replicate samples were obtained from each time point, for 15 samples (including an additional three samples from day 0).

Metabolite Extraction

Metabolite extraction was conducted according to Nurazah et al. (2021b). The culture fluids containing *G. boninense* extracellular metabolites were freeze-dried using a freeze dryer for two days using a FreeZone® Freeze Drier System (Labconco, USA). About 0.1 g of the powdered culture media was dissolved in 3 mL of methanol (Merck, Germany), vortexed for 1 min and sonicated for 30 min. The mixture was then filtered through a 0.2 µm cellulose acetate Minisart syringe filter (Merck, Germany). A volume of 150 µL of individual methanolic extract was placed into a 250 µL micro-insert and dried under nitrogen for at least an hour. Next, a total of 15 dried samples were subjected to gas chromatography/quadrupole-time-of-flight (GC/Q-TOF) derivatisation procedure according to Rozali et al. (2021). The dried samples were derivatised in 80 µL of 20 mg mL^{-1} methoxyamine hydrochloride in pyridine and incubated at 37ºC for 90 min, followed by derivatisation with 80 µL N-methyl-N-trimethylsilyltrifluoroacetamide (MSTFA) for 30 min. The derivatised samples were allowed to rest for 60 min before injection. All the derivatisation procedures were conducted using an automated GERSTEL multipurpose sampler (MPS) XT 4.2.0 (GERSTEL GmbH & Co. KG, Germany).

Gas Chromatography/Quadrupole-Time-of-Flight Mass Spectrometry

The derivatised samples of 1 µL each were injected into the GC/Q- mass spectrometer (MS) system comprising a Gerstel Autosampler, a 7890B Agilent gas chromatography, and a 7200B Agilent Q-TOF MS (Agilent Technologies, USA). The GC was operated with helium as the carrier gas (1 mL min^{-1}) with VF-5ms (10 m guard column) column (30 m long x 0.25 mm inner diameter x 0.25 µm film thickness). The injection temperature was set at 260ºC with the following temperature program; injection at 80ºC, held for 2 min, followed by a 10ºC oven temp, ramp to 325ºC held for 9 min with a run time of 46 min. Raw data were deconvoluted using the Unknown Analysis tool from MassHunter Quantitative Analysis Software Version B.07.01 (Agilent Technologies, USA) and the identification of compounds was performed by comparison of the spectra to the G1676AA Agilent Fiehn 2013 GC/MS Metabolomics Retention Time Locking (RTL) Library with 70% mass spectrum similarity.

Data Processing and Multivariate Data Analysis

The generated bucket data was uploaded to the MetaboAnalyst ver. 6.0 online platform (Xia et al., 2009) for principal component analysis (PCA) and partial least squares-discriminant analysis (PLS-DA)). The uploaded data were normalised by sum, log-transformed and Pareto scaled. Finally, the model was validated as described by Nurazah et al. (2021a), where the prediction accuracy was based on 5-fold CV (cross-validation), R^2 (predictive quality), and Q^2 (goodness of fit) values. The R^2 values demonstrate model fitness by explaining the variation inside the model and the Q^2 values denote model predictability, illustrating the model's capacity to predict a new data set. The highest R^2 values indicate a robust fit of the data, ranging from 0.0–1.0. R^2 levels approaching 1.0 signify an excellent model description, whereas Q^2 values over 0.5 denote proficient prediction, and values above 0.9 imply excellent prediction (Rozali et al., 2023; Eriksson et al., 2005). Permutation tests measured the significance ($p < 0.01$) of class discrimination: i) Prediction accuracy during training and ii) separation distance based on the ratio between group sum of squares (B/W ratio). The *p*-values less than 0.05 signify a statistically significant model (Khan et al., 2022). Variable importance was also determined using the variable importance in projection (VIP) analysis to detect metabolites that are responsible for the separation in the PLS-DA score scatter plot. Metabolites exhibiting a VIP larger than one (VIP > 1) were selected for further investigation (Liu et al., 2020).

Metabolic Pathway Analysis

Metabolite features with VIP values exceeding 1.0 was selected from the PLS-DA (Yang et al., 2021). It is provided as input for the KEGG pathway mapping tools, giving an overview of their positions within metabolic pathways using KEGG Mapper ver. 5.0 (https://www.kegg.jp/kegg/mapper) based on organism-specific, *Saccharomyces cerevisiae* (budding yeast) (Kanehisa et al., 2022). Pathway analysis was conducted by integrating pathway enrichment and topology analyses using MetaboAnalyst version 6.0 (Xia & Wishart, 2010, 2011) to identify the pathways most significantly affected by the selected metabolites. The KEGG database ID was used to map the names of the compounds with the following parameters: (1) Enrichment analysis was performed using the over-representation analysis hypergeometric test to identify differentially expressed metabolites within functionally related groups, (2) topology analysis was measured using out-degree centrality to assess the compound significance in a specific metabolic network and (3) pathway library

code *sce* (*S. cerevisiae*) in the KEGG database were used as reference metabolic pathways. The multiple testing-Holm-Bonferroni method and false discovery rate (FDR) were then applied to the statistically significant *p*-values obtained from the pathway enrichment analysis. The metabolic pathways with significant *p*-values ($p < 0.05$) were selected.

RESULTS AND DISCUSSION

Visualisation of *G. boninense* Extracellular Metabolite Profiles

Using different days for the extraction of metabolites is essential for capturing the dynamic changes in the metabolic profile of *G. boninense* throughout its growth stages. Each time point corresponds to a specific stage of growth, allowing us to observe the varying production of extracellular metabolites, as the organism progresses from one stage to another. Studies by Ellstrom et al. (2015) and Nagappan et al. (2024) highlight that these different growth stages of fungi exhibit distinct metabolic activities, which can influence the production of metabolites. By assessing the metabolic profiles at various time points, we gain a comprehensive understanding of the metabolic responses of an organism and potential adaptations during its lifecycle. This approach ultimately provides insights into the ecological and functional roles of the metabolites in relation to the growth phases of *G. boninense*.

PCA allows the visualisation of underlying patterns in GC/Q-TOF data, highlighting the intergroup variability of *G. boninense* metabolites associated with cultivation time. The PCA scores plot indicated that the principal component (PC)1 (30.0%) and PC2 (25.5%) contributed to 55.5% of the total variation in the dataset. Sample groupings were observed along PC1 and PC2 according to the days of cultivation (*Figure 1a*). A close sample grouping was observed for day 6 and 8, separated from days 2 and 4 by PC1, while the sample grouping for day 2 was separated from day 4 by PC2. Further analysis of the observed sample groupings was conducted using PLS-DA. As depicted in the scores plot, individual groupings of *G. boninense* samples cultivated at day 0, 2, 4, 6 and 8 were also observed with 42.8% of the total variation in the dataset from PC1 and PC2 (*Figure 1b*). The metabolite profiles were distributed along PC1, suggesting the time-dependent metabolic changes in *G. boninense*. The assessment of the classification significance was done by the 5-fold cross-validation (CV) that showed excellent fitting (R^2) and predictive (Q^2) values of 0.9951 and 0.9605, respectively. The model also showed significance at $p < 0.01$ according to the permutation tests. The variable importance in projection (VIP > 1.0) identified from the loadings plot was sorted by importance, and has identified 39 discriminant metabolites ($p < 0.05$) with greater contributions to the observed groupings, and also identified the changes in *G. boninense* according to cultivation time (*Figure 2*).

Pathway Analysis by KEGG Mapper and MetaboAnalyst

Metabolic perturbations examined using GC-Q/TOF showed distinct changes in metabolic pathways in *G. boninense* which may explain

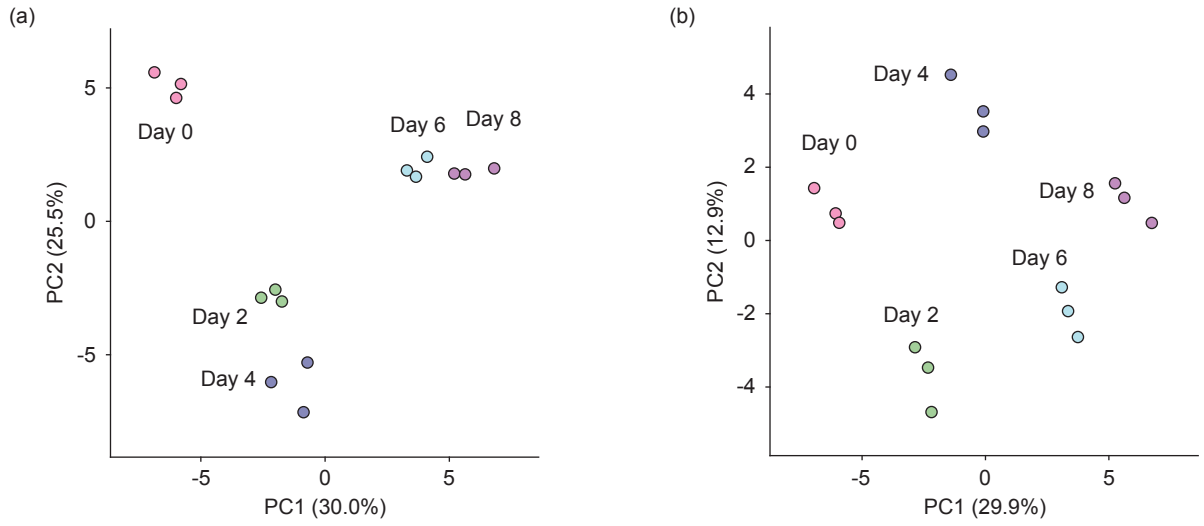

Figure 1. The scores plot of (a) principal component analysis (PCA) and (b) partial least squares-discriminant analysis (PLS-DA) for Ganoderma boninense *metabolites at day 0, 2, 4, 6 and 8 of cultivation.*

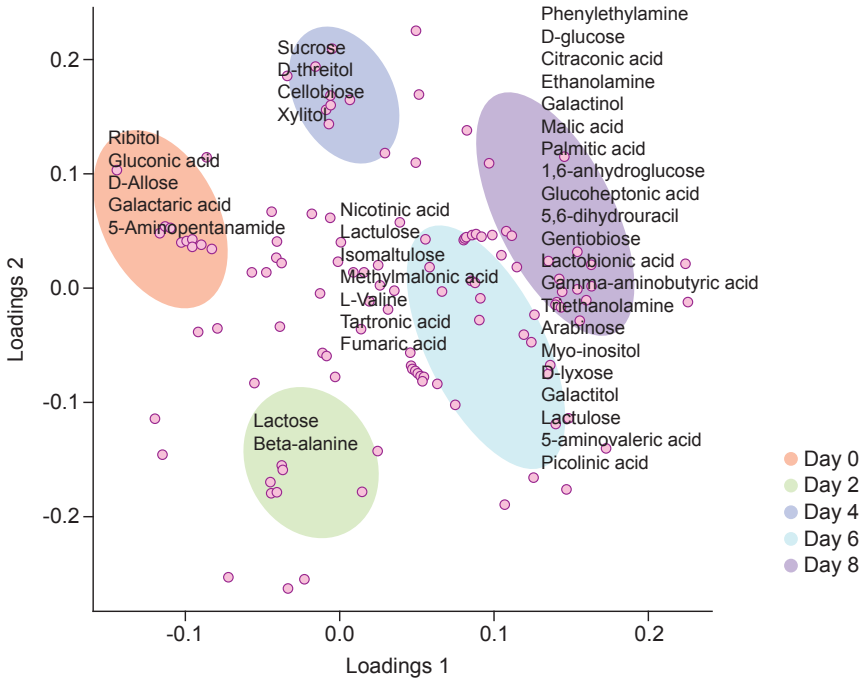

Figure 2. The loadings plot of partial least squares-discriminant analysis (PLS-DA) for Ganoderma boninense *metabolites at day 0, 2, 4, 6 and 8 of cultivation.*

the continuous metabolic perturbation across the cultivation time. By scrutinising the data through the lenses of PCA and PLS-DA, we gained an overview of metabolite patterns and identified key metabolites associated with *G. boninense* cultivation time. Beginning with the list of discriminant metabolites, the pathway analysis served as the next step that allowed us to distinguish the biological pathways that enlighten the collective behaviour of metabolites, in response to the experimental conditions (Karnovsky & Li, 2020).

Global visualisation of the differential metabolites according to the cultivation time of *G. boninense* in their specific biological pathways was viewed using KEGG Mapper (Kanehisa & Sato, 2020). KEGG Mapper comprises a set of mapping tools, designed to link molecular (such as genes, proteins, metabolites and glycans) with higher-level objects (such as pathways, modules, hierarchical structures, taxonomy and diseases) (Kanehisa et al., 2017). Based on the identified metabolites from the multivariate analysis, KEGG Mapper revealed 51 PATHWAYS and 31 MODULES. The specific metabolic pathways respective to the differential metabolites are highlighted (*Figure 3*). Details of the KEGG metabolic pathways according to the mapped metabolites are shown in *Table 1*. The result shows the involvement of fungal primary metabolism, with possible pathways altered in *G. boninense*, which include glycan metabolism, biosynthesis of terpenoids and polyketides, lipid metabolism, carbohydrate metabolism, energy metabolism, amino acid metabolism, nucleotide metabolism and the metabolism of cofactors and vitamins. The KEGG pathway classification shows changes in carbohydrate metabolism across the cultivation time, which could potentially have significant roles during the growth of *G. boninense* (*Figure 4*) (Zhou et al., 2021).

KEGG Mapper is particularly useful for exploring individual pathways and understanding the roles of specific metabolites within those pathways. However, it may not include some of the more advanced statistical analyses. Thus, implementing Pathway Analysis in MetaboAnalyst provides a more comprehensive and systematic approach that incorporates various statistical methods, namely enrichment and pathway topology analysis (Xia & Wishart, 2010). To better understand the dynamics of the observed alterations, we depicted 17 altered metabolic pathways in the pathway analysis plot, using a criterion of pathway impact values exceeding 0.01 (Wang et al., 2022) (*Figure 5*). Among these, four metabolic pathways were found to be significant in the enrichment analysis (highlighted as dark red circles at the top of the pathway analysis plot). These pathways include starch and sucrose metabolism, galactose metabolism, valine, leucine and isoleucine degradation and citrate (TCA) cycle (*Table 2*). These findings highlighted the potential pathways in primary metabolism, underlying the time-dependent metabolic alterations that occur during the growth, reproduction and survival of *G. boninense*.

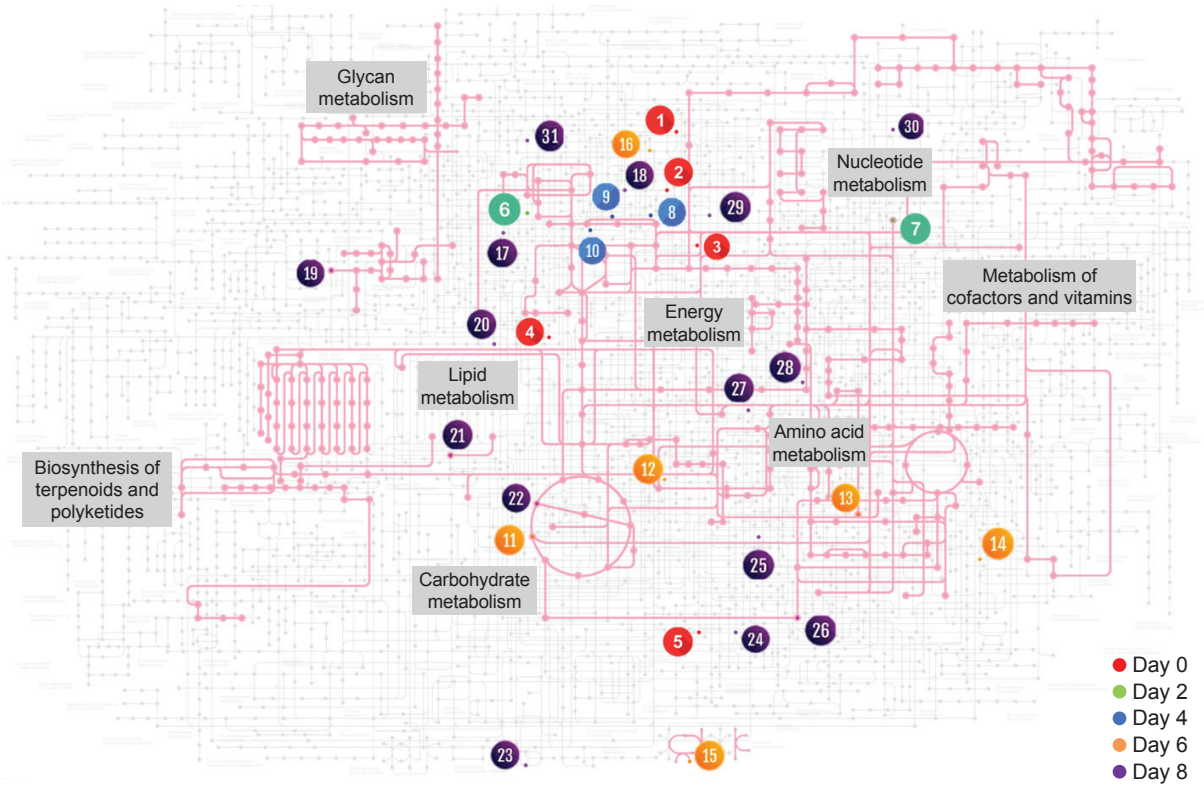

Figure 3. The KEGG metabolic map of Ganoderma boninense *metabolites associated with specific metabolic pathways. The coloured numbers represent the differential metabolites at day 0, 2, 4, 6 and 8 of cultivation. The nodes represent metabolites and the edge links two nodes if they are involved in a reaction as substrate and product.*

TABLE 1. THE KEGG METABOLIC PATHWAYS ASSOCIATED WITH *Ganoderma boninense* METABOLITES

Time points (day)	Putative metabolite (KEGG ID)	Metabolic pathway
0	1. Galactaric acid (C00879) 2. Gluconic acid (C00257) 3. Ribitol (C00474) 4. D-Allose (C01487) 5. 5-Aminopentanamide (C00990)	• Carbohydrate metabolism • Metabolism of cofactors and vitamins • Amino acid metabolism
2	6. Lactose (C00243) 7. Beta-alanine (C00099)	• Carbohydrate metabolism • Nucleotide metabolism
4	8. Xylitol (C00379) 9. Cellobiose (C00185) 10. Sucrose (C00089)	• Carbohydrate metabolism
6	11. Fumaric acid (C00122) 12. Methylmalonic acid (C02170) 13. L-Valine (C00183) 14. Nicotinic acid (C00253) 15. Fumaric acid (C00122) 16. Isomaltulose (C00252)	• Carbohydrate metabolism • Energy metabolism • Amino acid metabolism • Nucleotide metabolism • Metabolism of other amino acids • Metabolism of cofactors and vitamins
8	17. Galactitol (C01697) 18. D-Glucose (C00031) 19. Ethanolamine (C00189) 20. Inositol (C00137) 21. Palmitic acid (C00249) 22. Malic acid (C00149) 23. Malic acid (C00149) 24. 5-aminovaleric acid (C00431) 25. Phenylethylamine (C05332) 26. γ -aminobutyric acid (C00334) 27. Picolinic acid (C10164) 28. Citraconic acid (C02226) 29. Arabinose (C00259) 30. 5,6-dihydrouracil (C00429) 31. Galactinol (C01235)	• Carbohydrate metabolism • Lipid metabolism • Amino acid metabolism • Nucleotide metabolism

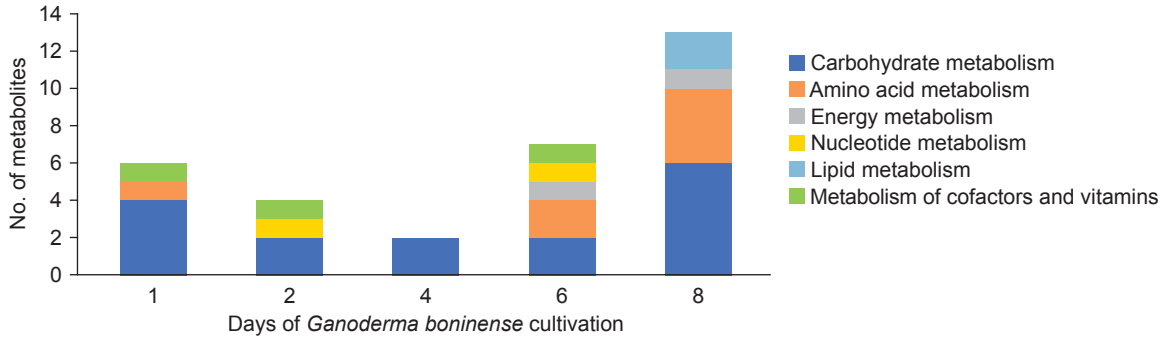

Figure 4. The specific KEGG pathways associated with the metabolites expressed during Ganoderma boninense *cultivation.*

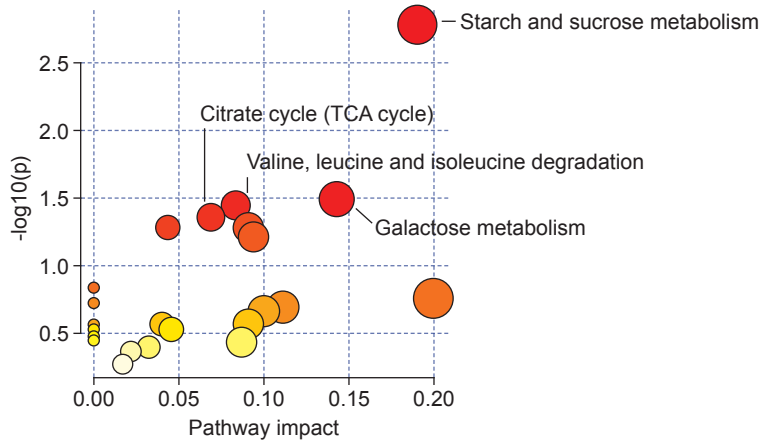

Figure 5. Pathway analysis plot that represents the pathway impact values (x-axis) and p*-value from the enrichment analysis (y-axis). The x-axis represents the pathway impact values computed from the pathway topological analysis, and the y-axis is the -log of the* p*-value obtained from the pathway enrichment analysis. Larger circles represent nodes with greater pathway enrichment and darker colors represent more significance.*

TABLE 2. OVERVIEW OF KEY METABOLIC PATHWAYS IN *Ganoderma boninense*

No.	Metabolic pathway	Putative matched metabolite	*p*-value	FDR	Pathway impact
1.	Starch and sucrose metabolism	Sucrose (C00089[a]) D-Glucose (C00031) Isomaltose (C00252)	0.0016	0.1203	0.1905
2.	Galactose metabolism	Sucrose (C00089) D-Glucose (C00031)	0.0322	0.6340	0.1429
3.	Valine, leucine and isoleucine degradation	L-Valine (C00183) Methylmalonic acid (C02170)	0.0360	0.6340	0.0833
4.	Citrate cycle (TCA cycle)	Malic acid (C00149) Fumaric acid (C00122)	0.0437	0.6340	0.0690

Note: [a] Letters in parentheses indicate the KEGG ID.

Primary metabolism, i.e. carbohydrate and amino acid metabolisms in fungi plays a vital role in biochemical processes required for growth, energy production, and cellular maintenance. Carbohydrate metabolism in *G. boninense*, including starch and sucrose metabolism, galactose metabolism, and citrate cycle (TCA cycle) exhibit enriched metabolic activities. This observation highlights the response of *G. boninense* to the availability of carbohydrates as a source of nutrients, supplying the energy and essential coenzymes needed to sustain growth and reproduction (Wisecaver et al., 2014; Zhou et al., 2021). Furthermore, the presence of key metabolites, i.e., sucrose, glucose, malic acid and fumaric acid in these metabolic activities, play important roles in energy production and maintenance of the cellular metabolism of *G. boninense* (Chroumpi et al., 2020). As reported by Li et al. (2024), a monosaccharide namely, glucose is consumed by microbes during the early and middle stages of growth. The upregulation of the carbohydrate metabolism pathway was also observed in a previous study by

Nagappan et al. (2024), highlighting *G. boninense*'s ability to degrade polysaccharides found in the oil palm cell walls. Being a hemibiotrophic fungus, this finding suggests the key importance of carbohydrate metabolism in *G. boninense'*s survival and pathogenicity, which may support its early biotrophic lifestyle in accessing nutrients (Chong et al., 2017).

G. boninense also exhibits enriched metabolic activities in the amino acid metabolism pathway, i.e. valine, leucine and isoleucine degradation. The alteration in this metabolism emphasises the crucial role of L-valine and methylmalonic acid in this pathway, facilitating nutrient absorption and balance, producing the building blocks of cells, and providing the energy necessary for life-sustaining functions (Borin & Oliveira, 2022). Moreover, this pathway linked to the TCA cycle, serves as an alternative route for energy generation, especially when primary carbon sources are limited (Berger et al., 2007). This observation suggests that as the nutrient levels decline, *G. boninense* may enter the growth stage where its primary metabolism shifts towards sustaining cell survival.

CONCLUSION

Although the biological interpretation of metabolites, which are involved in multiple roles within a biological system, remains challenging, our findings demonstrated utilising the pathway analysis tools, i.e. KEGG Mapper and pathway analysis in MetaboAnalyst, which enhance the visualisation and interpretation of metabolomics data in relation to biological processes. As in the context of *G. boninense*, several metabolic pathways, i.e. starch and sucrose metabolism, galactose metabolism, valine, leucine and isoleucine degradation and TCA cycle were found significantly enriched during cultivation. The pathway analysis tools enhanced biological interpretation, offering deeper insights into the dynamics of *in vitro* metabolite production, and temporal primary metabolic pathways associated with *G. boninense* development, energy production and cellular functions over time. These findings highlight the crucial role of pathway analysis in metabolomics workflow, revealing hidden biological insights within the complex metabolite profiles and advancing our understanding of *G. boninense* developmental processes.

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REFERENCES

Abdullah, S., Oh, Y. S., Kwak, M. K., & Chong, K. (2021). Biophysical characterization of antibacterial compounds derived from pathogenic fungi *Ganoderma boninense*. *Journal of Microbiology*, *59*(2), 164–174. https://doi.org/10.1007/s12275-021-0551-8

Berger, S., Sinha, A. K., & Roitsch, T. (2007). Plant physiology meets phytopathology: Plant primary metabolism and plant-pathogen interactions. *Journal of Experimental Botany*, *58*(15-16), 4019–4026. https://doi.org/10.1093/jxb/erm298

Borin, G. P., & Oliveira, J. V. D. C. (2022). Assessing the intracellular primary metabolic profile of *Trichoderma reesei* and *Aspergillus niger* grown on different carbon sources. *Frontiers in Fungal Biology*, *3*, 998361. https://doi.org/10.3389/ffunb.2022.998361

Chen, Y., Li, E. M., & Xu, L. Y. (2022). Guide to metabolomics analysis: A bioinformatics workflow. *Metabolites*, *12*(4), 357. https://doi.org/10.3390/metabo12040357

Chong, K. P., Dayou, J., & Alexander, A. (2017). Pathogenic nature of *Ganoderma boninense* and basal stem rot disease. In K. P. Chong, J. Dayou, & A. Alexander (Eds.), *Detection and control of* Ganoderma boninense *in oil palm crop* (pp. 5–12). Springer. https://doi.org/10.1007/978-3-319-54969-9_2

Chroumpi, T., Mäkelä, M. R., & De Vries, R. P. (2020). Engineering of primary carbon metabolism in filamentous fungi. *Biotechnology Advances*, *43*, 107551. https://doi.org/10.1016/j.biotechadv.2020.107551

Ellström, M., Shah, F., Johansson, T., Ahrén, D., Persson, P., & Tunlid, A. (2015). The carbon starvation response of the ectomycorrhizal fungus *Paxillus involutus*. *FEMS Microbiology Ecology*, *91*(4), fiv027. https://doi.org/10.1093/femsec/fiv027

Eriksson, L., Johansson, E., Antti, H., & Holmes, E. (2005). Multi- and megavariate data analysis:

Finding and using regularities in metabonomics data. In D. G. Robertson, J. Lyndon, J. K. Nicholson, & E. Holmes (Eds.), *Metabonomics in toxicity assessment* (pp. 263–336). CRC Press.

Fiehn, O. (2016). Metabolomics by gas chromatography-mass spectrometry: Combined targeted and untargeted profiling. *Current Protocols in Molecular Biology*, *114*, 30.4.1–30.4.32. https://doi.org/10.1002/0471142727.mb3004s114

Hailini, Z. H., Seman, I. A., Noor, M. A., Aripin, S. M. (2020). A feasibility study on volatile organic compounds profiling of oil palm-*Ganoderma* infected wood for basal stem rot detection. *Malaysian Journal of Analytical Sciences*, *24*, 599–614.

Isha, A., Yusof, N. A., Shaari, K., Osman, R., Abdullah, S. N. A., & Wong, M. Y. (2020). Metabolites identification of oil palm roots infected with *Ganoderma boninense* using GC-MS-based metabolomics. *Arabian Journal of Chemistry*, *13*(7), 6191–6200. https://doi.org/10.1016/j.arabjc.2020.05.026

Kanehisa, M., Furumichi, M., Tanabe, M., Sato, Y., & Morishima, K. (2017). KEGG: New perspectives on genomes, pathways, diseases, and drugs. *Nucleic Acids Research*, *45*(D1), D353–D361. https://doi.org/10.1093/nar/gkw1092

Kanehisa, M., & Sato, Y. (2020). KEGG Mapper for inferring cellular functions from protein sequences. *Protein Science*, *29*(1), 28–35. https://doi.org/10.1002/pro.3711

Kanehisa, M., Sato, Y., & Kawashima, M. (2022). KEGG mapping tools for uncovering hidden features in biological data. *Protein Science*, *31*(1), 47–53. https://doi.org/10.1002/pro.4172

Karnovsky, A., & Li, S. (2020). Pathway analysis for targeted and untargeted metabolomics. In S. Li (Ed.), *Computational methods and data analysis for metabolomics* (pp. 387–400). Springer. https://doi.org/10.1007/978-1-0716-0239-3_19

Khan, M. B. N., Iftikhar, F., Ali, M., Danish, A., Shamsi, T., Musharraf, S. G., & Siddiqui, A. J. (2022). XMN polymorphism along with HU administration renders alterations to RBC membrane lipidome in β-thalassemia patients. *Chemistry and Physics of Lipids*, *244*, 105195. https://doi.org/10.1016/j.chemphyslip.2022.105195

Khoo, Y. W., & Chong, K. P. (2023). *Ganoderma boninense*: General characteristics of pathogenicity and methods of control. *Frontiers in Plant Science*, *14*, 1156869. https://doi.org/10.3389/fpls.2023.1156869

Li, R., Wang, T., Bo, N., Wang, Q., Chen, Q., Liang, Z., Guan, Y., Jiang, B., Ma, Y., & Zhao, M. (2024). The carbohydrate metabolism and expression of carbohydrate-active enzyme genes in *Aspergillus luchuensis* fermentation of tea leaves. *Frontiers in Microbiology*, *15*, 1408645. https://doi.org/10.3389/fmicb.2024.1408645

Li, Y., Wang, C., & Chen, M. (2023). Metabolomics-based study of potential biomarkers of sepsis. *Scientific Reports*, *13*(1), 585. https://doi.org/10.1038/s41598-022-24878-z

Liu, L., Zuo, Z. T., Xu, F. R., & Wang, Y. Z. (2020). Study on quality response to environmental factors and geographical traceability of wild *Gentiana rigescens* Franch. *Frontiers in Plant Science*, *11*, 1128. https://doi.org/10.3389/fpls.2020.01128

Liu, Z., Kang, B., Duan, X., Hu, Y., Li, W., Wang, C., Li, D., & Xu, N. (2022). Metabolomic profiles of the liquid-state fermentation in co-culture of *Aspergillus oryzae* and *Zygosaccharomyces rouxii*. *Food Microbiology*, *103*, 103966. https://doi.org/10.1016/j.fm.2021.103966

Murphy, D. (2014). The future of oil palm as a major global crop: Opportunities and challenges. *Journal of Oil Palm Research*, *26*(1), 1–24.

Nagappan, J., Ooi, S. E., Chan, K. L., Kadri, F., Nurazah, Z., Halim, M. A. A., Angel, L. P. L., Sundram, S., Chin, C. F., May, S. T., & Low, E. T. L. (2024). Transcriptional effects of carbon and nitrogen starvation on *Ganoderma boninense*, an oil palm phytopathogen. *Molecular Biology Reports*, *51*(1), 212. https://doi.org/10.1007/s11033-023-09054-4

Nurazah, Z., Idris, A. S., Mohd Din, A., Manaf, M. A. A., Othman, A., & Ramli, U. S. (2021a). Metabolite fingerprinting of oil palm (*Elaeis guineensis* Jacq.) root for the identification of altered metabolic pathways associated with basal stem rot (BSR) disease. *Physiological and Molecular Plant Pathology*, *115*, 101647. https://doi.org/10.1016/j.pmpp.2021.101647

Nurazah, Z., Othman, A., & Ramli, U. S. (2021b). Principal component analysis (PCA) evaluation of liquid chromatography-mass spectrometry (LC-MS) datasets of *Ganoderma boninense* intracellular metabolites. *Journal of Oil Palm Research*, *33*(3), 555–564. https://doi.org/10.21894/jopr.2020.0103

Oh, S. W., Imran, M., Kim, E. H., Park, S. Y., Lee, S. G., Park, H. M., Jung, J. W., & Ryu, T. H. (2023). Approach strategies and application of metabolomics to biotechnology in plants. *Frontiers in Plant Science*, *14*, 1192235. https://doi.org/10.3389/fpls.2023.1192235

Olaniyi, O. N., & Szulczyk, K. R. (2020). Estimating the economic damage and treatment cost of basal stem rot striking the Malaysian oil palms. *Forest Policy and Economics*, *116*, 102163. https://doi.org/10.1016/j.forpol.2020.102163

Pang, Z., Lu, Y., Zhou, G., Hui, F., Xu, L., Viau, C., Spigelman, A. F., MacDonald, P. E., Wishart, D. S., Li, S., & Xia, J. (2024). MetaboAnalyst 6.0: Towards a unified platform for metabolomics data processing, analysis, and interpretation. *Nucleic Acids Research*, *52*(W1), W398–W406. https://doi.org/10.1093/nar/gkae253

Pinu, F. R., & Villas-Boas, S. G. (2017). Extracellular microbial metabolomics: The state of the art. *Metabolites*, *7*(3), 43. https://doi.org/10.3390/metabo7030043

Qiao, Y., Liu, G., Lv, X., Fan, X., Zhang, Y., Meng, L., Ai, M., & Feng, Z. (2020). Metabolic pathway profiling in intracellular and extracellular environments of *Streptococcus thermophilus* during pH-controlled batch fermentations. *Frontiers in Microbiology*, *10*, 3144. https://doi.org/10.3389/fmicb.2019.03144

Qiu, S., Cai, Y., Yao, H., Lin, C., Xie, Y., Tang, S., & Zhang, A. (2023). Small molecule metabolites: Discovery of biomarkers and therapeutic targets. *Signal Transduction and Targeted Therapy*, *8*(1), 132. https://doi.org/10.1038/s41392-023-01399-3

Rozali, N. L., Azizan, K. A., Singh, R., Syed Jaafar, S. N., Othman, A., Weckwerth, W., & Ramli, U. S. (2023). Fourier transform infrared (FTIR) spectroscopy approach combined with discriminant analysis and prediction model for crude palm oil authentication of different geographical and temporal origins. *Food Control*, *146*, 109509. https://doi.org/10.1016/j.foodcont.2022.109509

Rozali, N. L., Tahir, N. I., Hassan, H., Othman, A., & Ramli, U. S. (2021). Identification of amines, amino and organic acids in oil palm (*Elaeis guineensis* Jacq.) spear leaf using GC- and LC/Q-TOF MS metabolomics platforms. *Biocatalysis and Agricultural Biotechnology*, *37*, 102165. https://doi.org/10.1016/j.bcab.2021.102165

Rozali, N. L., Yarmo, M. A., Idris, A. B., Kushairi, A., & Ramli, U. S. (2017). Metabolomics differentiation of oil palm (*Elaeis guineensis* Jacq.) spear leaf with contrasting susceptibility to *Ganoderma boninense*. *Plant Omics*, *10*(2), 45–52. https://doi.org/10.21475/poj.10.02.17.pne364

Rupaedah, B., Wachid, W. A., Safarrida, A., Purwoko, D., & Masruri, M. (2024). Volatile organic compounds (VOCs) produced by indigenous bacterium strain BS1727 as antifungal agents against *Ganoderma boninense*. *Journal of the Saudi Society of Agricultural Sciences*, *23*(5), 345–351. https://doi.org/10.1016/j.jssas.2024.02.002

Santiago, K. A. A., Wong, W. C., Goh, Y. K., Tey, S. H., & Ting, A. S. Y. (2024). Pathogenicity of monokaryotic and dikaryotic mycelia of *Ganoderma boninense* revealed via LC-MS-based metabolomics. *Scientific Reports*, *14*, 5330. https://doi.org/10.1038/s41598-024-56129-8

Wahab, M. A. A. (2016). *Ganoderma stem rot of oil palm: Epidemiology, diversity and pathogenicity* (Doctoral dissertation). University of Bath.

Wang, D., Zhao, L., Hao, Z., Huang, Y., Liao, Y., Wang, L., Zhang, J., Cao, S., & Liu, L. (2022). High-throughput and untargeted metabolic profiling revealed the potential effect and mechanisms of paeoniflorin in young asthmatic rats. *Frontiers in Pharmacology*, *13*, Article 829780. https://doi.org/10.3389/fphar.2022.829780

Wieder, C., Frainay, C., Poupin, N., Rodríguez-Mier, P., Vinson, F., Cooke, J., Lai, R. P., Bundy, J. G., Jourdan, F., & Ebbels, T. (2021). Pathway analysis in metabolomics: Recommendations for the use of over-representation analysis. *PLoS Computational Biology*, *17*(9), e1009105. https://doi.org/10.1371/journal.pcbi.1009105

Wieder, C., Lai, R. P. J., & Ebbels, T. M. D. (2022). Single sample pathway analysis in metabolomics: Performance evaluation and application. *BMC Bioinformatics*, *23*, 481. https://doi.org/10.1186/s12859-022-05005-1

Wisecaver, J. H., Slot, J. C., & Rokas, A. (2014). The evolution of fungal metabolic pathways. *PLoS Genetics*, *10*(12), e1004816. https://doi.org/10.1371/journal.pgen.1004816

Xia, J., Psychogios, N., Young, N., & Wishart, D. S. (2009). MetaboAnalyst: A web server for metabolomic data analysis and interpretation. *Nucleic Acids Research*, *37*, W652–W660. https://doi.org/10.1093/nar/gkp356

Xia, J., & Wishart, D. S. (2010). MetPA: A web-based metabolomics tool for pathway analysis and visualization. *Bioinformatics*, *26*(18), 2342–2344. https://doi.org/10.1093/bioinformatics/btq418

Xia, J., & Wishart, D. (2011). Web-based inference of biological patterns, functions, and pathways from metabolomic data using MetaboAnalyst. *Nature Protocols*, *6*, 743–760. https://doi.org/10.1038/nprot.2011.319

Yang, X. L., Li, L., Zhang, T. F., Deng, J., Lin, X. L., Li, Y. M., Xia, B. H., & Lin, L. M. (2021). GC-MS-based serum metabolomic investigations on the ameliorative effects of polysaccharide from *Turpinia folium* in hyperlipidemia rats. *Oxidative Medicine and Cellular Longevity*, *2021*, 9180635. https://doi.org/10.1155/2021/9180635

Yang, H., Tian, L., Qiu, H., Qin, C., Ling, S., & Xu, J. (2023). Metabolomics analysis of sporulation-associated metabolites of *Metarhizium anisopliae* based on gas chromatography-mass spectrometry. *Journal of Fungi*, *9*(10), 1011. https://doi.org/10.3390/jof9101011

Zhou, S., Zhang, X., Ma, F., Xie, S., Tang, C., Tang, Q., & Zhang, J. (2021). Integrative analysis of selected metabolites and the fungal transcriptome during the developmental cycle of *Ganoderma lucidum* strain G0119 correlates lignocellulose degradation with carbohydrate and triterpenoid metabolism. *Applied and Environmental Microbiology*, *87*(13), e0053321. https://doi.org/10.1128/AEM.00533-21