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Original Article

Microbial biofertilizer-induced fatty acid and squalene modulation for enhancing plant resilience under CBRNE-related abiotic stress

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Abstract—Abiotic stress is one of the main factors that cause a decline in plant growth and productivity. The use of microorganism-based biofertilizers can be an environmentally friendly alternative to enhance plant resistance to extreme environmental conditions. This study aims to analyze the role of microorganisms in biofertilizers on the profile of fatty acids and squalene related to plant abiotic stress resistance. Metabolite analysis was carried out using a metabolomic approach and metabolic pathway mapping through the KEGG database. Identification results showed the presence of important compounds such as palmitic acid, oleic acid, stearic acid, phytol, squalene, alpha-tocopherol, stigmasterol, and sitosterol. These compounds play a role in cell membrane stability, antioxidant activity, and protection against oxidative stress. Pathway analysis shows the involvement of the Glycosylphosphatidylinositol (GPI)-anchor biosynthesis pathway, which is related to membrane metabolism and the cellular response to environmental stress. The research results indicate that microorganisms in biofertilizers have the potential to enhance plant adaptation to abiotic stress through the regulation of lipid metabolism and the production of bioactive compounds. Thus, microorganism-based biofertilizers can be developed as an environmentally friendly alternative to support sustainable agriculture.

Keywords— *Abiotic Stress; Biofertilizer; Fatty Acid; Microorganism; Squalene*

1. INTRODUCTION

Problems in plants are very diverse, one of which is caused by abiotic stress factors. Abiotic stress is an environmental condition that can inhibit the growth, development, and productivity of plants. Factors that influence abiotic stress include soil salinity, extreme temperatures, soil pH, heavy metals, and excessive radiation [1-4].

Nitrogen is one of the essential macronutrients that plants need in the process of growth and metabolism. Nitrogen plays an important role in the formation of chlorophyll, proteins, amino acids, enzymes, as well as various other metabolite compounds that support vegetative growth in plants [5-6,3]. However, the availability of nitrogen in the soil is often limited because it easily undergoes leaching, volatilization, and denitrification, especially under environmental conditions experiencing abiotic stress. Continuous use of inorganic nitrogen fertilizers can also cause a decline in soil quality and environmental pollution [7].

One environmentally friendly approach that can be used to increase plant resistance to abiotic stress is the use of biofertilizers. Biofertilizers are organic fertilizers that contain various beneficial microorganisms that act as plant growth promoters and maintain soil fertility and health sustainably. Plant Growth-Promoting Rhizobacteria (PGPR) microorganisms play an important role in enhancing plant resistance to abiotic stress. PGPR can increase nitrogen efficiency through biological nitrogen fixation, improve nutrient uptake, produce phytohormones, and generate antioxidants and osmoprotectants that help plants cope with environmental stress [1].

Previous research reported that several bacteria such as Azotobacter sp, Pseudomonas fluorescens, Bacillus thuringiensis, and other rhizosphere bacteria are able to improve the quality of biofertilizers as well as the nutrient content of the soil, especially nitrogen, phosphorus, and potassium [8-11].

Research on the relationship between nitrogen-based biofertilizers and changes in fatty acid profiles and squalene production in plants experiencing abiotic stress is still limited. Given the crucial role of fatty acids and squalene in maintaining cell membrane fluidity and physiological stability, investigating the impact of nitrogen-based biofertilizers on these profiles is essential for developing sustainable strategies to enhance plant abiotic stress resistance [12].

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2. EXPERIMENTAL SECTION

2.1. Materials

The materials used in this study included samples of biofertilizers based on microorganisms containing a consortium of Plant Growth Promoting Rhizobacteria (PGPR). The chemicals used consisted of methanol, n-hexane, ethyl acetate, distilled water, as well as standard compounds of fatty acids and squalene for metabolite analysis. All chemicals used had pro analysis purity levels.

2.2 Instrumentation

The instruments used in this study include an analytical balance, vortex, mixer, centrifuge, and laboratory glassware. Metabolite profile analysis was conducted using Gas Chromatography-Mass Spectrometry (GC-MS). Metabolic pathway analysis was performed using the Kyoto Encyclopedia of Genes and Genomes (KEGG) database, Human Metabolome Database (HMDB), and PubChem for compound identification and pathway analysis. Metabolomic data were analyzed using metabolomics pathway analysis software to identify pathway impact and the relationship of metabolites to plant abiotic resistance.

2.3 N Fertilizer Production

Biofertilizer is made using microorganisms consisting of nitrogen-fixing bacteria and PGPR bacteria produced from potato fermentation. Liquid biofertilizer is made using liquid organic materials as a fermentation medium, such as molasses, coconut water, and organic material extracts. For the N fertilizer formulation, an organic nitrogen source is added. The microorganism suspension is inoculated into the fermentation medium and then incubated for 7 days at room temperature with aeration and periodic stirring until a homogeneous liquid biofertilizer is obtained.

2.4 Metabolite Extraction from Liquid Biofertilizer

Metabolite extraction was carried out to obtain bioactive compounds and secondary metabolites present in liquid biofertilizer [13]. A liquid fertilizer sample of 50-100 mL was centrifuged at 8000 rpm for 10 minutes to separate microbial biomass and the supernatant. The supernatant was extracted using an organic solvent of n-hexane and ethyl acetate in a 1:1 ratio. The mixture was extracted using the maceration method and homogenized using a vortex for 10 minutes, then left to stand until two phases formed. The organic phase was separated and evaporated using a rotary evaporator at 40°C until crude metabolite extract was obtained. The extract was then stored at 4°C before GC-MS analysis.

2.5 GC-MS Analysis

Metabolite analysis was conducted using Gas Chromatography-Mass Spectrometry (GC-MS) following Fendiyanto et al. (2025a, 2025b) [14-15]. A total of 1 µL of metabolite extract was injected into the GC-MS system using a nonpolar capillary column. Helium gas was used as the carrier gas at a constant flow rate. The GC-MS analysis conditions included an injector temperature of 250°C, an initial oven temperature of 50°C for 2 minutes, then gradually increased to 300°C. Mass detection was carried out over an m/z range of 40-600. Metabolite compounds were identified based on mass spectrum patterns compared with the NIST, HMDB, PubChem ID, and KEGG ID databases. The metabolite identification results, including hydrocarbon fatty acids, sterols, squalene, and other bioactive compounds, are presented in the form of a metabolite identification table that includes the compound name, compound match, HMDB ID, PubChem ID, and KEGG ID.

2.6 Metabolomic Analysis

GC-MS data results were analyzed metabolomically using metabolite pathway analysis software such as MetaboAnalyst and the KEGG Pathway Database. Compounds that were successfully identified were first converted into HMDB, PubChem, or KEGG compound ID formats for metabolic pathway mapping. The analyses conducted were the identification of significant metabolites, metabolic pathway analysis, enrichment and pathway impact analysis, metabolic pathway visualization, and biological interpretation.

3. RESULT AND DISCUSSION

3.1 Identification of Metabolite Compounds

Metabolite analysis results (**Table 1**) showed that treatment with microorganism-based biofertilizers produced various bioactive compounds dominated by fatty acids, sterols, terpenoids, and antioxidant compounds. Based on identification results, several important compounds were obtained, such as palmitic acid, oleic acid, stearic acid, phytol, squalene, alpha-tocopherol, stigmasterol, and sitosterol. These compounds are known to be associated with mechanisms of cell protection against abiotic stress through membrane stabilization, antioxidant activity, and regulation of lipid metabolism [16]. The presence of fatty acid compounds such as palmitic acid, oleic acid, and stearic acid indicates a fairly high lipid metabolism activity in the treatment given [17-18]. Fatty acids play an important role in maintaining the fluidity and integrity of cell membranes when plants experience environmental stress. In addition, the detection of squalene and alpha-tocopherol indicates an increase in antioxidant mechanisms to reduce oxidative damage caused by the formation of reactive oxygen species (ROS) under abiotic stress conditions. Sterol compounds such as stigmasterol and sitosterol were also found in metabolite analysis results. Sterols function in maintaining cell membrane stability as well as assisting in the physiological adaptation processes of plants to environmental changes. The presence of epoxysqualene in the identification results indicates a connection between sterol and terpenoid biosynthesis pathways related to the plant's adaptive response to stress [19].

Table 1. Metabolite identification results

No	Query	Match	HMDB	PubChem	KEGG
1	Tetradecanoic acid	Myristic acid	HMDB0000806	11005	C06424
2	Hexathiiane	Hexathiepane	HMDB0035871	87012	NA
3	Neophytadiene	beta-Myrcene	HMDB0038169	31253	C06074
4	Tetramethyl hexadec	Phytanoyl-CoA	HMDB0001359	439640	C02060
5	n-Hexadecanoic acid	Palmitic acid	HMDB0000220	985	C00249
6	Phytol	Phytol	HMDB0002019	5280435	C01389
7	9-Octadecenoic acid	Oleic acid	HMDB0000207	445639	C00712
8	Octadecanoic acid	Stearic acid	HMDB0000827	5281	C01530
9	Eicosane	Eicosane	HMDB0059909	8222	NA
10	Squalene	Squalene	HMDB0000256	638072	C00751
11	Tetracosane	Lignocerane	HMDB0034282	12592	NA
12	Heptadecane	Heptadecane	HMDB0059830	12398	C01816
13	1-Hexacosene	1-Hexacosene	HMDB0040527	29303	NA
14	Vitamin E	alpha-Tocopherol	HMDB0001893	14985	C02477
15	Oxirane	(S)-2,3-Epoxysqualene	HMDB0001188	5459811	C01054
16	Stigmasterol	Stigmasterol	HMDB0000937	5280794	C05442
17	1-Heptacosanol	14-Heptacosanol	HMDB0031037	3084559	NA
18	Sitosterol	Clionasterol	HMDB0000649	457801	C19654
19	Cholest epoxy hydroxy	NA	NA	NA	NA
20	pyrrolidin-2-ylidenemine	(3Z)-phytochromobilin	HMDB0303975	NA	C05913
21	Glutinol	Glucinol	HMDB0039237	NA	NA
22	hexyloctahydro butyl	NA	NA	NA	NA
23	6-Octen-1-ol	3,7-dimethyl-acetate-Citronellyl acetate	HMDB0034160	9017	C12298

3.2 Metabolism Pathway Analysis

The pathway impact analysis results (**Figure 1**) show that several metabolic pathways experience significant changes due to the treatment of microorganism-based biofertilizers. Based on the pathway impact values and statistical significance, metabolic pathways related to the biosynthesis of lipids, sterols, and terpenoid compounds show a considerable influence.

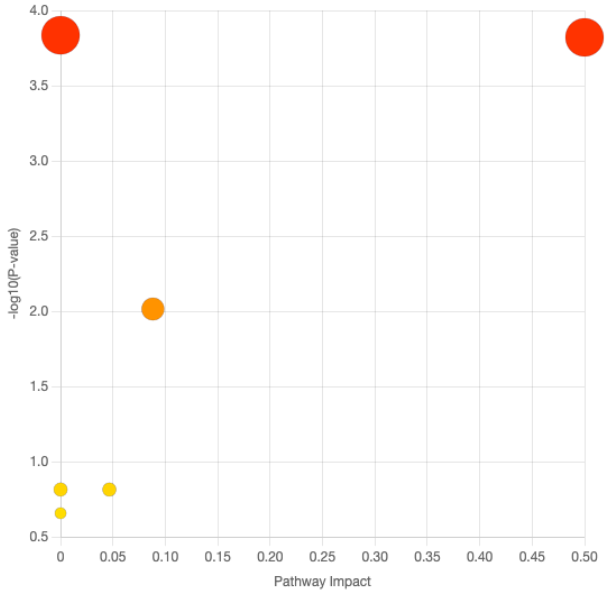

Figure 1. Metabolite pathway impact analysis results

The pathway analysis graph illustrates that several pathways have high impact values accompanied by high significance indicated by the size and color of the circles. This indicates that the detected metabolites play an important role in the cell's adaptation mechanism to abiotic stress conditions [20,4]. The increase in lipid metabolism is suspected to be related to the activity of PGPR microorganisms in modulating plant physiological responses through the increased production of cell-protective metabolites [21]. Compounds such as squalene, phytol, and alpha-tocopherol are known to act as natural antioxidants that help suppress the accumulation of ROS, thereby minimizing cell membrane damage during plant exposure to environmental stress [16].

3.3 KEGG Pathway Mapping

The results of metabolic pathway mapping using KEGG (**Figure 2**) showed that the detected compounds are involved in the Glycosylphosphatidylinositol (GPI)-anchor biosynthesis pathway. This pathway is related to the biosynthesis of membrane components and cell surface proteins, which play an important role in maintaining membrane stability as well as signal transduction processes during plant environmental stress [22]. The involvement of the GPI-anchor biosynthesis pathway indicates that the treatment with microorganism-based biofertilizers likely affects the regulation of cell membrane metabolism. This condition is related to the plant's ability to maintain cell integrity when experiencing abiotic stress such as salinity and extreme temperatures. Additionally, the connection of the GPI-anchor pathway with lipid metabolism indicates a relationship between the increase in fatty acid and sterol compounds and the cell defense mechanism [23-24]. PGPR microorganisms are suspected to help improve plant adaptation through the regulation of lipid metabolites that function to maintain membrane fluidity, membrane protein activity, and protection against oxidative damage [25].

3.4 The Role of Biofertilizer Microorganisms in Abiotic Stress Tolerance

Overall, the research results indicate that microorganisms in biofertilizers have the potential to

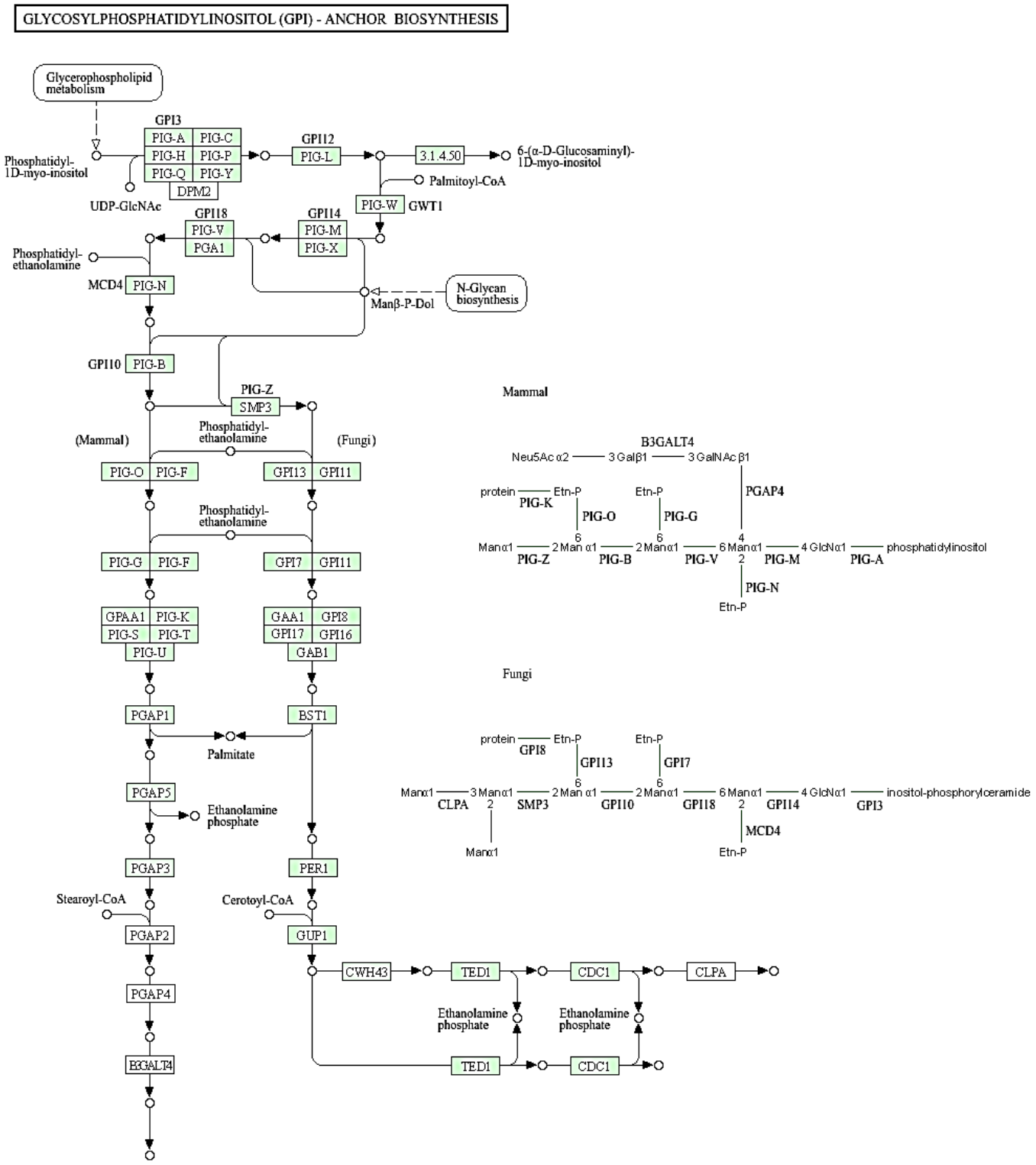

Figure 2. KEGG pathway mapping in GPI-anchor biosynthesis

enhance plant resistance to abiotic stress through the modification of lipid metabolism and the production of bioactive compounds. The presence of fatty acids, squalene, sterols, and other antioxidant compounds indicates a mechanism of cellular protection against oxidative stress triggered by extreme environmental conditions [12]. PGPR microorganisms are known to be able to enhance nutrient absorption, produce phytohormones, and modulate plant metabolism through the production of bioactive compounds [26]. These activities contribute to the improvement of cell membrane stability, the reduction of oxidative damage, and the enhancement of plant adaptation ability to abiotic stress. Therefore, microorganism-based biofertilizers have great potential to be developed as an environmentally friendly

alternative in supporting sustainable agriculture under suboptimal environmental conditions [27].

4. CONCLUSION

Microorganisms in biofertilizers have the potential to enhance plant resistance to abiotic stress through the regulation of lipid metabolism and the production of bioactive compounds such as fatty acids, squalene, sterols, and antioxidants. Pathway analysis shows the involvement of the Glycosylphosphatidylinositol (GPI)-anchor biosynthesis pathway, which plays a role in membrane stability and cell stress response. This indicates that microorganism-based biofertilizers can be an environmentally friendly alternative to improve plant adaptation to extreme environmental conditions.

5. AUTHOR'S DECLARATION

5.1. Supporting Information

Supporting information includes metabolite identification data, pathway analysis results, and KEGG metabolic pathway mapping used in this study.

5.2. Acknowledgements

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5.3. Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

5.4. Author Contributions

All of the authors contributed to conceptualization, data analysis, manuscript preparation, and final approval of the manuscript.

5.5. AI Statement

Gemini AI was performed to illustrate the graphical abstract. Chat GPT was utilize to enhance the clarity, grammar, and overall readability of this manuscript. All technical content, data interpretation, and conclusion were solely developed and verified by the authors. The final version of the manuscript was thoroughly reviewed to ensure accuracy, coherence, and alignment with the study's findings.

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