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Molecular Identification of *Trichoderma* Isolated from the Rhizosphere of Cacao (*Theobroma cacao* L.) in Central Sulawesi, Indonesia

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ABSTRACT

Background: Morphology-based identification methods, especially in cocoa, often have limitations, especially in similar overlapping symptoms. DNA-based molecular identification is required to ascertain effective fungal biocontrol species. This study aimed to identify and characterize *Trichoderma* isolates from the rhizosphere of cacao from three different altitudes in Central Sulawesi using a molecular DNA barcoding approach.

Methods: The method used in this study consists of various stages, namely sampling, isolation, purification of *Trichoderma*, DNA extraction, DNA amplification, electrophoresis, sequencing and phylogenetic analysis. Quick-DNA Magbead Plus Kit (Zymo Research, D4082) was used for genomic DNA extraction, primers ITS-1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS-4 (5′-TCCTCCGCT TATTGATATGC-3′) were used for PCR amplification and DNA sequence analysis using *Basic Local Alignment and Search Tool (BLAST)* on *NCBI* database.

Result: The results obtained showed that the amplified DNA electrophoresis had high quality with thick and clear bands with a size range of 575-578 bp. The five *Trichoderma* isolates based on sequencing analysis were identified as *Trichoderma asperellum* with different strains, namely *T. asperellum* strain NG125, *T. asperellum* strain NECC30406, *T. asperellum* strain IIPR-81, *T. asperellum* strain BHU203 and *T. asperellum* strain WZ-184, with a similarity rate (query cover and identity percentage) of 100%. The results of this study enrich the sequence data of *Trichoderma asperellum* in NCBI and can be a reference for the development of *Trichoderma*-based biological agents.

Key words: ITS, PCR, Phylogeny analysis, Rhizosphere fungi, *Trichoderma asperellum.*

INTRODUCTION

Cocoa production in Indonesia, especially in Central Sulawesi, plays an important role in supporting the national economy. Central Sulawesi is known as one of the main cocoa producing regions, contributing the most to the total cocoa production in Indonesia. Favorable climatic conditions and fertile soil create an ideal environment for cocoa cultivation (Amponsah-Doku *et al*., 2022).

Pest and disease attacks often cause declining cocoa productivity in this region (Delgado-Ospina *et al.,* 2021; Ratnawati *et al*., 2024). Similarly, the biocontrol agents inhabiting the cacoa plant also differs in their potential of biocontrol ability. Characterization of efficient *Trichoderma* species to be used as biocontrol agents is an eco-friendly approach.

The morphological identification process alone is not enough to accurately prove a species (Mirta and Rasyid, 2023). Central Sulawesi, with its diverse agroecosystems, is a strategic area to explore *Trichoderma* diversity from the cocoa rhizosphere. Furthermore, Khatun *et al.* (2021) and Elita *et al.* (2022) observed that conventional methods of *Trichoderma* identification that rely on morphological characteristics are often difficult to distinguish species that have similar appearances in their morphology.

The approach through DNA sequence analysis of the Internal Transcribed Spacer and Translation Elongation Factor (TEF-1α) genes has now developed into the most reliable and accurate method used by various global researchers. Molecular-based identification methods have evolved into a more accurate and reliable approach. One such method involves DNA sequence analysis of the Internal Transcribed Spacer (ITS) and Translation Elongation Factor (TEF-1α) genes (Pakshir *et al.,* 2020; Salehi *et al.,* 2020;

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Hussein and Saadullah, 2023; Weaver *et al.,* 2024). Genetic variation of *Trichoderma* using molecular approaches can be revealed with more depth and clarity, thus allowing for the identification of species and strains that are potentially superior as biocontrol agents.

The genetic diversity of *Trichoderma* isolated from the rhizosphere of cocoa plants has significant implications for the management of biological resources. These strains show certain advantages such as their ability to produce specific enzymes or bioactive compounds, which can be used as basic ingredients in the development of biofungicide and biofertilizer products (Abdul-Halim *et al.,* 2023; Xiao *et al.,* 2023). This study aims to identify *Trichoderma* species isolated from the rhizosphere of cocoa plants at several different altitude locations in Central Sulawesi. It's hoped that this research can provide in-depth insight into the potential of *Trichoderma* strains as biocontrol agents and biofertilizers and support effective management of local microorganisms that contribute positively to sustainable cocoa cultivation in Central Sulawesi.

MATERIALS AND METHODS

Location and time of research

This research was conducted in Sigi Regency, Central Sulawesi, on cocoa farms representing three different altitudes: lowland in Labuan Subdistrict, Donggala Regency 50 m asl (0.650627°, 119.86215°), medium altitude in Sigi Biromaru Subdistrict of Sigi Regency 200 masl (-1.091568°, 119.906393°) and high altitude in Palolo Subdistrict of Sigi Regency 1,106 masl (-1.132666°, 120.037956°) Central Sulawesi Province, Indonesia (Fig 1). *Trichoderma* isolation was carried out at the Microbiology Laboratory of Alkhairaat University Palu Central Sulawesi Indonesia, while molecular identification was carried out at the Molecular Biotechnology Laboratory, Tangerang, Indonesia. This research lasted for 3 months, from July to September 2024.

Sampling, isolation, purification of *Trichoderma*

Soil samples from the cocoa rhizosphere were taken using the Stratified Random Sampling method (Susila *et al.,* 2023). For each location, three replicate samples weighing 300 grams each were taken from the soil around the cocoa root at a depth of 20 cm (Ratnawati *et al.,* 2022; Sudewi *et al.,* 2024). Subsequently, soil samples were stored in sterile plastic clips and brought to the laboratory for fungal isolation. The plate culture technique and serial dilution method on Potato Dextrose Agar medium (enriched with chloramphenicol (100 mg/L) were applied to prevent contamination from bacteria. The diluted rhizosphere samples were placed into petri dishes containing PDA media and incubated for 5-7 days at 28°C. *Trichoderma* spp colonies with specific morphological characteristics having whitish green hyphae were purified on new PDA media.

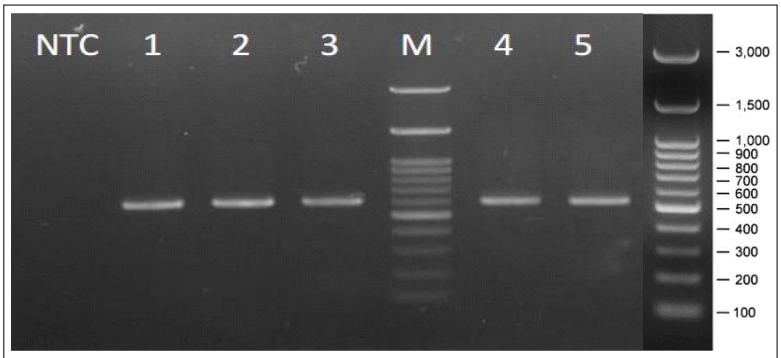
A total of 5 *Trichoderma* isolates obtained from the screening were then cultured on PDA media and purified. Seven days, old purified isolate diluted to a density of 10 v CFU mL^{-1} were plated onto PDA medium observed for 7 morphological characters such as color, elevation, edges (margins) of mycelium, coniodophores and conidia and growth rate of colonies for seven days (Widiati *et al.,* 2022).

DNA extraction

Quick-DNA Fungal/Bacterial Miniprep Kit (Zymo Research, D6005) was used for *Trichoderma* spp genomic DNA extraction according to standard procedures. Bashing BeadTM buffer and bead-beating were used to lyse 50 mg fungal samples to break down their cell walls. After centrifugation, it was mixed with fungal DNA binder and loaded onto a Zymo-Spin™ II purification column (Gand *et al.,* 2023; Li *et al.,* 2022). Additionally, the DNA was washed twice to remove contaminants, after which the extracted product was stored at 200°C for use in further analysis (Dilhari *et al.,* 2017).

DNA amplification and electrophoresis

Trichoderma fungi were identified through DNA amplification using 2X MyTaq HS red mix (Bioline, BIO-



M = 100 bp DNA ladder (in 2.5 µL); NTC: Negative control amplification; Sample code corresponds to the sequence number in the fungal sample information table. Isolates N05 (575 bp); A03 (578 bp); SA04 (577 bp); RAN16 (576 bp); NK3 (577 bp).

Fig 1: DNA amplification results of *Trichoderma* fungus isolates using ITS-1 and ITS-4 primers.

25048) using primers ITS-1 (5′-TCCGTAGG TGAAC CTGCGG - 3′) and ITS-4 (5′-TCCTCCGCTTATTGATATGC - 3′) (Sukapiring *et al.,* 2024). The DNA extract was used as a template in PCR. To obtain a total volume of 25 µL, mixed 12.5 µL MyTaq HS Red Mix (2X), 1 µL forward primer, 1 µL reverse primer, 1 µL template DNA and 9.5 µL nuclease-free water. Initial denaturation (1 cycle) for 1 minute at 95°C, followed by 35 cycles of denaturation at 95°C for 10 seconds and at 72°C for 5 minutes for final extension was done.

Electrophoresis was done at 75 volts for ± 90 minutes, then visualized with a UV Transilluminator and documented using a digital camera. PCR results were then confirmed through 0.8% agarose gel electrophoresis in 1X TBE buffer. Agarose gel solution of 50 mL was heated until dissolved, cooled and then add GelRed as a DNA dye (Lee *et al.,* 2012). The results obtained were compared with marker DNA to confirm the success of amplification. The names and sample codes of fungal isolates are presented in Table 1.

Sequencing and phylogenetic analysis

Sequencing uses the two-way Sanger Capillary Electrophoresis method with several steps. Previous PCR results in the form of DNA chain samples were analyzed using Bioedit software then aligned with ClustalW to compare with DNA chain sequences available in the Gene Bank database through blast on the web https://www.ncbi.nlm.nih.gov/ which was accessed on August 26, 2024. Phylogenetic tree analysis constructed with 1000 bootstraps using MEGA 10 software combined with the NCBI Blast Tree neighbor joining (NJ) approach (An *et al.,* 2022).

RESULTS AND DISCUSSION

DNA amplification and sequencing

The electrophoresis showed that the DNA obtained from PCR with ITS-1 and ITS-4 primers was of good quality as characterized clear visible bands on the electrophoregram for the five isolated samples (Fig 2). Leite *et al.* (2021) reported that ITS primers are able to amplify DNA optimally, making species identification easier. Mustafa and Rostam, (2021) suggested that fungal isolates that successfully amplify the ITS zone well are characterized by thick and sharp amplification results (Fig 1).

Based on the results of DNA amplification of *Trichoderma* fungus isolates with sample codes N05, A03, SA04, RAN16 and NK3 from 3 different altitudes showed very clear DNA bands with sizes of 575 bp, 578 bp, 577 bp, 576 bp and 577 bp respectively. DNA amplification of the 5 isolates using ITS-11 and ITS-4 primers, resulted in obtained amplicons in the range of 500-600 bp (Op De Beeck *et al.,* 2014). The DNA amplification looked very clear, stuck optimally and had, thick lines without the effect of shadows.

The sequence results of the fragments of *Trichoderma* fungus isolates based on the NCBI nucleotide database in Table 1 showed that the five fungal isolates were identified as *Trichoderma asperellum* strains (NG125, NECC30406, IIPR-81, BHU203 and WZ-184) with a similarity identity

Table 1: Summary of sequence results from fragments of *Trichoderma* fungus isolates based on NCBI nucleotide database.

Sampel code	Matched organism	% Query cover	E-value	% Identity	NCBI accession N°	Link
N05	*Trichoderma asperellum* strain NG125	100.00	0.00	100.00	MW287256.1	https://shorturl.at/Nfzj2
A03	*Trichoderma asperellum* strain NECC30406	100.00	0.00	100.00	MH153622.1	https://shorturl.at/iXJKU
SA04	*Trichoderma asperellum* strain IIPR-81	100.00	0.00	100.00	MK841019.1	https://shorturl.at/dBCBq
RAN16	*Trichoderma asperellum* strain BHU203	100.00	0.00	100.00	JN618346.1	https://shorturl.at/ZfWVF
NK3	*Trichoderma asperellum* strain WZ-184	100.00	0.00	100.00	MN856318.1	https://shorturl.at/XW4V4

percentage and query cover of 100%. The ratio indicated the degree of similarity between sample DNA sequences and target DNA sequences as percentage identity (Weaver *et al.,* 2024; Salini and Dhandapani, 2021). This result can be attributed to many factors, one of which is the conservative nature of the ITS (Internal Transcribed Spacer) gene. ITS is commonly used to identify fungi due to low variation within the same species (Bradshaw *et al.,* 2023). The isolates, although originated from different strains, may have a small degree of genetic variation within the amplified ITS region, however identified as the same species with a high percentage of identity.

Another factor for getting 100% similarity could be the use of reference databases such as NCBI BLAST, which had many *T. asperellum* entries from various strains. This is likely since the isolates obtained from the same place/ having a close evolutionary relationship so that genetic differences between strains are not large enough to affect identification results. The length and quality of DNA sequences can also help the matching with the database to be more accurate (Abedulridha and Al-Shamery, 2018). The similarity of the five fungal isolates belongeds to the same species, although differences between strains can only be found through further genetic analysis, using methods such as multilocus sequence typing (MLST) or whole genome sequencing.

Phylogenetic analysis

The know the relationship between the isolates the phylogenetic tree analysis of the five *Trichoderma* fungus isolates was done based on the NCBI database Fig 2. The phylogenetic tree analysis indicates that *Trichoderma* isolate 1 (N05) belongeds to the same cluster as several *T. asperellum* species and other related species. The sequence identity percentage reaching 100% strengthens the hypothesis that this isolate has a close genetic relationship with *T. asperellum*. This suggests that the isolate may share similar biological characteristics with species in its group, such as functioning as a biocontrol agent or playing a crucial role in soil ecosystems (Jaya *et al.,* 2023) as reflected by its position in the phylogenetic tree (Malik and Verma, 2025). These findings reveal that *Trichoderma* isolate 2 (A03) is related to the *T. asperellum* species, known as a plant growth-promoting fungus and biocontrol agent.

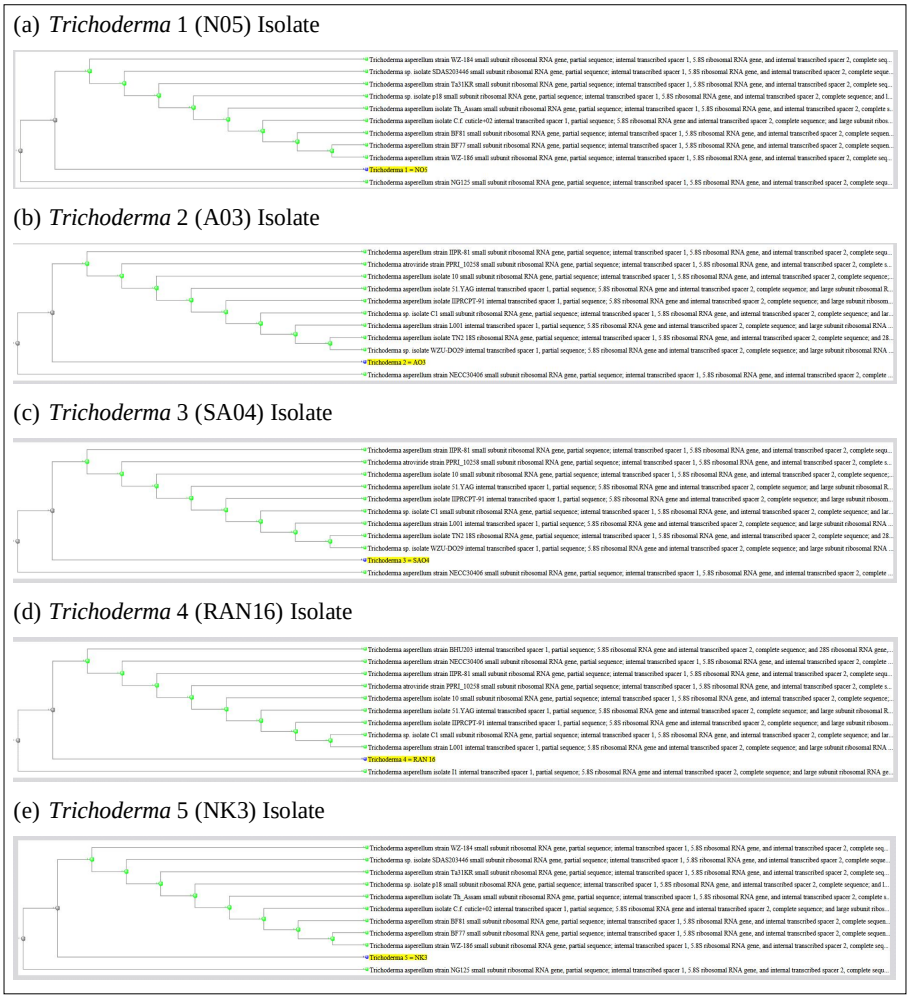


Fig 2: Phylogenetic tree based on DNA barcoding with ITS locus from cocoa rhizosphere fungi isolates (N05, A03, SA04, RAN16, NK3).

Similar findings were reported by Budi *et al.* (2023), *T. asperellum*, isolated from oil palm plantations, was found to inhibit *Ganoderma boninense* growth by 93.9% in a dual culture assay. Additionally, Youassi *et al.* (2024) reported that *T. asperellum* extract effectively inhibited the growth of *Phytophthora*, the cacao pod rot disease. All these findings confirm that *T. asperellum* has the potential to be used as a biocontrol agent, biofumog, or biopesticide.

The *Trichoderma* isolates 3 (SA04) and 4 (RAN16) were phylogenetically highly related with *T. asperellum*, *T. atroviride*s and other *Trichoderma* species (Baltzis *et al.,* 2025). The similarity of the tested isolates to sequence data stored in the NCBI database confirms that molecular identification using ITS sequences will produce strain data with a high degree of accuracy and clarity (Pere *et al.,* 2024; Kanthiya *et al.,* 2025). The bootstrap with green dots indicates a high level of validity for the species identified (Lemoine *et al.,* 2018), confirming the reliability of the molecular identification method in determining phylogenetic relationships between test isolates.

The same cluster is also seen in the Trichoderma 5 (NK3) isolate in Fig 2, which appears to be related to the *T. asperellum* isolate. The confidence level is indicated by the branches generated with green bootstrap dots. Meanwhile, the NK3 isolate is closely related to the *T. asperellum* strains WZ-186 and NG125, as seen in the phylogenetic tree structure, both of which are known to have similar potential as biocontrol agents.

CONCLUSION

Five isolates of the *Trichoderma* fungus (N05, A03, SA04, RAN16 and NK3) originating from the rhizosphere of cocoa plants at 3 different altitudes showed good amplification quality, a clear DNA band with a size of 575–578 bp based on the results of DNA electrophoresis. The use of primers ITS-1 and ITS-4 is recommended as good molecular markers for phylogenetic analysis and species recognition. Based on DNA sequence analysis supported by matching results to the NCBI nucleotide database with query cover of 100%, the five *Trichoderma* fungus isolates were identified as *T. asperellum* with different strains.

Phylogenetic analysis based on ITS sequences shows that the genetic closeness of these isolates has possibly the same biological and ecological characteristics. Strain variation within the same species can be caused by selection pressures, environmental factors and geographic conditions. Therefore, further research is needed to determine the physiological properties and ability of each strain to be used in various biotechnological applications, as a biocontrol agent or biofertilizer to promote plant growth.

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Disclaimers

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Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish, or preparation of the manuscript.

REFERENCES

Abdul-Halim, A.M.A.A., Shivanand, P., Krishnamoorthy, S. and Taha, H. (2023). A review on the biological properties of *Trichoderma* spp. as a prospective biocontrol agent and biofertilizer. *Journal of Applied Biology and Biotechnology*. **11(5):** 34-46. https://doi.org/10.7324/JABB.2023.11504.

Abedulridha, S.Q. and Al-Shamery, E.S. (2018). Optimal pair DNA Sequence alignment based on matching regions and multi-zone genetic algorithm. *International Journal of Engineering* and *Technology*. **7(4.19):** 751-756. https://doi.org/10.14419/ijet.v7i4.19.27993.

Amponsah-Doku, B., Daymond, A., Robinson, S., Atuah, L. and Sizmur, T. (2022). Improving soil health and closing the yield gap of cocoa production in Ghana-A review. *Scientific African*. **15:** e01075. https://doi.org/10.1016/j.sciaf.2021.e01075.

An, X. Y., Cheng, G. H., Gao, H. X., Li, X. F., Yang, Y., Li, D. and Li, Y. (2022). Phylogenetic analysis of trichoderma species associated with green mold disease on mushrooms and two new pathogens on *Ganoderma sichuanense*. *Journal of Fungi*. **8(7)**. https://doi.org/10.3390/jof8070704.

Baltzis, A., Santus, L., Langer, B.E., Magis, C., de Vienne, D.M., Gascuel, O., Mansouri, L. and Notredame, C. (2025). Multistrap: Boosting phylogenetic analyses with structural information. *Nature Communications*. **16(1):** 293. https://doi.org/10.1038/s41467-024-55264-0.

Bradshaw, M.J., Aime, M.C., Rokas, A., Maust, A., Moparthi, S., Jellings, K., Pane, A.M., Hendricks, D., Pandey, B., Li, Y. and Pfister, D.H. (2023). Extensive intragenomic variation in the internal transcribed spacer region of fungi. *IScience*. **26(8):** 107317. https://doi.org/10.1016/j.isci.2023.107317.

Budi, M.B.S., Giyanto and Tondok, E.T. (2023). *Trichoderma yunnanense* and *T. asperellum* as potential biological agents for control of basal stem rot disease in oil palm. *IOP Conference Series: Earth and Environmental Science*. **1133(1)**. https://doi.org/10.1088/1755-1315/1133/1/012036.

Delgado-Ospina, J., Molina-Hernández, J.B., Chaves-López, C., Romanazzi, G. and Paparella, A. (2021). The role of fungi in the cocoa production chain and the challenge of climate change. *Journal of Fungi*. **7(3)**. https://doi.org/10.3390/jof7030202.

Dilhari, A., Sampath, A., Gunasekara, C., Fernando, N., Weerasekara, D., Sissons, C., McBain, A. and Weerasekera, M. (2017). Evaluation of the impact of six different DNA extraction methods for the representation of the microbial community associated with human chronic wound infections using a gel-based DNA profiling method. *AMB Express*. **7(1)**. https://doi.org/10.1186/s13568-017-0477-z.

Elita, N., Agustamar and Susila, E. (2022). The Effects of the application of trichoderma asperellum and biochar on growth and productivity of rice cultivated by the SRI method and on soil quality. *IOP Conference Series: Earth and Environmental Science*. **1097(1):** 012018. https://doi.org/10.1088/1755-1315/1097/1/012018.

Gand, M., Bloemen, B., Vanneste, K., Roosens, N.H.C. and De Keersmaecker, S.C.J. (2023). Comparison of 6 DNA extraction methods for isolation of high yield of high molecular weight DNA suitable for shotgun metagenomics nanopore sequencing to detect bacteria. *BMC Genomics*. **24(1):** 1-14. https://doi.org/10.1186/s12864-023-09537-5.

Hussein, L.F. and Saadullah, A.A.M. (2023). DNA marker identification of *Trichoderma* and *Fusarium* level species. *IOP Conference Series: Earth and Environmental Science*. **1252(1)**. https://doi.org/10.1088/1755-1315/1252/1/012175.

Jaya, K., Ratnawati, S.S., Jumardin, A. and Zainal, M. (2023). The effect of various carrier mediums on the growth of *Trichoderma asperellum* TR3 and Trichoderma sp. *IOP Conference Series: Earth and Environmental Science*. **1230(1):** 3-7. https://doi.org/10.1088/1755-1315/1230/1/012090.

Kanthiya, R., Verma, R.K., Bharti, D., Kanthiya, S. and Chourey, H. (2025). The molecular identification of isolated fluoride-resistant plant growth-promoting fungi. *Agricultural Science Digest-A Research Journal.* 1-6. doi: 10.18805/ag.d-6315.

Khatun, M., Rahman, A., Quadir, Q., Rion, M. and Zakir, H. (2021). Isolation and characterization of plant associated rhizobacteria for plant growth promoting traits. *Fundamental and Applied Agriculture*. **6(1):** 1. https://doi.org/10.5455/faa.46616.

Lee, P.Y., Costumbrado, J., Hsu, C.Y. and Kim, Y.H. (2012). Agarose gel electrophoresis for the separation of DNA fragments. *Journal of Visualized Experiments*. **62:** 1-5. https://doi.org/10.3791/3923.

Leite, B.R., Vieira, P.E., Troncoso, J.S. and Costa, F.O. (2021). Comparing species detection success between molecular markers in DNA metabarcoding of coastal macroinvertebrates. *Metabarcoding and Metagenomics*. **5:** 249-260. https://doi.org/10.3897/MBMG.5.70063.

Lemoine, F., Wilkinson, E., Correia, D., Oliveira, D., Gascuel, O., Evolutive, B., Town, C., Africa, S., Sciences, M., Africa, S. and Africa, S. (2018). Renewing Felsenstein's Phylogenetic Bootstrap in the Era of Big Data. In Nature. **556(7702)**. https://doi.org/10.1038/s41586-018-0043-0.Renewing.

Li, Y., Bu La, Z. A., Cao, L. and Zhao, S. (2022). Establishment of a DNA barcoding database for legume and grass species identification. *Legume Research*. **45(6):** 659-668. doi: 10.18805/AF-727.

Malik, S. and Verma, A. (2025). Morphological and molecular characterization of the fungi isolated from infected wheat plant. *Agricultural Science Digest-A Research Journal.* 1-7. doi: 10.18805/ag.D-6459.

Mirta, B. and Rasyid, B. (2023). Exploration of Mahogany and Cocoa Rhizosphere Fungi as Agroforestry Land Cover in Herlang District, Bulukumba Regency. *IOP Conference Series: Earth and Environmental Science*. **1230(1).** https://doi.org/10.1088/1755-1315/1230/1/012073.

Mustafa, K. and Rostam, S.K. (2021). Molecular identification of local isolates *Aspergillus nidulans* from erbil province using internal transcribed spacers. *International Journal of Applied Biology*. **5(2):** 24-42. https://journal.unhas.ac.id/index.php/ijoab/article/view/14745.

Op De Beeck, M., Lievens, B., Busschaert, P., Declerck, S., Vangronsveld, J. and Colpaert, J.V. (2014). Comparison and validation of some ITS primer pairs useful for fungal metabarcoding studies. *Plos One*. **9(6)**. https://doi.org/10.1371/journal.pone.0097629.

Pakshir, K., Farazmand, F., Ghasemi, F., Mirhendi, H., Zomorodian, K., Kharazi, M., Pour, R.A., Golestani, H. and Motamedi, M. (2020). Translation elongation factor 1-alpha gene as a marker for diagnosing of candidal onychomycosis. *Current Medical Mycology*. **6(1):** 15-21. https://doi.org/10.18502/CMM.6.1.2503.

Pere, K., Mburu, K., Muge, E. K., Wagacha, J.M. and Nyaboga, E.N. (2024). Molecular identification, genetic diversity and secondary structure predictions of *Physalis species* using ITS2 DNA barcoding. *BMC Plant Biology*. **24(1).** https://doi.org/10.1186/s12870-024-05889-6.

Ratnawati, R., Arfan, A., Jaya, K. and Annisa, A. (2024). Potential of cocoa derived-endophytic fungi in the biocontrol of phytophthora palmivora. *International Journal of Design and Nature and Ecodynamics*. **19(6):** 2179-2185. https://doi.org/10.18280/ijdne.190634.

Ratnawati, Sudewi, S., Jaya, K. and Saleh, A.R. (2022). Microbial exploration from two different ecosystems in Central Sulawesi, Indonesia. *Biodiversitas*. **23(12):** 6089-6096. https://doi.org/10.13057/biodiv/d231201.

Salehi, Z., Shams-Ghahfarokhi, M. and Razzaghi-Abyaneh, M. (2020). Internal transcribed spacer rDNA and TEF-1α gene sequencing of pathogenic dermatophyte species and differentiation of closely related species using PCR-RFLP of the topoisomerase II. *Cell Journal*. **22(1):** 85-91. https://doi.org/10.22074/cellj.2020.6372.

Salini, A.P. and Dhandapani, M. (2021). Study on analysis of molecular diversity and trait association for zinc deficiency tolerance in rice under submerged conditions. *Indian Journal of Agricultural Research*. **59(1):** 1828-1834. doi: 10.18805/ijare.a-5722.

Sudewi, S., Saleh, A.R., Bangkele, L.I. and Dewi, E.S. (2024). Isolasi dan karakterisasi rizobakteri penambat nitrogen dari ekosistem padi sawah organik. *Jurnal Agrotek Tropika*. **12(3):** 642-654.

Sukapiring, D.N., Novianty, L. and Harahap, S.N. (2024). Molecular identification of fungi carried by chili seeds (*Capsicum annuum* L.) from Tanjung Morawa B Village, North Sumatra. *Jurnal Pembelajaran Dan Biologi Nukleus*. **10(2):** 379-388. https://doi.org/10.36987/jpbn.v10i2.5626.

Susila, E., Maulina, F. and Emilda, D. (2023). Characterization and identification of *Trichoderma* on shallots isolated from three elevation regions in West Sumatra, Indonesia. *Biodiversitas*. **24(4):** 2064-2071. https://doi.org/10.13057/biodiv/d240416.

Weaver, D., Novak-Frazer, L., Palmer, M., Richardson, M., Bromley, M. and Bowyer, P. (2024). Development of a novel mycobiome diagnostic for fungal infection. *BMC Microbiology*. **24(1):** 1-18. https://doi.org/10.1186/s12866-024-03197-5.

Widiati, B.R., Herwati, A. and Sofyan, S. (2022). Identifikasi cendawan rhizosfer tanaman jagung (*Zea mays* L.) dan uji efektivitas media perbanyakan *Trichoderma sp*. *Jurnal Galung Tropika*. **11(3):** 262-274. https://doi.org/10.31850/jgt.v11i3.1000.

Xiao, Z., Zhao, Q., Li, W., Gao, L. and Liu, G. (2023). Strain improvement of *Trichoderma harzianum* for enhanced biocontrol capacity: Strategies and prospects. *Frontiers in Microbiology*. **14(4):** 1-16. https://doi.org/10.3389/fmicb.2023.1146210.

Youassi, Y.O.Y., Ambata, H.T.A., Jiogue, M.B., Dikongue, F.J.N., Ayong, M.N.A., Bedine, M.A.B., Tchameni, S.N. and Sameza, M.L. (2024). The potential of *Trichoderma asperellum* organic extract and its emulsion to inhibit cocoa (*Theobroma cacao* L.) black pod disease and induce biochemical defenses against *Phytophthora megakarya*. *Journal of Plant Protection Research*. **64(1):** 19-28. https://doi.org/10.24425/jppr.2024.149159.