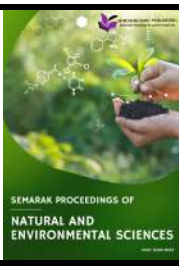
SEMARAK ILMU
PUBLISHING

Seminar Biologi Kebangsaan 2025

https://semarakilmu.my/index.php/spnes/index
ISSN: 3083 - 8193



Isolation and Characterisation of High Value Microbes from Rhizosphere Soil of Dipterocapaceae Plants

Omar Ayman[1], Noh Abdul Latif[2], Abdul Hamid Azzmer Azzar[1,4], Md Jani Haza Hanurhaza[3], Ahmad Zamri Huzaifah[1], Tukiman Izawati[3], Zakariya Khalilah[3], Mohamad Mohd Azrul Naim[1,4], Sharif Mohd Faez[1], Yahaya Muhammad Hadif Ridwan[1], Fauzi Muhammad Iman[1], Nizarudin Norhanis Diyana[3], Muhamad Tajudin Nur Shuhada[2], Ibrahim Putri Haryati[3], Mohd Hussain Ramzi[3], Othman Rashidi[3], Suhaimi Syahida Emiza[5], Zakaria Zahiruddeen[1], Shamsul Harumain Zakuan Azizi[1,4,♥]

[1] Department of Biotechnology, Kulliyyah of Science, International Islamic University Malaysia (IIUM), 25200, Kuantan, Pahang, Malaysia
[2] Department of Plant Science, Kulliyyah of Science, International Islamic University Malaysia (IIUM), 25200, Kuantan, Pahang, Malaysia
[3] Kulliyyah of Architecture and Environmental Design, International Islamic University Malaysia, Jalan Gombak, 53100, Kuala Lumpur, Malaysia
[4] Research Unit for Bioinformatics & Computational Biology (RUBIC), Kulliyyah of Science, International Islamic University Malaysia, 25200, Kuantan, Pahang, Malaysia
[5] Forest Biodiversity Division, Forest Research Institute Malaysia, Jalan FRIM, 52109 Kepong, Kuala Lumpur, Selangor Malaysia, Forest Research Institute Malaysia, Kuala Lumpur, Malaysia

ABSTRACT

The rhizosphere soil of Dipterocapacea supports diverse microbial communities with functional potentials relevant to forest conservation, agriculture and environmental sustainability. This study investigated the microbial functional capabilities of rhizosphere soil from four selected dipterocarp species (*Dipterocarpus oblongifolius, Neobalanocarpus heimii, Shorea sumatrana* and *Dryobalanops aromatica*) from FRIM Dipterocarp Arboretum, Gamuda Valencia (Township) and Wetland Arboretum Centre (WAC) (Nursery). Culture-dependent approaches successfully isolated diverse functional isolates. 62 cellulose-degrading bacteria were isolated using three selective media, with carboxymethyl cellulose (CMC) agar proving most effective (28 isolates). Four Actinomycetes isolates displaying characteristics of filamentous morphology and pigments diffusion were recovered, with Actinomycetes Isolation Agar (AIA) yielding optimal results. Plant growth-promoting bacteria (PGPB) demonstrated significant capabilities in indole-3-acetic acid synthesis, phosphate solubilization and zinc solubilization, with combined PGPB-fertiliser treatments producing better growth outcomes for *Brassica juncea* seedlings. Heavy metal resistance screening identified 20 bacterial isolates capable of tolerating elevated concentrations of Ni (4000 mg/L), Pb (1500 mg/L), Cr (1500 mg/L), Cd (550 mg/L) and As (600 mg/L). These findings demonstrate the rich functional diversity of dipterocarp rhizosphere soil microbiomes and their potential for sustainable agriculture, bioremediation and pharmaceutical applications.

Keywords: Heavy metals-Resistant; Plant Growth Promoting Bacteria; Cellulose-Degrading Bacteria; Actinomycetes

1. Introduction

Rhizosphere soil is the region of soil which surrounds the plant roots and is a crucial interface where complex and beneficial interactions occur between the soil microbiome and the plants. In Dipterocarpaceae family that comprises numerous tropical tree species, the bacterial population in the rhizosphere is paramount for efficient nutrient cycling, promoting plant health and ensuring

---

[♥] *Corresponding author.*
E-mail address: zak@iium.edu.my

ecosystem functioning. The functional microbial composition of rhizosphere influenced by the habitat conditions which includes the heterogenous disturbance from environmental and human activities. In this study, three representatives of habitats were studied i.e. Dipterocarp Arboretum at Forest Research Institute Malaysia (FRIM) as undisturbed habitat, Wetland Arboretum Centre Gamuda Cove (WAC) as reclaimed habitat from a mining area and now soil amendment was done and Gamuda Valencia as representative for habitat that is heavily influenced by human activities as it is a residential area. This diverse habitat background is representative of its different soil physicochemical composition. Previous study by Khan *et al.,* [1] Some rhizobacteria may degrade hydrocarbons and immobilize heavy metals, showing great promise for bioremediation. The presence of heavy metal, especially in residential areas and former mining areas, accentuates the need to unravel the microbial community's presence in the rhizosphere soil. Through nutritional and hormonal balance of plants, development of systemic tolerance to biotic and abiotic sources of stress, and promotion of plant development, rhizosphere bacteria weaken the negative effects of stress on plants [2]. Widiyatno *et al.* [3] signified the survivability of dipterocarp species up to 12.5 years. This can be attributed to the plant growth promoting microbes in its rhizosphere soil. The presence of cellulose-degrading bacteria in the rhizosphere of Dipterocarpaceae can have significant implications for the decomposition of organic matter, nutrient availability, and plant growth [4]. Actinomycetes, soil-dwelling bacteria known for producing bioactive compounds, are particularly noteworthy due to their role in biogeochemical processes and potential for antimicrobial discovery. This study aims to isolate and characterize heavy metal-resistant bacteria, plant growth promoting bacteria (PGPB), potential actinomycetes and cellulose degrading bacteria (CDB) from *Dipterocarpus oblongifolius, Neobalanocarpus heimii, Shorea sumatrana* and *Dryobalanops aromatica* found at those locations.

2. Methodology

2.1 Rhizosphere soil sampling

Any debris was cleaned before sampling. Fine roots were collected by tracing lateral roots 2 – 10 m from the trunk, and samples were taken at 5 – 30 cm depth [5].

2.2 Isolation of heavy metal-resistant bacteria

1 g of soil was mixed in 10 mL of distilled water and vortexed to obtain a soil suspension, then 1 mL of suspension was serially diluted in 9 mL of sterile PBS [6]. 100 µL of each diluted suspension was plated onto different nutrient agar supplemented with cadmium chloride (550 mg/L), nickel sulfate (4000 mg/L), sodium arsenite (600 mg/L), lead nitrate (1500 mg/L), and chromium (III) chloride (1500 mg/L). All plates were incubated at 30°C for 7 days.

2.3 Isolation of Plant Growth Promoting Bacteria (PGPB) and screening of indole-3-acetic acid (IAA), zinc and phosphate solubilization

Diluted soil suspension was inoculated onto minimal salt medium (MSM) agar and incubated for two days at 30°C. Isolates obtained were screened for IAA production using the Gordon Weber assay [7]. Then, the isolates were screened for zinc and phosphate solubilization by culturing the isolates on selective ZSB and PSB agar, then incubated at 30°C for 2 days [8], [9]. The halo zone was observed and measured. The solubility index then calculated by dividing the diameter of the colony and diameter of the halo zone with the diameter the colony.

2.4 Effectiveness of PGPB on the growth of Brassica juncea

The 10 highest producers of IAA, as well as zinc and phosphate solubilizer from the screened isolates were selected. 3 formulations were prepared; +PGPB, +AB (Formulation 1), +PGPB, -AB (Formulation 2), -PGPB, +AB (Formulation 3) with 1:1 ratio. Distilled water was used as a negative control. The formulation was added to soil containing the Brassica juncea seedlings [10]. For 14 days, the plant was watered daily then harvested, weighed the plant and root and measured the shoot length. The weight ratio was calculated by dividing the weight of the root by the total weight of the plant.

2.5 Isolation of cellulose-degrading bacteria (CDB)

10 g of soil was pretreated by enriching in 100 mL CMC broth incubated at 30°C for 7 days and shaken at 150 rpm. Then, the pretreated samples were serially diluted and spread onto selective media i.e. CMC agar, microcrystalline cellulose agar and soil extract agar for 7 days at 30°C [11], [12].

2.6 Isolation of Actinomycetes

The soil was heat-treated at 120°C for an hour to eliminate microbes other than actinomycetes. The treated soil was mixed with 100 mL of sterile distilled water and serially diluted. The diluted sample was inoculated onto selective media, i.e. starch casein agar (SCA), actinomycetes isolation agar (AIA), and tap water yeast extract agar (TWYEA). The inoculated plates were incubated at 28°C for 7 days. The colonies obtained were selected and cultured onto ISP2 agar.

3. Results & discussion

Isolates obtained in Table 1 from the rhizosphere soil of FRIM showed the highest number of heavy metal resistance at 9 isolates and were representative of all heavy metals tested. This may be due to the acidic condition of the soil that increases the solubility of heavy metals [13]. Valencia and WAC have relatively similar resistant isolates at 5 and 6 isolates, respectively and are only resistant towards several of the heavy metal tested.

Table 1
Heavy metal resistant isolates from each location based on type of heavy metal

Location	**No. of isolates**					**Total**
	Type of heavy metal					
	Nickle (4000 mg/L)	**Lead (1500 mg/L)**	**Cadmium (550 mg/L)**	**Chromium (1500 mg/L)**	**Arsenite (600 mg/L)**	
FRIM	3	1	2	1	2	9
Valencia	0	2	1	0	2	5
WAC	4	0	1	0	1	6
Total	7	3	4	1	5	**20**

In Table 2, the isolates listed are the top 10 ranked according to the concentration of IAA produced and solubilization of zinc and phosphate. Those isolates were selected and formulated with fertilizers A and B as shown in Table 3. The formulation containing both PGPB and fertilizer AB promoted highest shoot growth in length compared with the other two formulations. However, the root dry-weight ratio was as higher in the absence of PGPB and when plants were only treated with fertilizer AB. This suggests that although the incorporated PGPB improves the growth, the usage of fertilizer is necessary to maximize the effectiveness.

Table 2
Concentration of IAA produced and solubility index of zinc and phosphate of selected PGPB isolates (±SEM)

Isolate	[IAA] (µg/mL)	Solubility Index (cm)	
		Zinc	Phosphate
F1B	11.247±7.417	7.49±0.691	1.67±0.136
F1C	23.467±3.030	4.48±2.740	1.62±0.289
F2B	11.923±6.330	3.81±0.621	1.88±0.123
F3A	27.773±2.731	3.56±3.417	1.61±0.168
F3B	30.372±3.167	1.80±1.848	-
F3D	27.509±4.581	4.36±0.332	2.54±0.463
F3E	27.760±6.622	-	-
F4B	29.815±5.105	3.72±0.535	1.40±0.113
F4C	14.876±4.740	3.46±1.837	2.14±0.171
V3A	25.451±3.783	2.87±0.339	1.81±0.023
V4B	10.185±6.050	4.34±0.050	1.97±0.302
W1B	93.705±8.665	3.77±1.712	1.20±0.059
W2B	32.849±4.501	4.11±0.173	2.22±1.145
W2C	40.374±4.719	-	-
W3A	27.196±2.193	2.47±0.522	2.63±1.127
W3B	29.948±6.993	1.66±0.096	-
W3C	27.399±3.310	2.51±0.291	1.51±0.160
W4B	13.488±4.113	3.55±1.856	-

Table 3
Average plants' dry weight ratio and shoots length according to subjected treatment (±SEM)

Formulation	-PGPB, +AB	+PGPB, +AB	+PGPB, -AB	(-) Control
Dry-weight ratio (g)	0.185±0.084	0.167±0.041	0.132±0.040	0.201±0.041
Shoot length (cm)	6.83±1.583	7.96±1.288	6.81±1.292	7.89±1.763

In Figure 1, the highest number of isolates was cultivated using CMC agar with 28 isolates. Subsequently, 27 isolates were cultivated using SEM agar whilst the least number of isolates were cultivated using microcrystalline cellulose agar (7 isolates). Since CMC is a simpler form of cellulose and microcrystalline cellulose is more recalcitrant form of cellulose, the capability of bacteria to hydrolyze the CMC is higher as depicted by the number of isolates from CMC agar when compared to the total number of isolates [14].

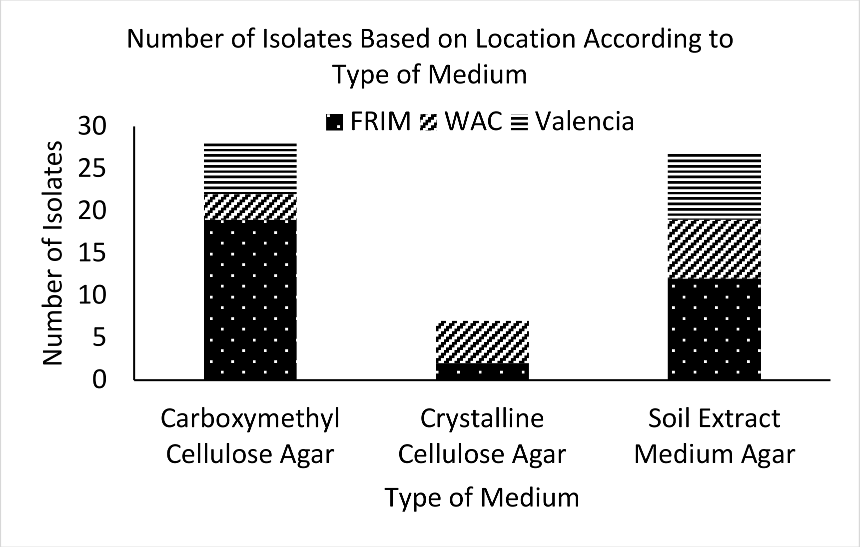


Fig. 1. Graph of number of CDB isolates based on location according to type of medium

In Figure 2, the isolates were identified based on their filamentous structures, which included branched hyphae resembling the mycelium of fungi, a defining feature of actinomycetes [15]. Three out of four of the isolated actinomycetes were obtained using AIA which indicates that it provides more favourable condition for actinomycetes [16].

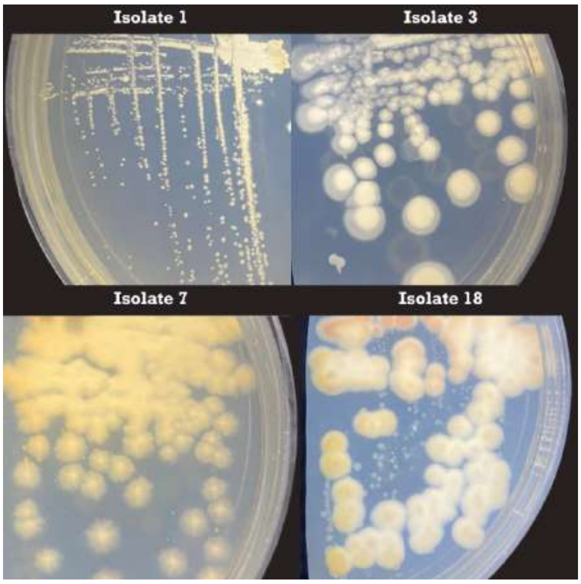


Fig. 2. Actinomycetes isolates obtained by using AIA (Isolate 1,3,7) and SCA (Isolate 18)

4. Conclusions

Rhizosphere soil bacteria isolated from dipterocapaceae plants possess significance in various aspects including bioremediation potential, agricultural improvement, and medicinal applications. This is shown by the cultivated bacterial isolates with heavy metal resistant, promotion of plant growth by production of IAA and solubilization of zinc and phosphate, the degradation of cellulosic materials as well as actinomycetes with its potential in antibiotic production. Ultimately, this study highlights the importance of rhizosphere soil as reservoir of various valuable microbes.

Acknowledgement

This project was supported by and received financial funding from Gamuda Parks (GAMUDAPARKS/2505-001). The authors sincerely thank the Forest Research Institute Malysia (FRIM) for all the support and cooperation. The main author would also like to acknowledge the research members for their diligent work and commitment.

References

[1] Khan, Abdullah, Yibin Wei, Muhammad Adnan, Izhar Ali, and Muqing Zhang. "Dynamics of rhizosphere bacterial communities and soil physiochemical properties in response to consecutive ratooning of sugarcane," *Front Microbiol*, vol. 14, Jul. 2023. https://doi.org/10.3389/fmicb.2023.1197246

[2] Pantigoso, Hugo A., Derek Newberger, and Jorge M. Vivanco. "The rhizosphere microbiome: Plant–microbial interactions for resource acquisition," *J Appl Microbiol*, vol. 133, no. 5, pp. 2864–2876. Nov. 2022. https://doi.org/10.1111/jam.15686

[3] Widiyatno *et al.* "Selection of dipterocarp species for enrichment planting in a secondary tropical rainforest," *Forest Sci Technol*, vol. 16, no. 4, pp. 206–215. 2020. https://doi.org/10.1080/21580103.2020.1831620

[4] Cavalieri, Andrea, Frederik Bak, Adriana M. Garcia-Lemos, Jacob Weiner, Mette Haubjerg Nicolaisen, and Ole Nybroe. "Effects of Intra- and Interspecific Plant Density on Rhizosphere Bacterial Communities," *Front Microbiol*, vol. 11, p. 531372. May 2020. https://doi.org/10.3389/fmicb.2020.01045

[5] Vishal, V., Munda, S., Singh, G. and Lal, S. "Cataloguing the bacterial diversity in the active ectomycorrhizal zone of Astraeus from a dry deciduous forest of Shorea," *Biodivers Data J*, vol. 9, May 2021. https://doi.org/10.3897/BDJ.9.e63086

[6] Wang, T. *et al.* "Screening of Heavy Metal-Immobilizing Bacteria and Its Effect on Reducing Cd2+ and Pb2+ Concentrations in Water Spinach (Ipomoea aquatic Forsk.)," *Int J Environ Res Public Health*, vol. 17, no. 9, p. 3122. Apr. 2020. https://doi.org/10.3390/ijerph17093122

[7] Gang, S., Sharma, S., Saraf, M., Buck, M. and Schumacher, J. "Analysis of indole-3-acetic acid (IAA) production in Klebsiella by LC-MS/MS and the Salkowski method," *Bio Protoc*, vol. 9, no. 9, 2019. https://doi.org/10.21769/BioProtoc.3230

[8] R. Gupta, A. Kumari, S. Sharma, O. M. Alzahrani, A. Noureldeen, and H. Darwish, "Identification, characterization and optimization of phosphate solubilizing rhizobacteria (PSRB) from rice rhizosphere," *Saudi J Biol Sci*, vol. 29, no. 1, pp. 35–42. Jan. 2022. https://doi.org/10.1016/j.sjbs.2021.09.075

[9] Othman, N.M.I. *et al.*, "Isolation, Characterization, and Identification of Zinc-Solubilizing Bacteria (ZSB) from Wetland Rice Fields in Peninsular Malaysia," *Agriculture*, vol. 12, no. 11, p. 1823. Nov. 2022. https://doi.org/10.3390/agriculture12111823

[10] Zaheer, A. *et al.*, "Isolation, characterization, and effect of phosphate-zinc-solubilizing bacterial strains on chickpea (Cicer arietinum L.) growth," *Saudi J Biol Sci*, vol. 26, no. 5, pp. 1061–1067. Jul. 2019. https://doi.org/10.1016/j.sjbs.2019.04.004

[11] Li, H. *et al.*, "Characterization of Cellulose-Degrading Bacteria Isolated from Silkworm Excrement and Optimization of Its Cellulase Production," *Polymers (Basel)*, vol. 15, no. 20, Oct. 2023. https://doi.org/10.3390/polym15204142

[12] Nguyen, T.M. *et al.*, "Effective Soil Extraction Method for Cultivating Previously Uncultured Soil Bacteria," 2018. https://doi.org/10.1101/322735

[13] Labouyrie, M. *et al.*, "Patterns in soil microbial diversity across Europe," *Nat Commun*, vol. 14, no. 1, p. 3311. Jun. 2023. https://doi.org/10.1038/s41467-023-37937-4

[14] Wilson, D.B. "Microbial diversity of cellulose hydrolysis," Jun. 2011. https://doi.org/10.1016/j.mib.2011.04.004

[15] Tistechok, S., Skvortsova, M., Luzhetskyy, A., Fedorenko, V., Parnikoza, I. and Gromyko, O. "Antagonistic and plant growth promoting properties of actinomycetes from rhizosphere Deschampsia antarctica E. Desv. (Galindez Island, Antarctica)," *Ukrainian Antarctic Journal*, no. 1(18), pp. 169–177. Dec. 2019. https://doi.org/10.33275/1727-7485.1(18).2019.140

[16] Sreevidya, M., Gopalakrishnan, S., Kudapa, H. and Varshney, R.K. "Exploring plant growth-promotion actinomycetes from vermicompost and rhizosphere soil for yield enhancement in chickpea," *Brazilian Journal of Microbiology*, vol. 47, no. 1, p. 85–95. Jan. 2016. https://doi.org/10.1016/j.bjm.2015.11.030