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Unveiling the Potential of Rhizosphere Bacteria from *Plumeria acuminata* Tree as Plant Growth-Promoting Rhizobacteria

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a b s t r a c t

The rizosphere bacteria are known to have the characteristics of *Plant Growth Promoting Rhizobacteria* (PGPR). From the rhizosphere of the Cambodian white tree at the Pracimaloyo Cemetery in Surakarta, 43 bacterial isolates have been obtained. Bacterial isolates have the ability to produce IAA up to 113.58 ppm. Publications related to the Cambodian tree rizospheric bacteria from the found burial site require further PGPR characterization tests because the characters as PGPR include IAA, N fixation, the antagonism of the pathogen, the ability to produce siderofores, ACC deaminases, and non-pathogens to host plants that are proven to have hypersensitive reactions, so this study aims to characterize the isolate of Cambodgian bacteria as the PGPR in improving the nitrogen and hypersensor reactions (HR). A total of 40 isolates of Cambodian tree rizosphere bacteria were tested for their ability to repair N_2 by measuring the concentration of ammonia formed using spectroscopic photometer methods, while the HR test used tobacco plants. The ability to measure nitrogen shows that as many as 11 isolates (26.82%) are capable of producing NH_3 (ammonia). The highest ammonia concentrations are produced by P8 isolates at 13.815 mM and P2 at 10.523 mM. Of the 41 rhizospheric bacterial isolates tested for hypersensitivity, 18 showed positive responses. A positive response to the HR test is characterized by symptoms of necrosis on tobacco leaves. The rhizosphere bacteria from the Cambodian trees in the cemetery have the potential to repair nitrogen, and 23 isolations show no symptoms of necrosis.

1. Introduction

Indonesia has many potentially great plants as a traditional medicine (Kadek et al., 2019), one of which is white Cambodia (*Plumeria acuminata*). This plant contains secondary metabolite compounds (Zulkifli et al., 2022) that are useful as drugs, such as saponins, flavonoids, polyphenols, alkaloids, and fulvoplumerins (Dermawan et al., 2021). The white Cambodian plant is identical to the imperial plant because it grows in a cemetery where its flowers are used as a symbol of imperiality (Sari & Sumadewi, 2021). The Public Cemetery (TPU) has a primary function as a place to bury bodies. Putra et al. (2020) states that the TPU was the site of the active demolition. The decomposition results are carbon and nitrogen (Sunniyyah, 2021; Wei et al.,

2022) that could support the growth of microbes, one of which is the Cambodian plant rizosphere bacteria. The population of Cambodian tree rizosphere bacteria in Pracimaloyo Surakarta TPU was 1.9–10.4 x 10^6 CFU/g (Putra et al., 2023). When compared to the diversity of bacteria in the rhizosphere of pioneering plants in the limestone mines, where only 21 different isolates were found (Sriwulan et al., 2022), the bacterial rhizosphere isolates of the Cambodian Tree in the TPU are much greater.

The risosphere is a burning zone that provides a variety of organic materials to stimulate the growth of microbes (Mirta et al., 2022). The risosphere is a burning zone that provides a variety of organic materials to stimulate the growth of microbes (Wulandari et al., 2019). The rizospheric bacteria are bacteria that exist around crop burns (Wahyudi et al., 2019) and are known as PGPR (*Plant Growth Promoting Rhizobacteria*). The rizospheric bacteria act as PGPR through direct and indirect mechanisms. Some characteristics of PGPR through direct mechanisms are: 1) the ability to synthesize several plant hormones, including indole-3-acetic acid (IAA), giberellate acid, cytokinin, and ethylene; 2) the provision of nutrients through siderophore production, phosphate dissolution, and nitrogen fixation, whereas the indirect mechanism is phytopathogen control (Safriani et al., 2020; Wahyudi et al., 2019).

The Cambodian tree rhizosphere bacteria that have been isolated from the Pracimaloyo TPU show the potential to produce IAA of 34.88%, with IAA concentrations reaching 113.58 ppm (Putra et al., 2023). The IAA concentrations produced by the rizospheric bacteria are higher compared to the palm coconut bacteria, which produced the highest concentrations of IAA at 58.50 ppm (Ariyani et al., 2021), bananas at 73.50 ppm (Rahayu, 2022) and tomatoes at 111.6 ppm (Dashti et al., 2021). Another characteristic of PGPR is the ability to fix nitrogen. To test the ability of bacteria to use N_2 air, use N-free media (Santoso et al., 2019) or measure ammonia resulting from N_2 retention air (Vionita et al., 2015). Isolates of nitrogen-fixing bacteria in the rizosphere have been extensively explored, including from Kakao mines (Jannah et al., 2022), Banana Kluthuk (Rahayu, 2022), *Cengkeh* (Ishak et al., 2018), respectively, with the highest ammonia concentrations of 0.52 mg. L^{-1}, 2, 49 mM, and 11.71 gL^{-1}.

Applications of bacterial isolates as PGPRs should consider whether the bacteria are potentially antagonistic agents and must be proven through hypersensitive reaction (HR) tests. Bacteria isolates that induce hypersensitivity reactions characterized by the appearance of necrotic symptoms. These symptoms are caused by death plant tissue at the site of infection, and limited multiplication and spread of pathogens (Amaria et al., 2023). Tobacco plants are used as an indicator plant to determine bacterial safety against plants because they can react very quickly to pathogenic bacteria in leaf tissue (Herdiyantoro et al., 2022). In contrast, absences symptoms on tobacco leaves indicate the presence of bacteria isolates are not pathogenic. Bacteria suspension infiltrated in tobacco leaves induces activation defense genes and antimicrobial production secondary metabolites that act as a plant barrier against pathogenic infection (Wang et al., 2016). Publications related to the characterization of the isolat of the Cambodian tree rizosphere bacteria from TPU as PGPR in Indonesia have not been found. From the initial characterization results as PGPR, these bacterial isolates are highly potential because they are capable of producing high IAAs (Putra et al., 2023) but still require characterization as other PGPRs because PGPR covers several characteristics including IAA, N fixation, antagonism of the pathogen, siderofor production ability, ACC deaminase, and being non-pathogenic to host plants that are proven to have hypersensitive reactions. The study aims to characterize the plant growth-promoting rhizobacteria (PGPR) in the nitrogen fixation and hypersensitivity tests. The research data is expected to complement the collection characteristics of the plant development-promoting rhizosphere bacteria as PGPR so that it can be used to support plant growth properly.

2. Method

The research was conducted at the Biology Education Laboratory of FKIP Muhammadiyah University of Surakarta. The research was conducted from January to April 2023.

Rejuvenation of rizospheric bacteria

The white Cambodian plant rizospheric bacterial isolate tested is a collection of the Microbiology Laboratory, Biology Education department, FKIP UMS. Before being used for testing, the isolate was remixed into the nutrient medium to form the new one and incubated in the incubator for 24 hours (Azzahra et al., 2021). After the bacterial isolate was reprogrammed, it tested its characterization as PGPR in fixing nitrogen and causing hypersensitive reactions on tobacco plants.

Testing nitrogen fixation capabilities

The rizospheric bacterial isolate is grown on a peptone water medium for 5 days at room temperature with a shake of 120 rpm. After five days, a 1 mL liquid bacterial culture is taken and centrifuged at a speed of 10,000 rpm for 10 minutes (Mahdi et al., 2020). A total of 0.2 mL of supernatural is taken and inserted into the reaction tube, plus 1 mL of *Nessler* reagent, and the volume is spread to 8.5 mL using aquabides. The mixture is homogenized using a vortex and left for 20–30 minutes to measure color absorption using a spectrophotometer at a wavelength of 450 nm. Blurred yellow indicates small ammonia production, and old yellow to brown indicates maximum ammonium production (Sharma et al., 2021). Ammonia concentrations are determined from standard ammonia sulfate curves with ranges of 0 mM, 5 mM, 10 mM, and 15 mM.

Hypersensitive reaction (HR) test to tobacco plants

The HR test was carried out by inoculating the rizospheric bacterial isolate on a *Nutrient Broth* (NB) medium incubated at a temperature of 28°C for 24 hours with a shake. After incubation, 2 mL of bacterial suspensions from the rizosphere are taken using a syringe and then injected into the tobacco leaf tissue (Amrulloh et al., 2021). The symptoms of hypersensitivity are seen if the infiltrated part of the bacterial suppression develops necrosis (Fanani et al., 2015) within 48 hours of incubation (Arimbawa et al., 2019).

3. Result and Discussion

A total of 40 isolates of the Cambodian tree rizosphere bacteria from TPU Pracimoloyo were tested for characterization as *Plant Growth Promoting Rhizobacteria* (PGPR) in nitrogen and hypersensitive reaction (HR) test, as well as in IAA production.

Table 1. Recapitulation of the Character of the Bacterial Rizosphere Isolate of the Cambodian Tree

Isolate code (P)	Characterization of *Plant Growth-Promoting Rhizobacteria* (PGPR)		
	IAA (ppm) (Putra et al., 2023)	Ammonia production (mM)	Hypersensitivity reaction test
P0	0	0	-
P1	0	0	-
P2	11.87	10.52	+
P4	0	3.79	+
P5	0	3.74	+
P6	0	0	+
P7	0	4.29	+

Isolate code (P)	Characterization of *Plant Growth-Promoting Rhizobacteria* (PGPR)		
	IAA (ppm) (Putra et al., 2023)	Ammonia production (mM)	Hypersensitivity reaction test
P8	0	13.82	-
P9	0	2.89	+
P10	0	0	+
P11	0	0	-
P12	0	0	-
P13	0	0	-
P14	0	0	+
P15	0	0	-
P16	0	0	+
P17	0	2.67	-
P18	0	2.99	-
P19	0	0	-
P20	0	0	+
P21	0	0	+
P24	55.69	0	+
P25	0	0	-
P26	70.10	0	-
P28	0	0	-
P29	0.54	0	-
P30	7.75	0	-
P31	24.66	0	-
P32	9.22	2.89	-
P33	0	3.09	+
P34	0	0	-
P35	0	0	-
P36	35.50	2.89	-
P37	113.58	0	+
P38	77.95	0	+
P39	12.41	0	+
P40	18.49	0	-
P41	12.70	0	-
P42	0	0	-
P43	0	0	+

Description:
(+) = positive necrosis
(-) = negative necrosis

Capacity to Fix N_2 (quantitative) Rizosphere Bacteria

The quantification of N_2 fixation capabilities began with the manufacture of standard ammonia sulfate curves. From the standard curve formed, obtain the equation Y= 0.0401x0.105 (Figure 1). The obtained absorption rate is then converted to the ammonia concentration value using the standard regression curve equation for the ammonium sulfate solution. Y is the absorption value, while X is the concentration of ammonia. Ammonia is a compound formed by the fixation of nitrogen (Chen et al., 2021), So the higher the concentration of ammonia formed, the greater the ability to fix N_2 (Leghari et al., 2016). In the nitrogen cycle, the N_2 gas will be inspected by both free-living and symbiotic nitrogen-fixing bacteria and ammonia-producing plants. Subsequently, the compound undergoes a process of nitrification into nitrates that will be assimilated by plants for growth. Some of the nitrate compounds will undergo denitrification to produce N_2 gases (Rafferty, 2023).

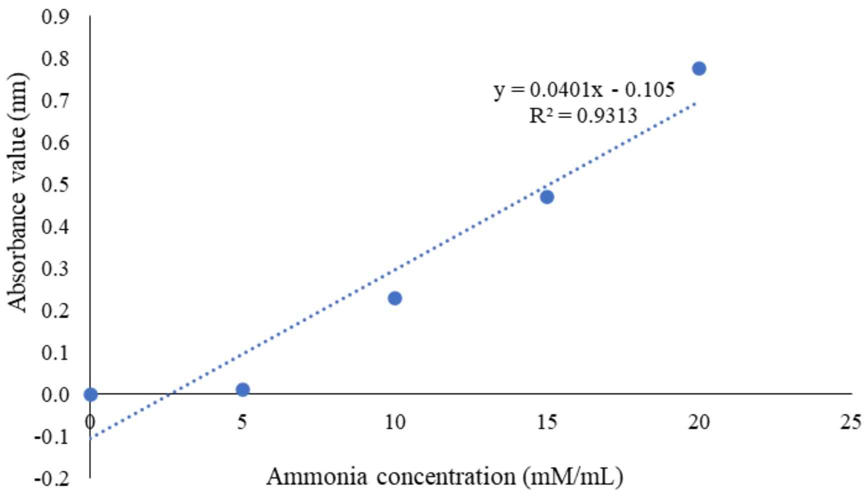

Figure 1. Standard curve for determining ammonia concentration

The results of the N_2 quantifiable capability test showed that 11 positive rhizospheric bacterial isolates (26.82%) were able to fix N_2 with ammonia concentrations ranging from 2.67 to 13.82 mM (Table 1). These variations in ammonia concentrations occur because each type of bacterium has a different potential. Some bacteria capable of fixing nitrogen include *Azospirillum*, *Gluconacetobacter diazotrophicus*, *Azoarcus*, *Beijerinckia*, *Enterobacter*, *Klebsiella*, *Pseudomonas*, *Azorhizobium*, *Herbaspirillum*, and *Azotobacterium* (Richard et al., 2018). In this study, P8 isolate produced the highest ammonia concentration of 0.449 mM, and P2 produced 0.317 mM (table 1). These results show that the rhizosphere of the Cambodian trees in the TPU holds potential as a nitrogen-fixing bacterial habitat. Similar studies have also shown that rizospheric bacteria are capable of fixing nitrogen: *Pantoea dispersa* and *Enterobacter asburiae* (Singh et al., 2021), *Herbaspirillum aquaticum*, *Serratia nematodiphila*, *Agrobacterium* sp., and *Acinetobacter baylyi* (Rahayu, 2022).

The ability to fix this nitrogen becomes one of the essential characteristics necessary for plant growth. The N element is one of the macronutrients that plants need in the production of proteins, nucleic acids, and chlorophyll (Armita et al., 2022), development and metabolism (Chen et al., 2022). Fertilizers contain a lot of nutrients that are needed by plants for their growth. The element contained in the fertilizer is nitrogen (N) (Yusmayanti & Asmara, 2019). The abundant availability of nitrogen in the atmosphere means that plants cannot use it directly. The forms of nitrogen that plants can extract from the soil are nitrate (NO_3^-) and ammonia (NH_4^+). Both forms of nitrogen can be derived from either urea or NPK fertilizers. In fact, they are environmentally unfriendly and can lead to environmental pollution, thus opening up the use of environmentally friendly biological agents (Astuti et al., 2021).

Hypersensitivity Reaction (HR) Test

The hypersensitivity test results showed that there were 18 isolates showing symptoms of necrosis and 23 isolates not showing any symptoms (figure 2). This hypersensitivity test is important to determine the potential pathogenicity of a bacterium characterized by a necrosis reaction on the leaves of tobacco plants. HR tests have also been carried out on isolated bacteria in rizospheric seedlings *Reutealis trisperma* (Amaria et al., 2019). Six isolates showed symptoms of necrosis, and 20 isolates reacted negatively. Herdiyantoro et al. (2022) who states that tobacco plants are used as indicator plants because they can react very quickly to pathogenic bacteria in leaf tissue. This reaction is a hypersensitive reaction that is a symptom of necrosis, the death of cells in plant tissue, which is the way tobacco plants prevent pathogenic bacteria from spreading throughout the tissue.

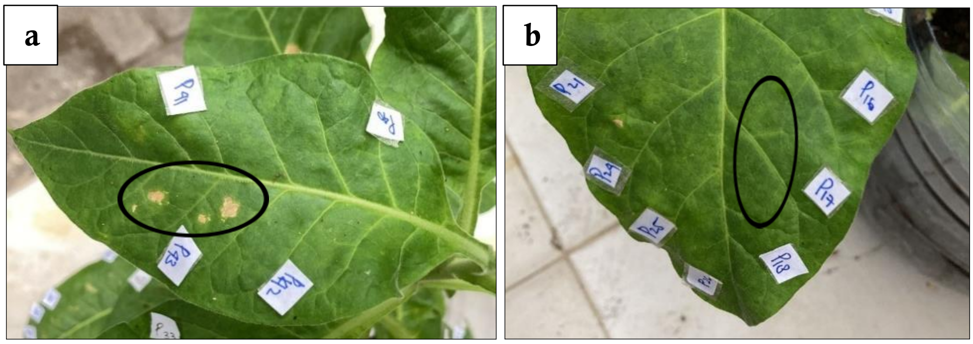

Figure 2. Test results for hypersensitivity to tobacco: (a) Positive Necrosis, (b) Negative necrosis

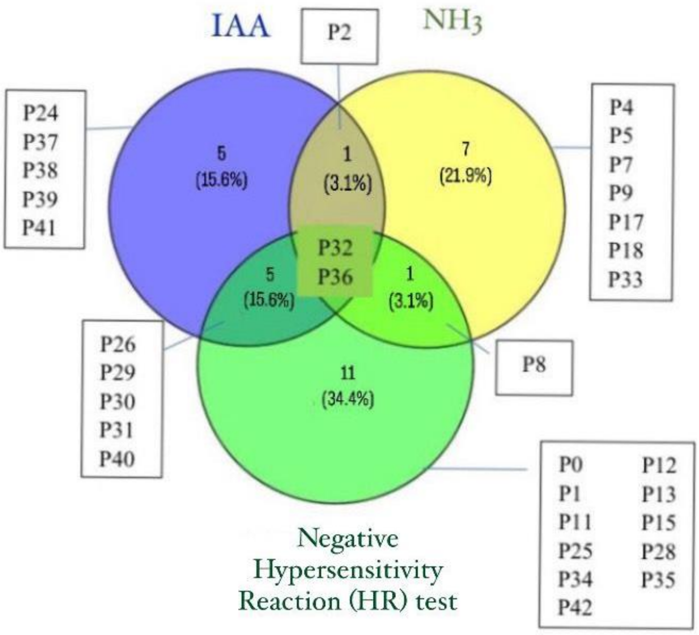

Figure 3. The PGPR character (IAA, NH3, and HR negative production) that the bacterial rhizosphere isolates of the Cambodian Tree possesses.

Based on the figure 3, we can briefly identify the character of each isolated bacterial rhizosphere of the Cambodian tree as PGPR. There are isolates that only have characters producing IAA or NH_3, and there are also those that have multiple characters. For example, P32 and P36 isolates are capable of producing IAA and NH_3 with negative HR tests, whereas P2 isolates can produce IAA and NH3 with positive HR tests. The selection of isolates to be applied to plants as growth supports can be based on character complementation. For example, a consortium of bacteria consisting of P24, P4, and P11 isolates emerged to have a more complete character as PGPR. Another way is to use a single isolate, such as P32 or P36, which has three expected characters.

The application of bacteria to support plant growth is suggested in the form of a *synthetic microbial community* (SynComs). *Synthetic microbial communities* (SynComs) are a more realistic understanding of the results of various biotic interactions in which microbes, plants, and environments play a role in multidimensional and complex space and time (Marín et al., 2021). The advantage obtained from SynComs is that it has low complexity, known community membership characteristics that are predictable for plant growth, produces different effects with a single culture, is stable, and its composition can be replaced according to purpose (Beltran-Garcia et al., 2021; Du et al., 2020). One example of rhizospheric syncom applications is the use of a mixture of bacteria such as *Bacillus* spp., *Acinetobacter* spp., *Enterobacter* sp., *Xanthomonas* sp., and *Burkholderia* sp., which can significantly increase wheat plant growth, root development, and biomass production (Liu et al., 2022).

4. Conclusion

The white Cambodian plant rhizosphere bacteria of the pracimoloyo TPU have the potential to fix 26.82% of nitrogen at the highest concentration of 13.815 mM (P8 isolates), and as many as 23 rhizosphere bacterial isolates reacted negatively (Negative necrosis) in the hypersensitivity reaction (HR) test, which showed that the white Cambodian crop rhizosphere bacteria are not potentially pathogenic to plants. Further development of bacterial isolates with plant growth-supporting characteristics can be applied to other plants to enhance growth and productivity.

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References

Amaria, W., Kasim, N. N., & Munif, A. (2019). Kelimpahan populasi bakteri filosfer, rizosfer, dan endofit tanaman kemiri sunan (*Reutealis trisperma* (Blanco) Airy Shaw), serta potensinya sebagai agens biokontrol. *Journal TABARO*, *3*(1), 305–317. http://dx.doi.org/10.35914/tabaro.v3i1.200

Amaria, W., Sinaga, M. S., & Mutaqin, K. H. (2023). Hemolysis and hypersensitive tests ease culture collection management of antagonistic bacteria. *Jurnal Hama Dan Penyakit Tumbuhan Tropika*, *23*(2), 24–30. https://doi.org/10.23960/jhptt.22324-30Amrulloh, M. K., Addy, H. S., & Sri Wahyuni, W. (2021). Characterization of Physiology And Biochemistry Causes Wood Treatment Bacteria Disease on Crops (*Syzygium aromaticum* L.) in PT Tirta Harapan. *Jurnal Proteksi Tanaman Tropis*, *2*(1), 1–7. https://doi.org/10.19184/jptt.v2i1.17919

Arimbawa, I. M., Wirya, G. N. A. S., Sudana, I. M., & Winantara, I. M. (2019). Isolasi dan seleksi bakteri antagonis untuk pengendalian penyakit busuk batang panili (*Vanilla planifolia Andrews*) Secara In Vitro. *Jurnal Agroekoteknologi Tropika*, *8*(2), 182–193. https://ojs.unud.ac.id/index.php/JAT

Ariyani, M. D., Dewi, T. K., Pujiyanto, S., & Suprihadi, A. (2021). Isolasi dan karakterisasi *plant growth promoting rhizobacteria* dari perakaran kelapa sawit pada lahan gambut. *Bioma*, *23*(2), 159–171. https://doi.org/10.14710/bioma.23.2.159-171

Armita, D., Wahdaniyah, Hafsan, & Al Amanah, H. (2022). Diagnosis visual masalah unsur hara esensial pada berbagai jenis tanaman. *Teknosains: Media Informasi Sains Dan Teknologi*, *16*(1), 139–150. https://doi.org/10.24252/teknosains.v16i1.28639

Astuti, L. A., Muslichah, D. A., Suprihadi, A., Rukmi, MG. I., Mulyani, N., & Sutisna, E. (2021). Karakterisasi Bakteri diazotrof dan pengaruhnya terhadap pertumbuhan tanaman kedelai (*Gylycine max* L. Merrill). *NICHE Journal of Tropical Biology*, *4*(1), 40–49. https://ejournal2.undip.ac.id/index.php/niche

Azzahra, S. C., Effendy, Y., & Slamet, S. (2021). Isolasi dan karakterisasi bakteri pemacu pertumbuhan tanaman (*Plant Growth Promoting Rhizobacteria*) asal Tanah Desa Akar-Akar, Lombok Utara. *Jurnal Al-Azhar Indonesia Seri Sains dan Teknologi*, *6*(2), 70. https://doi.org/10.36722/sst.v6i2.662

Beltran-Garcia, M. J., Martinez-Rodriguez, A., Olmos-Arriaga, I., Valdez-Salas, B., Chavez-Castrillon, Y. Y., Di Mascio, P., & White, J. F. (2021). Probiotic endophytes for more sustainable banana production. *Microorganisms*, *9*(9), 1–17. https://doi.org/10.3390/microorganisms9091805

Chen, H., Yuan, D., Wu, A., Lin, X., & Li, X. (2021). Review of low - temperature plasma nitrogen fixation technology. *Waste Disposal & Sustainable Energy*, *3*(3), 201–217. https://doi.org/10.1007/s42768-021-00074-z

Chen, R. Y., Jiang, W., Fu, S. F., & Chou, J. Y. (2022). Screening, evaluation, and selection of yeasts with high ammonia production ability under nitrogen free condition from the cherry tomato (*Lycopersicon esculentum* var. cerasiforme) rhizosphere as a potential bio-fertilizer. *Rhizosphere*, *23*, 1–19. https://doi.org/10.1016/j.rhisph.2022.100580

Dashti, N. H., Al-Sarraf-, N. Y. A., Cherian, V. M., & Montasser, M. S. (2021). Isolation and characterization of novel *Plant Growth-Promoting Rhizobacteria* (PGPR) isolates from tomato (*Solanum lycopersicum* L.) rhizospherical soil: A novel IAA producing bacteria. *Kuwait J.Sci*, *48*(2), 1–18. https://doi.org/10.48129/kjs.v48i2.8427

Dermawan, I. P., Dewi, I. K., & Putra, I. N. G. J. (2021). Effectiveness comparison of cambodian leaf extract (*Plumeria acuminata* Ait) with jatropha leaf extract (*Jatropha curcas* L) in healing of minor recurrent aphtosa stomatitis (RAS). *Interdental: Jurnal Kedokteran Gigi*, *17*(2), 132–138. https://doi.org/10.46862/interdental.v17i2.2933

Du, J., Li, Y., Yin, Z., Wang, H., Zhang, X., & Ding, X. (2020). High-throughput customization of plant microbiomes for sustainable agriculture. *Frontiers in Microbiology*, 11, 1–9. https://doi.org/10.3389/fpls.2020.569742

Fanani, A. K., Abadi, A. L., & Aini, L. Q. (2015). Eksplorasi bakteri patogen pada beberapa spesies tanaman kantong semar (*Nepenthes* sp.). *Jurnal HPT*, *3*(3), 104–110. https://jurnalhpt.ub.ac.id/index.php/jhpt/article/view/210

Herdiyantoro, D., Setiawati, R. M., & Simarmata, T. (2022). Reaksi hipersensitif daun tembakau oleh isolat bakteri pelarut kalium pada praformulasi pupuk hayati. *Soilrens*, *20*(2), 72–77. https://doi.org/10.24198/soilrens.v20i2.45266

Ishak, I., Ardyati, T., & Aini, L. Q. (2018). Screening of rhizosphere bacteria from clove (*Syzygium aromaticum*) in Tidore Island as *Plant Growth Promoting Rhizobacteria*. *Journal of Experimental Life Science,* 8(3), 153-160. https://doi.org/10.21776/ub.jels.2018.008.03.04

Jannah, N. A., Mustafa, I., & Jatmiko, Y. D. (2022). Isolation and identification of nitrogen-fixing rhizobacteria associated with Cocoa plantation (*Theobroma cacao* L) as biofertilizer agent. *J.Exp. Life Sci*, *12*(3), 75–80. https://doi.org/10.21776/ub.jels.2022.012.03.01

Leghari, S. J., Niaz, A. W., Laghari, G. M., HafeezLaghari, A., MustafaBhabhan, G., Talpur, K. H., Bhutto, T. A., Wahocho, S. A., & Lashari, A. A. (2016). Role of nitrogen for plant growth and development: A review. *Article in Advances in Environmental Biology*, *10*(9), 209–218. https://www.researchgate.net/publication/309704090

Liu, H., Qiu, Z., Ye, J., Verma, J. P., Li, J., & Singh, B. K. (2022). Effective colonisation by a bacterial synthetic community promotes plant growth and alters soil microbial community. *Journal of Sustainable Agriculture and Environment*, *1*(1), 30–42. https://doi.org/10.1002/sae2.12008

Mahdi, I., Fahsi, N., Hafidi, M., Allaoui, A., & Biskri, L. (2020). Plant growth enhancement using rhizospheric halotolerant phosphate solubilizing bacterium *Bacillus licheniformis* qa1 and *Enterobacter asburiae* qf11 Isolated From *Chenopodium quinoa* willd. *Microorganisms*, *8*(6), 1–21. https://doi.org/10.3390/microorganisms8060948

Marín, O., González, B., & Poupin, M. J. (2021). From microbial dynamics to functionality in the rhizosphere: a systematic review of the opportunities with synthetic microbial communities. *In Frontiers in Plant Science*, Vol. 12, 1–12. https://doi.org/10.3389/fpls.2021.650609

Mirta, B., rois, & Amelia, R. (2022). Isolasi dan karakteristik bakteri asal rhizosfer padi sawah intensif di Kabupaten Sigi. *E-J. Agrotekbis*, *10*(1), 17–29. http://103.245.72.23/index.php/agrotekbis/article/view/1177

Putra, A. B., Amin, I. F., Fitriyasa, R. D., Najma, & Safitry, O. (2020). Memperkirakan saat kematian (*Postmortem Interval*) menggunakan temuan mikrobiom pada setiap tahap-tahap penguraian (decomposition): An evidence-based case report. *Journal of Indonesian Forensic and Legal Medicine*, *2*(1), 111–122. http://jos.unsoed.ac.id/index.php/jfmi/article/view/3829/2165

Putra, S. S., Rahayu, T., & Tyastuti, E. M. (2023). Isolation and characterization of cambodian tree rhizosphere bacteria (*Plumeria acuminata*) at TPU Pracimaloyo as a producer of IAA. *Bioeduscience*, *7*(1), 15–23. https://doi.org/10.22236/jbes/7111375

Rafferty, J. P. (2023). *Nitrogen cycle biochemistry, animal & nature, britannica*. Britannica. https://www.britannica.com/Animals-Nature

Rahayu, T. (2022). Komunitas bakteri endofitik kultivar pisang kluthuk dan ambon serta potensi pemanfaatannya [disertasi]. Universitas Gadjah Mada Yogyakarta.

Richard, P. O., Adekanmbi, A. O., & Ogunjobi, A. A. (2018). Screening of bacteria isolated from the rhizosphere of maize plant (*Zea mays* L.) for ammonia production and nitrogen fixation. *African Journal of Microbiology Research*, *12*(34), 829–834. https://doi.org/10.5897/ajmr2018.8957

Safriani, S. R., Fitri, L., & Ismail, Y. S. (2020). Isolation of potential *Plant Growth Promoting Rhizobacteria* (PGPR) from Cassava (*Manihot esculenta*) rhizosphere soil. *Biosaintifika: Journal of Biology & Biology Education*, *12*(3), 459–468. https://doi.org/10.15294/biosaintifika.v12i3.25905

Santoso, K., Rahmawati., & Rafdinal. (2019). Eksplorasi bakteri penambat nitrogen dari tanah hutan mangrove Sungai Peniti, Kabupaten Mempawah. *Jurnal Protobiont*, *8*(1), 52–58. http://dx.doi.org/10.26418/protobiont.v8i1.30855

Sari, N. K. Y., Permatasari, A. A. A. P., & Sumadewi, N. L. (2019). Uji aktivitas anti fungi ekstrak daun kamboja putih (*Plumeria acuminata*) terhadap pertumbuhan jamur *Candida albicans*. *Jurnal Media Sains*, *3*(1), 28–31. https://jurnal.undhirabali.ac.id/index.php/jms/article/view/697

Sari, N. K. Y., & Sumadewi, N. L. U. (2021). Aktivitas Antifungi Saponin Bunga Kamboja Putih (*Plumeria acuminata*) pada *Candida albicans* ATCC 10231. *Metamorfosa: Journal of Biological Sciences*, *8*(1), 74–80. https://doi.org/10.24843/metamorfosa.2021.v08.i01.p07

Sharma, A., Dev, K., Sourirajan, A., & Choudhary, M. (2021). Isolation and characterization of salt-tolerant bacteria with *Plant Growth-Promoting* activities from saline agricultural fields of Haryana, India. *Journal of Genetic Engineering and Biotechnology*, *19*(1), 1–10. https://doi.org/10.1186/s43141-021-00186-3

Singh, R. K., Singh, P., Guo, D., Sharma, A., Li, D.-P., Li, X., Verma, K. K., Malviya, M. K., Soang, X.-P., Lakshmanan, P., Yang, L.-T., & Li, Y.-R. (2021). Root-derived endophytic diazotrophic bacteria *Pantoea cypripedii* AF1 and *Kosakonia arachidis* EF1 Promote Nitrogen Assimilation and Growth in Sugarcane. *Frontiers in Microbiology*, *12*, 119. https://doi.org/10.3389/fmicb.2021.774707

Sriwulan, S., Andriani, R., Anggraini, S. D., Kurniahu, H., & Rahmawati, A. (2022). Bakteri tanah di sekitar rhizosfer tumbuhan pioner pada lahan bekas tambang kapur. *Bioeksperimen*: *Jurnal Penelitian Biologi*, *8*(1), 30–35. https://doi.org/10.23917/bioeksperimen.v8i1.14414

Sunniyyah, D. (2021). Perubahan kadar nitrogen total pada tanah sebagai alternatif estimasi post-mortem interval. *Jurnal Biosains Pascasarjana*, *23*(1), 1–5. https://doi.org/10.20473/jbp.v23i1.2021.1-5

Vionita, Y., Rahayu, Y. S., & Lisdiana, L. (2015). Potensi isolat bakteri endofit dari akar tanaman ubi jalar (*Ipomoea batatas*) dalam penambatan nitrogen. *LenteraBio*, *4*(2), 124–130. http://ejournal.unesa.ac.id/index.php/lenterabio

Wahyudi, A. T., Priyanto, J. A., Afrista, R., Kurniati, D., Astuti, R. I., & Akhdiya, A. (2019). *Plant growth promoting* activity of *actinomycetes* isolated from soybean rhizosphere. *OnLine Journal of Biological Sciences*, *19*(1), 1–8. https://doi.org/10.3844/ojbsci.2019.1.8

Wahyudi, A. T., Priyanto, J. A., Fijrina, H. N., Mariastuti, H. D., & Nawangsih, A. A. (2019). *Streptomyces* spp. from rhizosphere soil of maize with potential as *plant growth promoter*. *Biodiversitas*, *20*(9), 2547–2553. https://doi.org/10.13057/biodiv/d200916

Wang, N., Liu, M., Guo, L., Yang, X., & Qiu, D. (2016). A novel protein elicitor (PeBA1) from bacillus amyloliquefaciens NC6 induces systemic resistance in Tobacco. *International Journal of Biological Sciences*, *12*(6), 757–767. https://doi.org/10.7150/ijbs.14333

Wei, X., Ling, X., Yang, L., Zhang, J., Cui, M., He, Z., & Lazar, C. S. (2022). Analysis of microbial community structure and diversity in Burial Soil of Yangguanzhai Cemetery. *Frontiers in Microbiology*, 13, 1–11. https://doi.org/10.3389/fmicb.2022.845870

Wulandari, N., Irfan, M., & Saragih, R. (2019). Isolasi dan karakterisasi *plant growth promoting rhizobacteria* dari rizosfer kebun karet rakyat. *Jurnal Dinamika Pertanian*, *35*(3), 57–64. https://doi.org/10.25299/dp.2019.vol35(3).4565

Yusmayanti, M., & Asmara, A. P. (2019). Analisis kadar nitrogen pada pupuk urea, pupuk cair dan pupuk kompos dengan metode Kjeldahl. *AMINA*, *1*(1), 28–34. https://doi.org/10.22373/amina.v1i1.11

Zulkifli, L., Rasmi, D. A. C., Sukarso, A., Andayani, Y., & Jekti, D. S. D. (2022). Isolation, molecular identification and antibacterial activity of endophytic bacteria from bark of the *Plumeria acuminata*. *Jurnal Penelitian Pendidikan IPA*, *8*(3), 1158–1165. https://doi.org/10.29303/jppipa.v8i3.2249