Yuzuncu Yil University
Journal of Agricultural Sciences
(Yüzüncü Yıl Üniversitesi Tarım Bilimleri Dergisi)
https://dergipark.org.tr/en/pub/yyutbd

ISSN: 1308-7576 e-ISSN: 1308-7584

Short Communication

Bioprospecting of Fragrant Ginger (*Zingiber aromaticum*) Endophytic Bacteria from Enggano Island, Indonesia as Antimicrobial Compounds Producer

Risky HADI WIBOWO*[1], Sipriyadi[2], Welly DARWIS[3], Eddy SUKMAWINATA[4], Masrukhin[5], Mashudi[6], Muhammad ASRIL[7], Thoriqul HIDAYAH[8], Aldy TRIANDA[9]

[1,2,3]Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Bengkulu, Kandang Limun, Bengkulu, Indonesia, 38112, Indonesia
[1,2,8]Master Study Program of Biology, Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Bengkulu, Jl. W.R. Supratman, Bengkulu, 38371, Indonesia
[4]Research Centre for Veterinary Science, Research Organization for Health, National Research and Innovation Agency, Bogor, West Java, 16911, Indonesia
[1]Research Centre of Sumatera Natural Products and Functional Materials, Universitas Bengkulu, Jl. W.R. Supratman, Bengkulu, 38371, Indonesia
[5]Biosystematics and Evolution Research, Cibinong Science Center, Cibinong, Indonesia, 16911, Indonesia.
[6]Department of Environmental Engineering, Institut Teknologi Sepuluh Nopember, Surabaya, Indonesia, 60111, Indonesia
[7]Department of Biology, Institut Teknologi Sumatera, Way Hui, Jati Agung, Lampung Selatan, Lampung, 35365, Indonesia
[9]Undergraduate Student, Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Bengkulu, Kandang Limun, Bengkulu, 38112, Indonesia

[1]https://orcid.org/ 0000-0002-5917-4625, [2]https://orcid.org/ 0000-0003-1042-2576, [3]https://orcid.org/0000-0002-5982-8983
[4]https://orcid.org/0000-0002-8749-0270, [5]https://orcid.org/0000-0001-8853-6034, [6]https://orcid.org/0009-0001-4819-505X
[7]https://orcid.org/0000-0001-8637-4190, [8]https://orcid.org/0009-0005-2719-3046, [9]https://orcid.org/0009-0004-2608-7527

*Corresponding author e-mail: riskyhadiwibowo80@gmail.com

Article Info

Received: 02.02.2024
Accepted: 22.03.2024
Online published: 15.06.2024
DOI: 10.29133/yyutbd.1429698

Keywords

Antimicrobial,
Endophytic,
Zingiber aromaticum

Abstract: Fragrant Ginger or Lempuyang wangi (*Zingiber aromaticum* Val.) is one of the plants from the Zingiberaceae family that Indonesians widely use as traditional medicine. Endophytic bacteria living in the healthy plant are potentially carrying antimicrobial properties and good secondary metabolites. This study aims to determine the potential of endophytic bacteria from fragrant ginger plants from Enggano Island as antimicrobial. Antimicrobial activity was analyzed using the disc diffusion method from pallets and supernatant of bacteria. The results showed that five of 44 isolates consisting of *Providencia* strain LWERG 29, *Stenotrophomonas* strain LWERG 30, *Bacillus* strain LWEBG 39, *Bacillus* strain LWEBG 41, and *Pseudomonas* strain LWEBG 42 isolates were able to suppress pathogenic bacteria such as *B. subtilis*, *P. aeruginosa*, and *E. coli*. Interestingly, those selected species could show their ability to inhibit tested pathogens with a strong category. This is the first study that showed the potential of endophytic bacteria as antimicrobial agents isolated from fragrant ginger (Lempuyang Wangi) in Enggano Island, Indonesia.

To Cite: Hadi Wibowo, R, Sipriyadi, Darwis, W, Sukmawinata, E, Masrukhin, Mashudi, Asril, M, Hidayah, T, Trianda, A, 2024. Bioprospecting of Fragrant Ginger (*Zingiber aromaticum*) Endophytic Bacteria from Enggano Island, Indonesia as Antimicrobial Compounds Producer. *Yuzuncu Yil University Journal of Agricultural Sciences*, 34(2): 263-270. DOI: https://doi.org/10.29133/yyutbd.1429698

1. Introduction

Lempuyang Wangi (*Zingiber aromaticum*), or fragrant ginger, is a Zingiberaceae family plant widely cultivated in the tropics, including Indonesia, but its origin is still uncertain (Leong-Skornickova et al., 2019). Enggano Island is an island located in the Indian Ocean, Indonesia. Geological data show that this island is oceanic and unique regarding its biogeography and evolution. Four genera of Zingiberaceae were reported from Enggano Island: Amomum, Etlingera, Zingiber, and Curcuma, mostly found along the rivers in Kuala Besar and Kali Jangkar districts (Hamidy et al., 2017). Lempuyang Wangi or fragrant ginger rhizome is commonly applied as a remedy for asthma, diarrhea, malaria, flu, anthelmintics, and appetite stimulants. Secondary metabolites found in Lempuyang Wangi include zerumbone, α-humulene, β-selinene dan (-)-caryophyllene oxide, saponins, tannins, flavonoids, and essential oils (Aji and Zakkiyah., 2021). The isolation of endophytic bacteria, which live inside plant tissues without causing harm to plants, and benefit their host by producing substances that regulate growth and disease resistance, is considered to have bioactive compounds such as antibiotics similar to those produced by the host plants (Achika Rori et al., 2020; Duhan et al., 2020; Fani et al., 2022; Uçar et al. 2023).

Since drug resistance has emerged, the discovery and development of new antibiotics have been challenging. Several genera of endophytic bacteria were known to produce secondary metabolites and potential sources of novel antimicrobials, such as *Streptomyces* sp. Tc022 was isolated from Zingberaceae (*Alpinia galanga*) as an actinomycin producer (Nurjannah et al., 2023). A recent study showed the potency of Zingiberaceae endophytic bacteria isolated from North Sumatera, Indonesia to inhibit pathogens such as Enteropathogenic *Escherichia coli*, *Staphylococcus aureus* ATCC 29213, *Listeria monocytogenes* BTCC B693, *Methicillin-Resistant S. aureus* ATCC 43300, *S. epidermidis* ATCC 12228 and *Proteus vulgaris* ATCC 13315 (Mamangkey et al., 2020). Recently, we successfully isolated 44 fragrant ginger endophytic bacteria from Enggano Island and grouping into the genera *Bacillus*, *Micrococcus*, *Amphibacillus*, *Pseudomonas*, and *Azotobacter* based on morphology, Gram-staining, and biochemical tests. (Andeas et al., 2023). Nevertheless, the detailed information on endophytic bacteria species from Indonesian fragrant ginger is poorly explored. In this study, we explored the potential of collected fragrant ginger endophytic bacteria, including their species, as a producer of antimicrobial compounds against pathogenic microbes.

2. Material and Methods

2.1. Bacteria isolates

In this study, 44 endophytic bacterial isolates from the collection of the Microbiology Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, University of Bengkulu were screened to see the potential of antimicrobial activity. Previously, those collections were isolated from fragrant ginger (*Zingiber aromaticum* Val.; local name: Lempuyang Wangi) from Enggano island, Bengkulu Province, Indonesia. Gram Staining and biochemical tests of those isolates have been done in our previous study (Andeas et al., 2023). All isolates were recultured to Nutrient Agar (NA; Oxoid CM0003B, UK) supplemented with nystatin (0.01% w/v) and incubated at 30 °C for 48-72 h (Andeas et al., 2023).

2.2. Antimicrobial test of endophytic bacteria against pathogenic microbes

The antimicrobial activity of endophytic bacterial isolates was tested against four pathogens consisting of three bacteria, *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, and a fungi *Candida albicans* based on Wibowo et al. (2023). First, overnight cultures of pathogen bacteria and fungi at 30 °C were prepared using tryptic soy broth (TSB; Oxoid CM0129B, UK) and potato dextrose broth (PDB; Oxoid CM0962, UK), respectively. Then, we prepared plate agar containing 1:100 of each pathogen culture mixed with tryptic soy agar (TSA; Oxoid CM0131, UK) for bacteria and potato dextrose agar (PDA; Oxoid CM0139, UK) for fungi. Subsequently, an inoculating loop of endophytic bacterial cultures was spotted on the agar plate containing the pathogen and incubated for 24 h at 30 °C. The endophytic bacterial isolates that were able to inhibit the growth of pathogens were observed from a clear zone surrounding the endophytic bacterial isolate and selected as antimicrobial producers.

To confirm and quantify the antimicrobial activity, 1.5 ml overnight culture from selected endophytic isolates was centrifuged at 10000 rpm for 5 min, and the pellets and supernatant were separated for use in the antagonist test against pathogenic microbes. The pallet from endophyte bacteria was resuspended with 150 µl supernatant to get a concentrate culture. Then, to observe the antimicrobial activity from bacteria and its metabolite, 20 µl of each supernatant and concentrate culture were dripped onto different paper discs, placed on the plate agar containing the pathogen, and incubated for 24 h at 30 °C. Chloramphenicol 30 µg (Oxoid CT0013B) and Nystatin 100 international units (Oxoid CT0073B) were used as a positive control for bacteria and fungi susceptibility, respectively. Antimicrobial activity was evaluated by measurement of the clear zone surrounding the paper disc. Then, the degree of intensity from the antimicrobial zone of inhibition (ZOI) was classified into four categories: very strong (>20 mm); strong (10-20 mm), medium (5-10 mm), and weak (<5mm) (Ouchari *et al.,* 2019). The assay was conducted in two independent experiments. The data analysis was conducted using the statistical data management application Statistical Product and Service Solution (SPSS) 25 with Analysis of Variance (ANOVA). If there were significant differences in the data, the analysis was continued with Duncan's multiple range test (Dahlan, 2014).

2.3. Species identification

The isolate that showed antimicrobial activity was identified to the species using sequencing analysis. Genomic DNA was extracted by using Presto™ Mini gDNA Bacteria Kit (Genaid, GBB100, Taiwan) based on manufacture protocol. All isolates were amplified using 16S rRNA to investigate the bacterial species based on minor modifications from Anggraini et al., (2018). All amplicon products were then sent to Genetika Sains Indonesia in Jakarta/Indonesia for sequencing. The construction of a phylogenetic tree based on the 16S rRNA coding gene was carried out using the Neighbor-Joining method with a bootstrap value of 1000 replications using MEGA 7.

3. Results

In this study, we found five species with antimicrobial activities that consisted of *Providencia* sp. LWERG29, *Stenotrophomonas rhizophila* LWERG30, *Bacillus altitudinis* LWEBG39, *Bacillus amyloliquefaciens* LWEBG41, and *Pseudomonas* sp. LWEBG42. Isolate identification is shown in Table 1 and Figure 1.

Table 1. Sequence alignment of 16S rRNA gene from Lempuyang Wangi isolates

Isolate	Source*	Homology	Query Cover (%)	E-Value	Similarity	Accession Number
LWERG29	Rhizome	*Providencia rettgeri* strain HSC-49S18	100 %	0.0	99.76 %	MK640700.1
LWERG30	Rhizome	*Stenotrophomonas rhizophila* strain KR2-13	100 %	0.0	100 %	MN753976.1
LWEBG39	Stem	*Bacillus altitudinis* strain P5.15	100 %	0.0	99.68 %	QQ295976.1
LWEBG41	Stem	*Bacillus amyloliquefaciens* strain K2-2	100 %	0.0	99.76 %	MH265986.1
LWEBG42	Stem	*Pseudomonas* sp. strain PAMC 27353	100 %	0.0	99.81 %	MT555388.1

*Bacteria were isolated by grinding method (Andeas et al., 2023).

In general, the antimicrobial activity showed by the inhibition zone from the pellet was higher than supernatants. Four species (*Providencia* sp. LWERG29, *S. rhizophila* LWERG30, *B. amyloliquefaciens* LWEBG41, and *Pseudomonas* sp. LWEBG42) in both pellet and supernatant had inhibitory activity against three pathogenic bacteria, *E. coli*, *P. aeruginosa*, and *B. subtilis*. Only *B. altitudinis* LWEBG39 did not have antimicrobial activity against *P. aeruginosa*. However, the *B.*

altitudinis LWEBG39 strain revealed antimicrobial activity against *C. albicans* in pellet form, while antimicrobial activity was not found in supernatant. Specifically, the highest antimicrobial activity of *Z. aromaticum* isolates pellet to *E. coli*, *P. aeruginosa*, and *B. subtilis* were shown by *S. rhizophila* LWERG30, *B. amyloliquefaciens* LWEBG41, and *Providencia* sp. LWERG29, respectively. Consistently, *S. rhizophila* LWERG30 and *B. amyloliquefaciens* LWEBG41 supernatants also showed the highest antimicrobial activity to *E. coli* and *P. aeruginosa,* respectively. When we categorized them based on their strong ability to inhibit bacteria from species pallets, *E. coli* inhibition was only shown by *S. rhizophila* LWERG30. Near all species showed strong intensity to inhibit *P. aeruginosa* and *B. subtilis,* except for *B. altitudinis* LWEBG39 which couldn't inhibit *P. aeruginosa* and *Pseudomonas* sp. LWEBG42 weakly inhibit *B. subtilis*. Interestingly, *B. amyloliquefaciens* LWEBG41 supernatant also revealed strong intensity to inhibit *P. aeruginosa*. The results of the antimicrobial activity test using the disc diffusion method of pellets and supernatant are summarized in Table 2, Table 3, and Figure 2.

Table 2. Antibacterial activity of endophytic *Zingiber aromaticum* pellet by disc diffusion method

Sample	Inhibition Zone (size ± s.d. in mm)			
	E. coli	***P. aeruginosa***	***B. subtilis***	***C. albicans***
LWERG 29	$3.25^{b} \pm 0.35$	$14.5^{d} \pm 0.15$	$16.80^{e} \pm 0.30$	$0.00^{a} \pm 0.00$
LWERG 30	$10.20^{f} \pm 0.10$	$13.95^{c} \pm 0.15$	$12.25^{c} \pm 0.05$	$0.00^{a} \pm 0.00$
LWEBG 39	$5.95^{c} \pm 0.75$	$0.00^{a} \pm 0.00$	$13.70^{d} \pm 0.10$	$4.80^{b} \pm 0.40$
LWEBG 41	$7.15^{d} \pm 0.55$	$16.75^{e} \pm 0.15$	$16.60^{e} \pm 0.10$	$0.00^{a} \pm 0.00$
LWEBG 42	$8.50^{e} \pm 0.20$	$11.45^{b} \pm 0.05$	$8.15^{b} \pm 0.05$	$0.00^{a} \pm 0.00$
Chloramphenicol	$28.00^{g} \pm 0.70$	$20.60^{f} \pm 0.85$	$29.45^{f} \pm 0.5$	NT
Nystatin	NT	NT	NT	$19.15^{c} \pm 0.5$

*Different superscripts indicated a significant difference in the group with $p<0.05$. s.d.: Standard deviation. NT: Not tested.

Table 3. Antibacterial activity of endophytic *Zingiber aromaticum* supernatant by disc diffusion method

Sample	Inhibition Zone (size ± s.d. in mm)			
	E. coli	***P. aeruginosa***	***B. subtilis***	***C. albicans***
LWERG 29	$5.70^{d} \pm 0.30$	$4.05^{c} \pm 0.35$	$5.90^{d} \pm 0.10$	$0.00^{a} \pm 0.00$
LWERG 30	$8.10^{e} \pm 0.30$	$8.55^{d} \pm 0.15$	$7.85^{f} \pm 0.05$	$0.00^{a} \pm 0.00$
LWEBG 39	$1.55^{b} \pm 0.45$	$0.00^{a} \pm 0.00$	$1.40^{b} \pm 0.20$	$0.00^{a} \pm 0.00$
LWEBG 41	$4.25^{c} \pm 0.05$	$14.95^{e} \pm 0.15$	$4.55^{c} \pm 0.25$	$0.00^{a} \pm 0.00$
LWEBG 42	$4.05^{c} \pm 0.35$	$1.55^{b} \pm 0.05$	$6.35^{e} \pm 0.05$	$0.00^{a} \pm 0.00$
Chloramphenicol	$28.00^{f} \pm 0.70$	$20.60^{f} \pm 0.85$	$29.45^{g} \pm 0.5$	NT
Nystatin	NT	NT	NT	$19.15^{b} \pm 0.5$

*Different superscripts indicated a significant difference in the group with $p<0.05$. s.d.: Standard deviation. NT: Not tested.

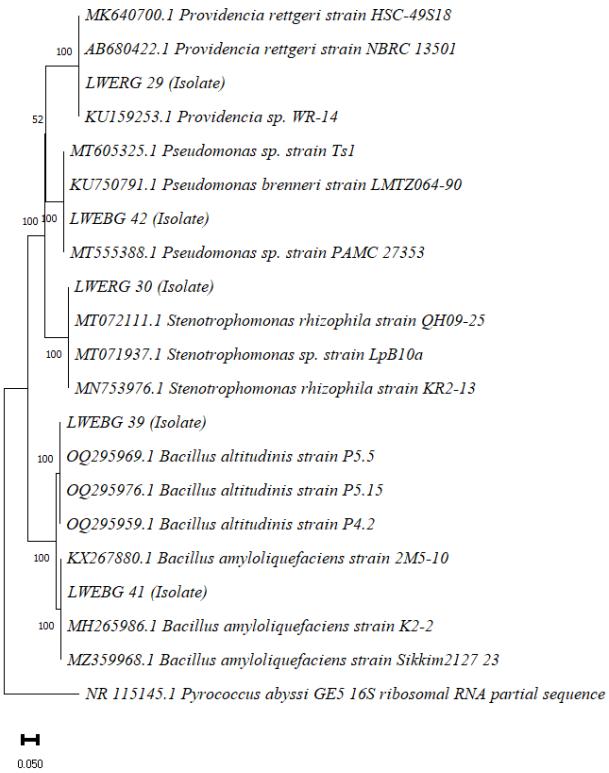

Figure 1. The phylogenetic tree of five sequenced isolates are LWERG29, LWERG30, LWEBG39, LWEBG41, and LWEBG42.

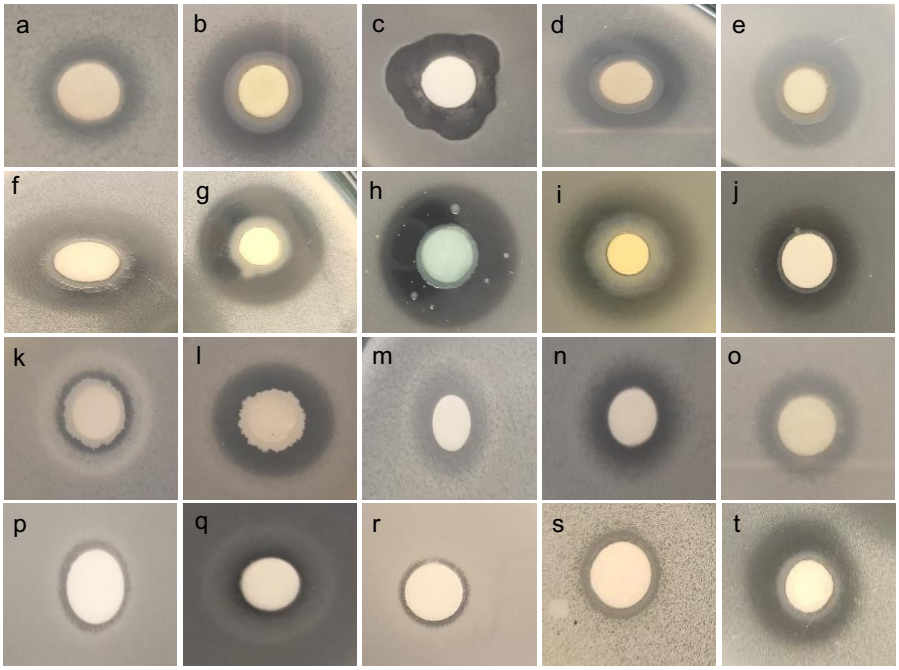

Figure 2. Antibacterial and antifungal activities of endophyte bacterial pellets using disc diffusion method sequentially from (a)-(j): (a) LWERG 29, (b) LWERG 30, and (c) LWEBG 39 against *E. coli,* (d) LWEBG 41, and (e) LWEBG 42 against *P. aeruginosa,* (f) LWERG 29, (g) LWERG 30, and (h) LWEBG 39 against *B. subtilis,*(i) LWEBG 39 and (j) LWEBG 41 against *C. albicans*, and supernatant sequentially from (k)-(t) : (k) LWERG 29, (l) LWERG 30, and (m) LWEBG 41 against *E. coli*, (n) LWERG 30 (o) LWEBG 41, and (p) LWEBG 42 against *P. aeruginosa,* (q) LWERG 29, (r) LWERG 30, and (s) LWEBG 39 against *B. subtilis*, (t) LWEBG 41 against *C. albicans.*

4. Discussion

The ability of the antimicrobial compounds produced by selected species to limit the growth of the pathogenic microorganisms differed between species. The inhibition zone resulting from supernatant administration proved that tested bacteria excreted antimicrobials into the medium (extracellular) by bacterial isolates, while bacterial pallets produced bioactive compounds and secreted intracellularly (Xie et al., 2021). The ability to create an inhibition zone varies among five potential species in pellet assays. In the stationary phase of bacterial growth, there is competition to obtain nutrients and defend themselves so that bacteria will produce bioactive compounds, such as antimicrobial compounds. (Cappucino and Welsh., 2018).

Providencia strain LWERG29, *Stenotrophomonas* strain LWERG30, *Bacillus* strain LWEBG39, *Bacillus* strain LWEBG41, and *Pseudomonas* strain LWEBG42 isolates were able to suppress nearly all pathogenic bacteria, including *B. subtilis*, *P. aeruginosa*, and *E. coli*. Some modes of action from antimicrobial compounds include suppressing bacterial cell wall formation, bacterial protein synthesis, folate synthesis, DNA synthesis, and modifying cell membrane permeability (Reygaert, 2018). On the other side, the pathogenic fungus *C. albicans* was suppressed by *Bacillus* strain LWEBG39. There are numerous mechanisms of antifungal action, including cell membrane damage, inhibition of ergosterol production, inhibition of protein synthesis, and inhibition of enzyme action (Hossain et al., 2022).

In our study, *Stenotrophomonas* strain LWERG30 is close to *S. rhizophila* species. Previously, this species was known to have antifungal activity against *Botrytis cinerea* infection in tomato leaves (Raio et al., 2023), while *Stenotrophomonas* LWERG30 had strong intensity in inhibit *E. coli* and *B. subtilis.* Only *Bacillus* strain LWEBG39 which is close to *B. altitudinis*, showed antifungal and antibactertia activities in this study. Consistent with our findings, *B. altitudinis* and *B. amyloliquefaciens* have also been reported as bacteriocins producers (Abednego et al., 2023; Hanafy et al., 2023). In addition, *Pseudomonas* strain LWEBG42 was able to inhibit *E. coli* and *B. subtilis* might be due to the activity of pyocins that are commonly produced by *Pseudomonas* as a previous report (Ghequire et al., 2023).

Conclusion

In conclusion, this study confirmed that a total of five best potential species of fragrant ginger endophytic bacteria successfully inhibit four testing pathogenic microbes using their cultures, supernatant, and pellets. Interestingly, selected species could show their ability to inhibit pathogens with a strong degree of intensity and that potential to develop as new antimicrobial compounds. These results also suggested that Enggano Island, Bengkulu, and Indonesia are sources of potential endophytic bacteria isolated from fragrant ginger (Lempuyang Wangi) that can be used as antimicrobial agents. This study was limited to identifying fragrant ginger endophytic bacteria that showed their potential to produce antimicrobial compounds. Based on the 16s rRNA gene, those 5 isolates were highly homolog to *Providencia, Stenotrophomonas, Bacillus,* and *Pseudomonas* genera. Further analysis is necessary to continue to identify the genes responsible for antimicrobial activities using whole genome sequencing.

Acknowledgments

We thank to Research and Community Services Centre (LPPM) and Faculty of Mathematics and Natural Sciences, University of Bengkulu through the research scheme (Hibah Unggulan FMIPA UNIB 2022) with Contract number: 2039/UN30.12/HK/2022. We also thank the people of Enggano Island, Bengkulu province, and all parties who have helped the completion of this research.

References

Abednego, R. N., & Silago, V. (2023). Antibacterial activity of soil-isolated Bacillus altitudinis/pumilus complex against methicillin-resistant Staphylococcus aureus from Mwanza, Tanzania. *African Journal of Laboratory Medicine, 12*, a2167.

Aji, O. R., & Zakkiyah, H. C. (2021). Antifungal Activity of 96% Ethanol Extract of Lempuyang Wangi Rhizome (Zingiber aromaticum Val.) against Pythium sp. Fungi in Vitro. *Biota: Jurnal Ilmiah Ilmu-Ilmu Hayati, 6*(1), 58–63.

Andeas, A. F., Wibowo, R. H., Darwis, W., Sipriyadi, Supriati, R., Hidayah, T., & Supriyanto, A. P. (2023). Isolation and identification of endophytic bacteria in lempuyang wangi (Zingiber zerumbeth var. aromaticum Val.) from Enggano Island, Bengkulu Province. *AIP Conference Proceedings, 2606*(1), 050007.

Anggraini, L., Marlida, Y., Wizna, W., Jamsari, J., Mirzah, M., Adzitey, F., & Huda, N. (2018). Molecular identification and phylogenetic analysis of GABA-producing lactic acid bacteria isolated from indigenous dadih of West Sumatera, Indonesia. *F1000Research, 7*, 1663.

Cappucino, J., & Welsh, C. (2018). *Microbiology: a laboratory manual* (17th ed.). Pearson Education Limited.

Dahlan, M. S. (2014). Statistics for Medicine and Health. *Jakarta: Epidemiology Indonesia. Indonesia Health Profile Data and Information, 6*, 7-33.

Duhan, P., Bansal, P., & Rani, S. (2020). Isolation, identification and characterization of endophytic bacteria from medicinal plant Tinospora cordifolia. *South African Journal of Botany, 134*, 43-49.

Fani, E., Rahmawati, R., & Kurniatuhadi, R. (2022). Identification And Detection Of Proteolytic Activity Of Endophite Bacterial Isolated From Avicennia Marina Leaves In Mempawah Mangrove Center. *LenteraBio: Berkala Ilmiah Biologi, 11*(2), 293–299.

Ghequire, M. G. K., & Öztürk, B. (2018). A Colicin M-Type Bacteriocin from Pseudomonas aeruginosa Targeting the HxuC Heme Receptor Requires a Novel Immunity Partner. *Applied and Environmental Microbiology, 84*(18), e00716-18.

Hamidy, A., Keim, A., Sihotang, V., Lupiyaningdyah, P., Irham, M., Ardiyani, M., Kertonegoro, A., Wiantoro, S., Khusniati, T., Susanti, R., Wardani, W., & Royyani, M. (2017). Ekspedisi Pulau Enggano. In I. Maryanto, A. Hamidy, A. Keim, V. Sihotang, P. Lupiyaningdyah, M. Irham, & M. Ardiyani (Eds.), *LIPI Press*.

Hanafy, A. M., Alreedy, R. M., Elbahloul, Y., & El-Sayed, W. S. (2023). Diversity of Bacteriocin-encoding Gene Families and the Activity Spectrum among Bacillus amyloliquefaciens Isolates. *Egyptian Journal of Botany, 63*, 69-83.

Hossain, C. M., Ryan, L. K., Gera, M., Choudhuri, S., Lyle, N., Ali, K. A., & Diamond, G. (2022). Antifungals and Drug Resistance. *Encyclopedia, 2*, 1722-1737.

Leong-Skornickova, J., Souvannakhoummane, K., & Tran, H. (2019). Zingiber zerumbet (Shampoo Ginger). *The IUCN Red List of Threatened Species 2019: E.T96816015A96816082*.

Mamangkey, J., Mendes, L. W., Sibero, M. T., & Nadapdap, P. E. (2020). Bioprospecting For Bacterial Endophytes Associated With Zingiberaceae Family Rhizomes In Sibolangit Forest, North Sumatera. *International Journal of Science, Technology & Management, 1*(1), 27-36.

Nurjannah, L., Azhari, A., & Supratman, U. (2023). Secondary Metabolites of Endophytes Associated with the Zingiberaceae Family and Their Pharmacological Activities. *Scientia Pharmaceutica, 91*, 3.

Ouchari, L., Boukeskasse, A., Bouizgarne, & Ouhdouch, Y. (2019). Antimicrobial Potential Of Actinomycetes Isolated From The Unexplored Hot Merzouga Desert and Their Taxonomic Diversity. *Biology Open, 8*(2), 1-7.

Raio, A., Brilli, F., Neri, L., Baraldi, R., Orlando, F., Pugliesi, C., Chen, X., & Baccelli, I. (2023). Stenotrophomonas rhizophila Ep2.2 inhibits growth of Botrytis cinerea through the emission of volatile organic compounds, restricts leaf infection and primes defense genes. *Frontiers in plant science, 14*, 1235669.

Reygaert, W. C. (2018). An overview of the antimicrobial resistance mechanisms of bacteria. *AIMS Microbiology, 4*(3), 482–501.

Rori, C. A., Kandou, F. E. F., & Agustina, M. T. (2020). Extracellular Enzyme Activity of Mangrove Plant Endophytic Bacteria Avicennia marina. *Jurnal Bios Logos, 10*(2), 48–55.

Uçar, C. P., Selem, E., Tunçtürk, R., Tunçtürk, M., & Akköprü, A. (2023). The effect of some endophytic bacteria on seedling growth and physiological properties of *Salvia officinalis* L. *Yuzuncu Yil University Journal of Agricultural Sciences*, *33*(1), 130-139.

Wibowo, R. H., Sipriyadi, Darwis, W., Putri, D. A., Yudha, S., Mashudi, Ilsan, N. A., Renta, P. P., & Masrukhin. (2023). Isolation, characterization and identification of sponge-associated bacteria producing antimicrobial compounds. *Biodiversitas, 24*, 3616-3623.

Xie, Y., Peng, Q., Ji, Y., Xie, A., Yang, L., Mu, S., Li, Z., He, T., Xiao, Y., Zhao, J., & Zhang, Q. (2021). Isolation and identification of antibacterial bioactive compounds from *Bacillus megaterium* L2. *Frontiers in Microbiology, 12*, 645484.