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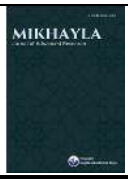

Isolation and Characterisation of Actinomycetes in the Rhizosfer *Pandanus ammaryllifolius* Plants in Gorontalo

Annisa Dwiyani[1*], Yuliana Retnowati[2],Abubakar Sidik[3], Novri Y. Kandowangko[4], Wirnangsi Din Uno[5]
Universitas Negeri Gorontalo, Jalan Prof. Dr. Ing. B.J. Habibie, Kabupaten Bone Bolango, 96554, Indonesia
Email: annisadwiyaniishak02@gmail.com

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ABSTRAK

Actinomycetes adalah salah satu kelompok bakteri gram positif diketahui memiliki kemampuan bioaktivitas yang tinggi, termasuk potensi sebagai penghasil antibiotik dan senyawa bioaktif lainnya. Penelitian ini bertujuan untuk mengisolasi dan mengkarakterisasi bakteri Actinomycetes dari rhizosfer Pandanus ammaryllifolius di Gorontalo. Rhizosfer, yang merupakan segmen tanah di sekitar akar tanaman, kaya akan mikroorganisme yang berperan penting dalam siklus hara dan kesehatan ekosistem. Pengambilan sampel dilakukan di dua lokasi, yaitu perkebunan Desa Pahu, Kecamatan Asparaga, dan Bone Bolango, Kecamatan Tilongkabila. Hasil isolasi menggunakan metode Spread Plate pada media Starch Casein Agar (SCA), menunjukkan keberadaan Actinomycetes yang diperoleh 9 isolat Actinomycetes. Jumlah isolat terbanyak ditemukan pada lokasi perkebunan Bone Bolango, yaitu sebanyak 6 isolat . Sementara itu, 3 isolat lainnya ditemukan di Desa Pahu. Kondisi lingkungan di lokasi Desa Pahu memiliki pH 4,4 dan kelembaban 5,7%, sedangkan tanah di lokasi perkebunan Bone Bolango memiliki pH 7,9 dan kelembaban 6,8%. Hasil karakterisasi morfologi menunjukkan variasi dalam bentuk, ukuran, dan warna koloni. Isolat Actinomycetes yang diperoleh berukuran Small dengan bentuk koloni yang Irregular (tidak beraturan) dan Circular (bulat). Warna miselium substrat koloni didominasi oleh warna kuning, orange, putih, kuning kehitaman, dan putih keabu-abuan. Karakteristik morfologi yang ditemukan pada isolat ini mengindikasikan tiga kelompok potensial Actinomycetes: Streptomyces, Micromonospora, dan Micrococcus. Seluruh isolat ditemukan bersifat Gram positif.

ABSTRACT

Actinomycetes are a group of gram-positive bacteria known to have high bioactivity, including the potential to produce antibiotics and other bioactive compounds. These microorganisms have cosmopolitan nature and adaptability in various soil habitats indicating that they can be found and adapt well in various types of soil. This study aims to isolate and characterise Actinomycetes bacteria from the rhizosphere of *Pandanus ammaryllifolius* plants in Gorontalo. The rhizosphere, which is the soil segment around plant roots, is rich in microorganisms that play an important role in nutrient cycling and ecosystem health. Sampling was conducted in two locations, namely the plantations of Pahu Village, Asparaga District, and Bone Bolango, Tilongkabila District. Isolation results using the spread plate method on Starch Casein Agar (SCA) media, showed the presence of Actinomycetes obtained 9 isolates of Actinomycetes. The highest number of isolates was found in the Bone Bolango plantation location, which was 6 isolates. Meanwhile, 3 other isolates were found in Pahu Village. Environmental conditions at the Pahu Village site had a pH of 4.4 and humidity of 5.7%, while the soil at the Bone Bolango plantation site had a pH of 7.9 and humidity of 6.8%. Morphological characterisation results showed variations in colony shape, size and colour. Actinomycetes isolates obtained were small with irregular and circular colony shapes. The colour of the colony substrate mycelium was dominated by yellow, orange, white, blackish yellow, and greyish white. The Morphological characteristics found in this isolate indicated three potential groups of Actinomycetes: *Streptomyces, Micromonospora*, and *Micrococcus*. All isolates were found to be Gram positive.

* *Corresponding Author: Annisa Dwiyani: annisadwiyaniishak02@gmail.com*

1. INTRODUCTION

Soil microorganisms consist of groups of bacteria, fungi, and protozoa. One soil segment that has many microbes that are useful for plant development is the rhizosphere. The rhizosphere is the soil segment located at the roots of plants. Rhizosphere microbes have an important role in nutrient cycling and soil formation processes, plant development, influencing microbial activity and as a biological control against root pathogens. According to Prayudyaningsih *et al*., (2015), the number of microorganisms in the rhizosphere is usually more numerous and varied than in non-rhizosphere soil. Among the three microorganisms, bacteria are the most abundant microbes in the soil. The bacterial community is called rhizobacteria (Saputri *et al*., 2021).

Rhizobacteria are bacteria that reside in the rhizosphere of plants, providing various benefits to plants during their growth and development cycle. This creates a symbiotic relationship between the two parties (Raza *et al*., 2016). Bacteria obtain nutrients from plant root exudates and microbes are able to support plant growth and development and influence soil pHysical and chemical properties (Yunus F, *et al*., 2017). One of the rhizobacteria found in the rhizosphere zone is the Actinomycetes type of bacteria.

Actinomycetes are gram-positive bacteria that have the ability of bioactivity in producing various bioactive compounds. Most of the bioactive compounds have potential as producers of antibiotics (antibacterial), antifungal (Nurjasmi. R., & Suryani, 2017), antiviral, chemotherapeutic (anticancer agents), enzymes and immunousuppressants (Alwi *et al*., 2020). Actinomycetes also have cosmopolitan properties and adaptability in various soil habitats indicating that they can be found and adapt well in various types of soil (Setyawati *et al*., 2021).

Gorontalo has very supportive environmental conditions for the exploration of soil microorganisms, with a tropical climate, high rainfall, and high biodiversity (Gorontalo Central Bureau of Statistics, 2020). Gorontalo has quite diverse soil types, ranging from fertile soils such as alluvial and grumoso to less fertile soils such as latosol, mediteran, podsolik, and lithosol, which are able to support the growth of various plants, including pandanus plants (Arum, 2017).

The *Pandanus ammaryllifolius,* which is widespread in the region, has an extensive root system that can provide a unique habitat for soil microorganisms, especially bacteria. Microbial interactions with plant roots involve microorganisms that can be symbiotic in nature such as Actinomycetes associated with pandanus plants. The rhizosphere of pandanus wangi plants is used as the focus of research because the rhizosphere of *Pandanus ammaryllifolius* plants provides a unique habitat for soil microorganisms. This rhizosphere environment is rich in nutrients and organic compounds produced by the roots of *Pandanus amaryllifolius* plants through the exudation process which has the potential to support the growth and activity of Actinomycetes (Raza *et al*., 2016).

Despite the huge potential of Actinomycetes in agriculture, research on the isolation and characterisation of these bacteria from the rhizosphere of *Pandanus amaryllifolius* plants in Gorontalo is still very limited. Most people still view bacteria as harmful organisms, even though some types of bacteria such as Actinomycetes have a very important role in supporting plant growth and ecosystem health.

Based on these considerations, this study aims to isolate and characterise Actinomycetes from the rhizosphere of *Pandanus amaryllifoliusplants* in Gorontalo. The results

of the study are expected to provide scientific contributions in the development of biofertilisers and environmentally friendly sustainable agricultural strategies.

2. METHOD

This research was conducted using a quantitative descriptive approach. The data obtained from the isolation and characterization included macroscopic and microscopic characteristics presented in tabular form. The study was carried out from june to november 2024. Rhizosphere soil sampling was conducted using a survey method at two locations, namely the plantations in Pahu village, asparaga subdistrict, and Bone Bolango. The isolation and characterization of Actinomycetes from the rhizosphere soil of *Pandanus ammaryllifolius* were performed at the biology laboratory, faculty of mathematics and natural sciences, Gorontalo State University, and at the agricultural plant protection laboratory. The isolation began with pre-treatment (Dhaneskaran and Jiang 2016). A 200 µl suspension of soil from each dilution was taken using the surface/spread plate technique with a duplicate method and inoculated on the surface of sca medium that had been supplemented with antifungal cycloheximide (50 µl/ml) or nystatin (50 µl/ml) to prevent the growth of contaminating fungal colonies. The samples were then incubated at 37°C for 7-14 days (Katili and Yuliana 2017). Morphological characterization was observed, including colony shape, size, and color of the mycelium of each colony. Subsequently, gram staining was performed to determine cell shape (Iwin *et al*., 2020). The population of Actinomycetes in each rhizosphere soil was determined by counting the number of colonies based on the plate count method. The selected and counted plates were those containing between 30-300 colonies. If none were found, the closest count was chosen. The number of bacteria was expressed as colony forming units (cfu) (Lestari *et al*., 2016). The formula for calculating total plate count (tpc) is as follows:

$$\text{Number of colonies per cup} = \frac{1}{Dilution\ factor}$$

3. RESULT AND DISCUSSION

In this study, soil samples came from the rhizosphere of *Pandanus amaryllifolius* plants. Sampling was carried out in two locations, namely in the Pahu Village Plantation, Asparaga District and Bone Bolango.

Table 3.1 *Pandanus amaryllifolius* plant soil environmental parameters

No	Location	Environmental Parameters		Coordinate point
		Average pH	Average humidity	
1.	Desa Pahu Kecematan Asparaga	4,4	5,7%	(0° 80’74.77’’N; 122 °43’68.45’’E)
2.	Bone Bolango	7,9	6,8%	(0°54’91.84’’N; 123°13’04.72’’E)

The results of measuring the soil environment of the rhizosphere of *Pandanus amaryllifolius* plants show that the 2 sampling locations tend to have acidic to neutral pH so that it is very good for microbial growth, especially Actinomycetes. While the humidity level is said to be very low but very good for the growth of Actinomycetes.

Isolation of Actinomycetes from the rhizosphere of *Pandanus amaryllifolius* plants

Isolation of Actinomycetes bacteria using the multistage dilution method up to 10^{-5}. Colony counts were carried out only on the 10^{-3}-10^{-5} dilution series to facilitate colony counting. So that the following results were obtained:

Table 3.2 Results of environmental soil parameter tests for *Pandanus amaryllifolius* plants

Location	Dilution	Numberof colonies	TPC (CFU/g)
Desa Pahu Kecematan Asparaga	10^{-3}	9	$7.49x10^5$
	10^{-4}	4	
	10^{-5}	7	
Bone Bolango	10^{-3}	-	$2.94x10^6$
	10^{-4}	4	
	10^{-5}	29	

The results of the analysis in the table above show that both locations have a significant number of bacterial colonies, where the Bone Bolango location shows a higher level of diversity than the Pahu village location, which is $2.94x10^6$.

Actinomycetes Morphological Characterisation

Morphological characteristics observed from Actinomycetes bacteria are the shape, size and colour of the colonies seen from the arial mycelium and substrat mycelium.

Table 3.3 Results of Morphological characterization of Actinomycetes

Isolate	Shape	Size	Mycelium areal	Substrate mycelium
Rzp-pI	Circular	Small	Yellowish white	Yellow
Rzp-p2	Circular	Small	Orange	Orange
Rzp-p3	Irregular	Small	White	White

The results of Morphological characterisation of Actinomycetes in the plantation location of Pahu Village, Asparaga Sub-district, obtained 3 types of Actinomycetes isolates that have different Morphological structures.

Table 3.4 Results of Morphological characterization of Actinomycetes

Isolate	Shape	Size	Mycelium areal	Substrate mycelium
Rzp-b4	Circular	Small	Blackish orange	White
Rzp-b5	Circular	Small	Dark grey	Yellow-black
Rzp-b6	Circular	Small	White-yellowish	Yellow
Rzp-b7	Circular	Small	Greyish yellow	Greyish white
Rzp-b8	Irregular	Small	Orange	Orange
Rzp-b9	Irregular	Small	White	White

The results of Morphological characterisation of Actinomycetes at the Bone Bolango location obtained 9 types of Actinomycetes isolates that have different Morphological structures.

Based on the table above, morphologically it can be explained that the Actinomycetes isolate with the code:

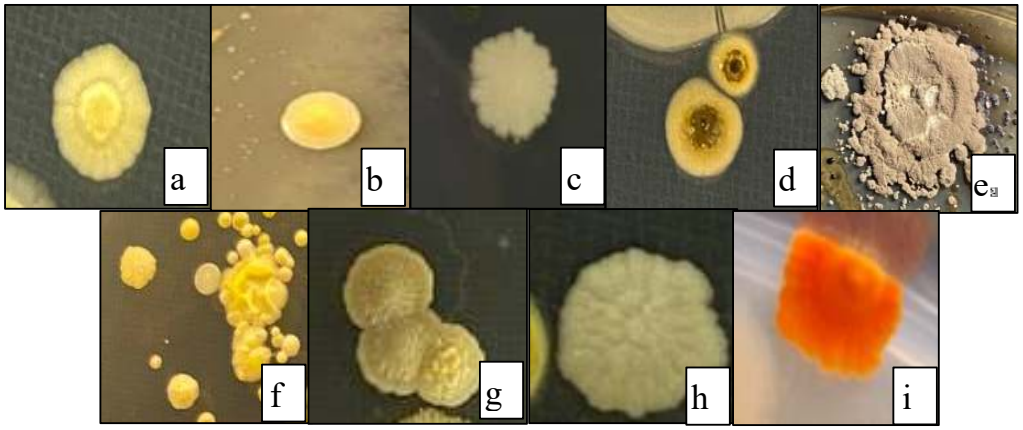

Figure 3.1 Macroscopic morphology of Actinomycetes. (a) Rzp-pI, (b) Rzp-p2, (c) Rzp-p3, (d) Rzp-b4, (e) Rzp-b5, (f) Rzp-b6, (g) Rzp-b7, (h) Rzp-b8, (i) Rzb-p9

Rzp-pI has a curcular colony shape, small size, and aerial mycelium colony colour yellowish white with yellow substrate mycelium. Rzp-p2 has Curcular colony shape, Small size, and Orange aerial mycelium colony colour with orange substrate mycelium. Rzp-p3 has Irregular colony shape, Small size, and White air mycelium colony colour with white substrate mycelium. Rzp-b4 has a Circular colony shape, Small size, and blackish Orange aerial mycelium colony colour with white substrate mycelium. Rzp-b5 has a circular colony shape, small size, and dark grey aerial mycelium colony colour with blackish yellow substrate mycelium. Rzp-b6 has a circular colony shape, small size, and aerial mycelium colony colour yellowish white with yellow substrate mycelium. Rzp-b7 has a circular colony shape, small size, and aerial mycelium colony colour greyish yellow with greyish white substrate mycelium. Rzp-b8 has an Irregular colony shape, Small size, and Orange aerial mycelium colony colour with orange substrate mycelium. Rzb-p9 has irregular colony shape, small size, and white aerial mycelium colony colour with white substrate mycelium. Actinomycetes have different colony colours due to differences in the pigment content of each constituent cell (Indriani, 2018).

The results of the Morphological characteristics of the 9 isolates obtained were then purified by grouping the types of Actinomycetes that had different mycelium arial and mycelium substrate.

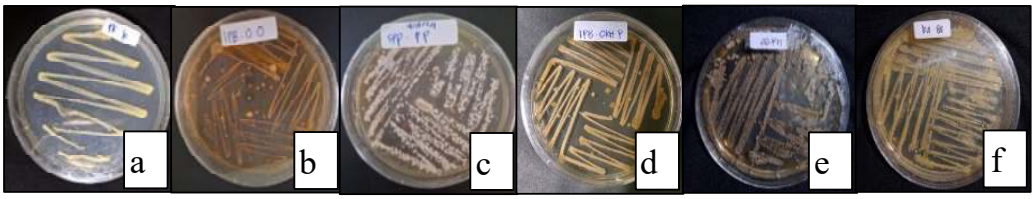

Figure 3.2. Actinomycetes purification results. (a) Rzp-pI; yellowish-white, (b) Rzp-p2; orange-orange, (c) Rzp-p3; white-white, (d) Rzp-b4; blackish-white orange, (e) Rzp-b5; dark grey-yellow, (f) Rzp-b7; greyish-yellow-white

The results of purification of Actinomycetes from the rhizosphere of *Pandanus amaryllifolius* plants obtained as many as 6 types of isolates, each of which has a different substrate and area. The 6 Actinomycetes isolates obtained from the purification results showed the characteristics of Actinomycetes bacteria. The isolates that have been purified are Gram stained to ensure that the isolates obtained are Actinomycetes which are Gram positive bacteria.

Table 3.5 Results of microscopic characteristics of Actinomycetes

Isolate codes	Gram Positif/Negatif
Rzp-pI	+
Rzp-p2	+
Rzp-p3	+
Rzp-b4	+
Rzp-b5	+
Rzp-b7	+

Characterization of the purified Actinomyecetes isolates was performed using the gram staining method. The gram staining test was conducted to determine whether the obtained isolates are gram-positive or gram-negative bacteria. The results of the gram staining of the Actinomyecetes isolates showed gram-positive, indicated by a purple color. Gram-positive bacteria contain a thicker layer of peptidoglycan compared to gram-negative bacteria, which are indicated by a pink color (Figure 3.3).

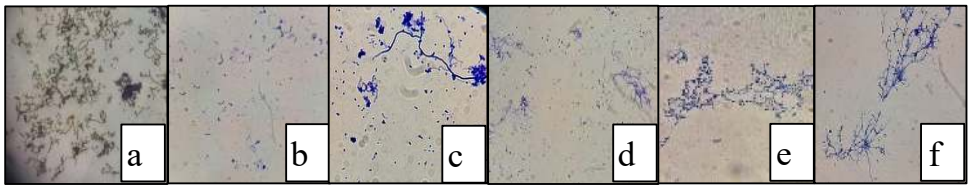

Figure 3.3 Microscopic observation of Actinomycetes (10x0.25)
(a) Rzp-pI; yellowish white, (b) Rzp-p2; orange-orange, (c) Rzp-p3; white-white, (d) Rzp-b4; blackish orange-white, (e) Rzp-b5; dark grey-yellow, (f) Rzp-b7; greyish yellow-greyish white

The results of microscopic observations made under a microscope using magnification (10x0.25) can be seen that the 6 isolates have a purple cell colour which is a gram-negative bacteria that shows the characteristics of Actinomycetes.

Based on the isolation results, this study succeeded in revealing the presence of Actinomycetes in the rhizosphere of *Pandanus amaryllifolius* plants in gorontalo which were distributed in two different locations, namely the plantations of pahu village, asparaga and bone bolango. Actinomycetes isolation began with pre-treatment with 60 °C wet heat heating for 15 minutes using a water bath. This pre-treatment aims to select actinomycetes groups by inhibiting or eliminating unwanted microorganisms (Dhaneskaran and Jiang 2016).

The results of isolating all sample suspensions using the pour plate method on starch-casein agar media with the addition of cyclohexamide or nystatin successfully cultivated Actinomycetes with various colony morphology characteristics (Figure 3.1). The number of isolates obtained included 6 Actinomycetes isolates from the plantation location in pahu

village, asparaga district (table 3.3), and 3 isolates from the Bone Bolango location (table 3.4). According to Astuty (2017), the number of Actinomycetes isolates successfully obtained is influenced by environmental factors, sample sources, pre-treatment, the medium used, and isolation techniques, which will affect the number of isolates obtained and the variation of different Actinomycetes. Additionally, Actinomycetes are not tolerant to acid, and their numbers decrease as soil pH decreases.

The location of Pahu Village has a pH of 4.4, indicating acidic soil characteristics with a moisture content of 5.7%, while Bone Bolango has a neutral pH of 7.9 with a moisture content of 6.8%. Although the moisture content is relatively low, this condition is still consistent with the statement by Setyawati *et al*. (2021), which emphasizes the cosmopolitan nature of Actinomycetes and their ability to adapt to various soil habitats. The cosmopolitan nature and adaptability of Actinomycetes in different soil habitats indicate that they can be found and adapt well to various types of soil.

The Actinomycetes population in the two locations showed differences. The highest Actinomycetes population was obtained from the rhizosphere of *Pandanus amaryllifolius* plants in the Bone Bolango location and the lowest Actinomycetes population was obtained from the rhizosphere of Pandanus amaryllifoliusplants in the plantation location of Pahu village, asparaga subdistrict. This is influenced by environmental factors that can affect the spread of an organism, especially bacteria. According to Sofariyan *et al*., (2019) environmental conditions with pH being a key factor in providing a good place for the growth of Actinomycetes. Actinomycetes are bacteria that cannot tolerate acidic conditions and can be anaerobic or facultative anaerobic (can grow either with or without the presence of free oxygen) (Murray *et al*., 2016). Actinomycetes are intolerant of acidic environments according to Lestari *et al*., (2019) the optimal pH of Actinomycetes to grow is the range between 5-7. Meanwhile, according to research by Tyas (2020) said that Actinomycetes can also live well in areas with pH levels of 6.5-8.0. The ecological context of this research supports the concept of symbiosis in the rhizosphere as proposed by Raza *et al*., (2016). Actinomycetes play a strategic role in the soil ecosystem, acting as decomposers, nutrient recyclers, and having the potential to support plant growth.

The characterization of macroscopic morphology reveals complex diversity, in line with the research by Bonanomi *et al*., (2016) on soil microorganisms. The obtained Actinomycetes isolates display variations in colony shape, with the majority being small and exhibiting circular and irregular morphologies. The diverse colors of the mycelium, ranging from white, orange, yellow, to gray, indicate cellular pigmentation diversity. The differences in color observed among the isolates may be due to each isolate having distinct biological roles. According to Buedenbender *et al*., (2017), the diversity of colors in Actinomycetes bacteria is attributed to the presence of spore chain pigments that these bacteria possess; the hyphae will change to a specific color upon spore formation, resulting in different colors. The pigments produced have biological capabilities such as antibiotic, antitumor, and vitamin properties. characteristics; these five isolates are capable of growing more quickly with smooth or rough surfaces and edges that are either flat or irregular, within a period of 3-5 days.

Isolates Rzp-p2, Rzp-b4, Rzp-b7, and Rzp-b9 exhibit Morphological similarities that classify them within the Micromonospora group, characterized by small, dense colonies with unusual aerial mycelium, glossy texture, flat surfaces, and round colonies with flat edges

(Gopalakrishnan *et al*., 2020). Isolates Rzp-p1 and Rzp-b6 show Morphological similarities that classify them within the Micrococcus group.

Isolates Rzp-p3, Rzp-b5, and Rzp-b8 share similar characteristics; these three isolates appear slowly and grow over a slightly longer period, with powdery colony surfaces within 10-14 days. This is consistent with the statement by Rozirwan *et al*., (2020) that Actinomycetes emerge slowly, showing a powdery consistency and adhering tightly to the agar surface, a characteristic that distinguishes them from bacteria in general, which are typically slimy and grow more quickly. Isolates Rzp-p3, Rzp-b5, and Rzp-b8 can be classified into the Streptomyces group due to their Morphological similarities with Streptomyces, characterized by white and gray aerial mycelium and yellow or orange substrate mycelium (Tan *et al*., 2015).

Based on the microscopic characteristics of Actinomycetes, they are marked by branched or rod-shaped filaments and possess non-septate hyphae. The mycelium can be branched or unbranched, straight or spiral in shape. Spores are spherical, cylindrical, or oval (Anggraini, 2015). The results show that all six purified isolates are Gram-positive (purple) (Figure 3.1.5). Perkmen (2021) explains that the cell structure of Gram-positive bacteria features a thick cell wall (peptidoglycan) and only one inner membrane without an outer membrane, as well as teichoic acid containing alcohol and phosphate. The purple dye that is not washed out by 96% alcohol in Gram-positive bacteria means that the addition of safranin red dye does not have an effect. Therefore, when the purple-iodine crystal complex enters the Gram-positive bacterial cells, it cannot be washed out by alcohol due to the robust peptidoglycan layer in the cell wall. The consistent Gram staining indicates a Gram-positive nature, confirming the taxonomic classification of Actinobacteria.

4. CONCLUSION

The isolation and characterization of Actinomycetes from the rhizosphere of *Pandanus ammaryllifolius* in Gorontalo yielded several significant findings. From the two locations studied, namely Pahu village and Bone Bolango, six Actinomycetes isolates were successfully isolated in Pahu village and three isolates in Bone Bolango. The analysis results showed that the soil pH in Pahu village was 4.4 (acidic) and in Bone Bolango was 7.9 (neutral), with low soil moisture, yet still supporting the growth of Actinomycetes. Morphological characteristics revealed colonies with irregular and circular shapes, small colony sizes, and variations in color including yellowish-white, orange, white, blackish-orange, dark gray-yellowish, and grayish-yellowish-white. The characterization results indicated three potential groups of Actinomycetes: streptomyces, micromonospora, and micrococcus, with gram staining results showing that all isolates are gram-positive bacteria.

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6. REFERENCES

Alwi M, Suharjono, Ardyati, Subandi. (2020). Eksplorasi Actinomycetes Sebagai Kandidat Antibakteri Patogen Yang Resisten Dari Rhizosfer Tumbuhan Leda (Eucalyptus Deglupta Blume.) Di Taman Nasional Lore Lindu, Indonesia. *Biocelebes*. 14(3): 253-267, https://doi.org/10.22487/bioceb.v14i3.15310

Anggraini W. (2015). Isolasi Actinomycetes dengan menggunakan metode skrining sebagai penghasil enzim kitinase. *Jurnal Ilmiah Pendidikan Fisika Al-Biruni*. 4(1):85-95. DOI: 10.24042/jpifalbiruni.v4i1.83

Arum W.G., (2017). Pemetaan Tahanan Pentanahan Berdasarkan Jenis Tanah Di Provinsi Gorontalo. *Skripsi*. Universitas Negeri Gorontalo: Teknil Elektro

Astuty E. 2017. Isolasi dan Karakterisasi Morfologi Aktinomisetes Indegenus Asal Tanah Gambut. *Jurnal Ilmu Alam dan Lingkungan*. 8(16):7-15.

Badan Standardisasi Nasional. (2014). *Agens Pengendali Hayati (Aph)-Bagian 3 : Trichoderma spp*. SNI 8027.3:2014

Bonanomi, G., De Filippis, F., Cesarano, G., La Storia, A., Ercolini, D., & Scala, F. (2016). Organic farming induces changes in soil microbiota that affect agro-ecosystem functions. *Soil Biology and Biochemistry*, 103(4): 327-336

Buedenbender, L., Carroll, A. R., Ekins, M., & Kurtböke, D. I. (2017). Taxonomic and metabolite diversity of Actinomycetes associated with three Australian ascidians. *Diversity*, 9(4), https://doi.org/10.3390/d9040053

Dhanasekaran D, Jiang Y. (2016). *Actinobacteria*: Basic and Biotenichal Applications. Ave4eva Movimix Records.
DOI: 10.1016/j.soilbio.2016.09.005

Gopalakrishnan, S., Srinivas, V., & Prasanna, S. L. (2020). *Streptomyces. In beneficial microbes in agro-ecology* (pp. 55-71). Academic Press. DOI: 10.1016/B978-0-12-823414-3.00005-8

Katili A.S., And Y. Retnowati. (2017). Isolation Of Actinomycetes From Mangrove Ecosystem In Torosiaje, Gorontalo, Indonesia. *Biodiversitas*. 18(2): 826- 833, https://doi.org/10.13057/biodiv/d180259

Lestari S, Mukarlina, Kurniatuhadi R. (2019). Identifikasi dan Deteksi Aktivitas Daya Hambat Bakteri Actinomycetes yang diisolasi dari Tanah Gambut di Desa Tajok Kayong Kalimantan Barat. *J Protobiont.*. 8(1):13–19. DOI: 10.26418/protobiont.v8i1.30843

Lwin HT, Yin ZZ, Mya TM. (2020). Isolation and Antimicrobial Activity of Actinomycetes From Chaung Tha Area And Biochemical Characterization of Selected Streptomyces (TR-2). *Journal Myanmar Acad. Arts Sci*. 18 (4B): 145-158.

Murray PR, Rosenthal KS, Pfaller MA. (2016). *Medical Microbiology. 8th ed.*. Kanada: Elseiver. 106–118 p

Nurjasmi, R., dan Suryani. (2017). Uji Antagonistik Actinomycetes Asal Limbah kulit Bawang Merah Terhadap Patogen Tanaman. *Jurnal ilmiah Respati Pertanian*. 11(2): 718-722, https://doi.org/10.52643/jir.v8i2.76

Prayudyaningsih, A., Nursyamsi dan S. Ramadana. (2015). Mikroorganisme Tanah Bermanfaat bagi Rizosfer Tanaman Umbi dibawah Tegakan Hutan Rakyat Sulawesi Selatan. *Balai Penelitian Hutan Makassar*. 1(4): 954-959.

Raza, W., Ling, N., Yang, L., Huang, Q., & Shen, Q. (2016). Response of tomato wilt pathogen Ralstonia solanacearum to the volatile organic compounds produced by a biocontrol strain Bacillus amyloliquefaciens SQR-9. *Scientific reports*. 6(1): 24856. DOI: 10.1038/srep24856

Rozirwan, Muda, H.I., Ulqodry, T. Z. (2020). Antibacterial Potential of Actinomycetes Isolated from Mangrove Sediment in Tanjung Api-Api South Sumatra. *Biodiversitas*. 21(12): 5723-5728, https://doi.org/10.13057/biodiv/d211232

Saputri, K. E., Idiawati, N., dan Sofiana, M. S. J. (2021). Isolasi dan Karakteristik Bakteri Penambat Nitrogen dari Rizosfer Mangrove di Kuala Singkawang. *Jurnal Laut Khatulistiwa.* 4(2): 17–21, http://dx.doi.org/10.26418/lkuntan.v4i2.45316

Setyawati, T.R., R. Kurniatuhadi dan A.H. Yanti. (2021). Karakter Morfologi Koloni Streptomyces spp. yang Diisolasi dari Substrat Habitat Cacing Nipah (Namalycastis rhodochorde) pada Medium Berbeda. *Seminar Nasional Penerapan Ilmu Pengetahuan dan Teknologi 2021.* Pontianak, Indonesia: Universitas Tanjungpura.

Sofariyanti AE, Sasongkowati R, Anggraini AD. (2019). Aktivitas antibakteri aktinomisetes di Hutan Mangrove Wonorejo Surabaya yang antagonis terhadap bakteri Staphylococcus aureus. *Anal Kesehat Sains*. 8(2):738–748.

Tan, G. Y., Peng, Y., Lu, C., Bai, L., & Zhong, J. J. (2015). Engineering validamycin production by tandem deletion of γ-butyrolactone receptor genes in Streptomyces hygroscopicus 5008. *Metabolic Engineering*, 28, 74–81, https://doi.org/10.1016/j.ymben.2014.12.003

Tyas SP. (2020). Optimasi Pertumbuhan Isolat Actinomycetes (Isolat TE 235) dan Uji Aktivitas terhadap Bakteri Escherichia coli dan Staphylococcus aureus. *Skripsi.* Program Studi Farmasi, Fakultas Ilmu Kesehatan. Universitas Muhammadiyah. Magelang.

Yunus, F., Lambui, O., & Suwastika, I. N. (2017). Kelimpahan mikroorganisme tanah pada sistem perkebunan kakao (Theobroma cacao L.) semi intensif dan non intensif. *Natural Science: Journal of Science and Technology*, 6(3), https://doi.org/10.22487/25411969.2017.v6.i3.9192