



Research Article

Actinobacteria from Mangrove Rhizosphere as a Source of Biocontrol Agents to Support Sustainable Agriculture

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ABSTRACT

Mangrove ecosystems harbor diverse microorganisms with important ecological and biotechnological roles. Actinobacteria, in particular, are well known as producers of bioactive compounds and potential biocontrol agents against plant pathogens, making their exploration relevant for sustainable agriculture. This study aimed to isolate and characterize Actinobacteria from the rhizosphere of mangroves in Bagek Kembar, West Lombok, and to evaluate their antagonistic potential against *Ralstonia solanacearum*, plant disease agent. Soil samples were collected using a quadrant method, while environmental parameters such as pH, temperature, humidity, and salinity were measured to assess habitat suitability. Actinobacteria were isolated and identified through Gram staining and morphological observation, their abundance was calculated using the Total Plate Count method, and antagonistic activity was tested using the agar well diffusion assay. The results showed that soil pH was relatively neutral, with temperature and salinity suitable for Actinobacteria growth. Five isolates (ACT R1–R5) were obtained, all Gram-positive with filamentous hyphae-like morphology. Total Plate Count analysis indicated high abundance (3.2×10^5 cfu/g), although the diversity of isolates recovered was relatively low. Antagonistic assays revealed that ACT R2 and ACT R4 moderate inhibitory effect, and ACT R1, ACT R3, and ACT R5 weak. Overall, these findings demonstrate that mangrove rhizospheres in West Lombok are a promising source of Actinobacteria with significant potential as environmentally friendly biocontrol agents.

Keywords: Mangrove rhizosphere, Actinobacteria, *Ralstonia solanacearum*

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INTRODUCTION

Mangrove ecosystems are among the most productive coastal ecosystems and have high biodiversity that supports various ecological and economic functions globally (Trégarot et al., 2021; Wang & Gu, 2021). In addition to acting as a natural barrier against abrasion and climate change, mangroves are also habitats for unique microorganisms, including Actinobacteria (Hu et al., 2020; Balakrishnan et al., 2016). These microorganisms are known to produce bioactive compounds, such as antibiotics, antifungals, and antibacterials, which can be utilized in various applications, including biocontrol of plant pathogens (Sangkanu et al., 2017; Kemung et al., 2020; Naligama et al., 2022). In a global context, the biocontrol approach is an important option in supporting sustainable agriculture, reducing dependence on chemical pesticides, and minimizing negative impacts on the environment and human health.

In Indonesia, which has the world's largest mangrove ecosystem (Miteva et al., 2015), research on mangrove microorganisms, including Actinobacteria, is gaining attention. The existence of largely untouched mangrove areas opens up great opportunities for the exploration of applicable local microorganisms. The mangrove environment is

known as an extreme ecosystem for microbial growth due to its high salinity, high humidity, and intense sunlight exposure. These conditions make the mangrove ecosystem a natural selection arena that encourages the emergence of microbes with unique abilities, including the potential to produce biocontrol compounds against various plant pathogens (Hao et al., 2019).

One important pathogen that is the focus of research is *Ralstonia solanacearum* (Xue & Lozano-dur, 2020). This bacterium causes bacterial wilt disease, which attacks various major horticultural crops such as tomatoes, peppers, and potatoes. Infection by this pathogen not only reduces agricultural productivity, but also causes significant economic losses for farmers in Indonesia (Setiawan, 2019; Wang et al., 2023). Therefore, the exploration of Actinobacteria from mangrove ecosystems is expected to provide an alternative source of biocontrol agents that support sustainable plant disease control.

Bagek Kembar mangrove area in Sekotong District, West Lombok, West Nusa Tenggara, is one of the areas with great potential for Actinobacteria exploration. This ecosystem is dominated by mangrove species such as *Rhizophora apiculata*, *Avicennia marina*, and *Sonneratia alba* (Hadiprayitno et al., 2024; Amini et al., 2024), which are known to support microbial diversity, including soil bacteria with important ecological functions. However, to date, there has been no research specifically examining the potential of Actinobacteria from the rhizosphere of mangroves in Bagek Kembar, especially in relation to the control of *Ralstonia solanacearum*, a bacterium that causes wilt disease in various horticultural crops. This research gap confirms that the coastal area of Lombok still holds unique microbial resources that have not been explored and have the potential to make a real contribution to plant disease control.

Previous studies have shown that rhizosphere bacteria, including Actinobacteria, play an important role as biocontrol agents through the production of bioactive compounds such as antibiotics and hydrolytic enzymes, which are effective in suppressing the development of various plant pathogens (Xie et al., 2024; Nur et al., 2024; Nurikhsanti et al., 2024). However, the majority of studies have focused on agricultural soils or forest ecosystems, so information on local Actinobacteria from mangrove ecosystems, particularly on the coast of Lombok Island, is still limited. Therefore, the exploration of Actinobacteria from the rhizosphere of the Bagek Kembar mangrove not only offers scientific novelty, but also opens up opportunities for its use in sustainable agricultural practices with an environmentally friendly biotechnology approach to overcome bacterial wilt caused by *Ralstonia solanacearum*.

This study aims to determine the characteristics of Actinobacteria isolated from the rhizosphere of mangroves in the Bagek Kembar area, Sekotong, West Lombok, and to evaluate their antagonistic potential against *Ralstonia solanacearum* as an environmentally friendly biocontrol agent. The exploration focused on coastal areas through soil sampling around the roots of *Rhizophora* sp. mangroves, which have extreme environmental parameters such as high salinity, high humidity, and intense light exposure, thereby promoting the existence of microbes with unique physiological and metabolic capabilities. With this approach, the study is expected to reveal the potential of mangrove Actinobacteria as a source of new biocontrol agents.

METHODS

This research method was designed with an exploratory and experimental approach to explore the abundance and characteristics of Actinobacteria from the rhizosphere of mangroves in the Bagek Kembar area, West Lombok, and to test their potential as biocontrol agents against *Ralstonia solanacearum*.

Sample Collection Locations and Environmental Factor Measurements

The method used was composite sampling, which involved combining several sub-samples from the same location to obtain a more representative sample. Samples were taken from around the roots of *Rhizophora* sp. in 5 – 10 cm from surface of soil in addition, environmental factors such as altitude, humidity, light intensity, temperature, soil pH, and soil salinity were also measured to understand the habitat conditions of the isolated microbe, using an altimeter, digital hygrometer, lux meter, thermometer, portable pH meter, and refractometer, respectively. Next, total plate count (TPC) was calculated at the Biology Education Laboratory of Mataram University.

Isolation and Morphological Characterization of Actinobacteria cultures

Soil samples were subjected to serial dilution and spread onto Starch Casein Agar (SCA) medium supplemented with 20 µg/mL nystatin and 10 µg/mL nalidixic acid to selectively isolate Actinobacteria colonies (Hidayatullah, 2019; Chiranjeevi & Vijayalakshmi, 2022). The colonies that grew were then incubated at 30°C for 7–14 days, and different isolates were purified for further characterization. After isolation, morphological characterization was performed by observing the shape, size, color, and texture of the colonies, as well as by Gram staining to determine Gram-positive or Gram-negative properties under a light microscope at 1000× magnification using oil immersion (Q. Li et al., 2016; Hadi et al., 2019).

Antagonism Test against *Ralstonia solanacearum*

Antagonistic tests against *Ralstonia solanacearum* were conducted using a completely randomized design (CRD) with three replicates for each treatment. Actinobacteria isolates were subcultured on Starch Casein Broth (SCB) medium with a volume of 50 mL and incubated at 30°C for seven days until they reached a density of approximately 10⁵ cfu/mL, while *R. solanacearum* was grown on Nutrient Broth (NB) medium to a concentration of 10⁵ cfu/mL. The pathogen suspension was then spread evenly on Nutrient Agar (NA) medium in Petri dishes, which were then left in the refrigerator for one hour to ensure adhesion to the agar surface. After that, wells with a diameter of approximately 4 mm were made on the surface of the medium with a depth of half the thickness of the NA medium, then each well was filled with 20 µL of Actinobacteria isolate suspension (Anith et al., 2021). The dishes were incubated at 30°C, and observations were made after five days.

The inhibition zone formed around the wellbore is measured using calipers or a precision ruler by recording the total diameter of the clear zone (D_{total}), then subtracting the wellbore diameter (D_{colony}) to obtain the inhibition zone width (IZ) using the formula: IZ = D_{total}/D_{colony}. The measurement results were then categorized into four activity levels, namely weak (<5 mm), moderate (5–10 mm), and strong (>10 mm). The inhibition zone data were statistically analyzed using Analysis of Variance (ANOVA) based on a Completely Randomized Design (CRD) and three replications for each treatment. The analysis was conducted at a significance level of $p < 0.05$ to determine whether there were significant differences in antagonistic activity among isolates. If the ANOVA results showed significant differences, a Tukey's HSD post hoc test was performed to compare the mean inhibition zones between treatments. (Nurikhsanti et al., 2024).

RESULTS AND DISCUSSION

Description of Sample Collection Locations and Environmental Parameters

The vegetation components of the mangrove ecosystem in Bagek Kembar, Sekotong District, West Lombok Regency, show variations in species and vegetation structure that are characteristic of tropical coastal ecosystems (Figure 1). Based on the results of vegetation inventory, there are a total of 4 families with 9 major mangrove species and 1 minor mangrove species. The major mangrove species consist of *Avicennia alba*, *Avicennia marina*, *Bruguiera gymnorhiza*, *Ceriops tagal*, *Rhizophora apiculata*, *Rhizophora mucronata*, *Rhizophora stylosa*, *Sonneratia alba*, and *Sonneratia caseolaris*. The minor mangrove species is *Excoecaria agallocha* (Sultana & Motaher Hossain, 2022).

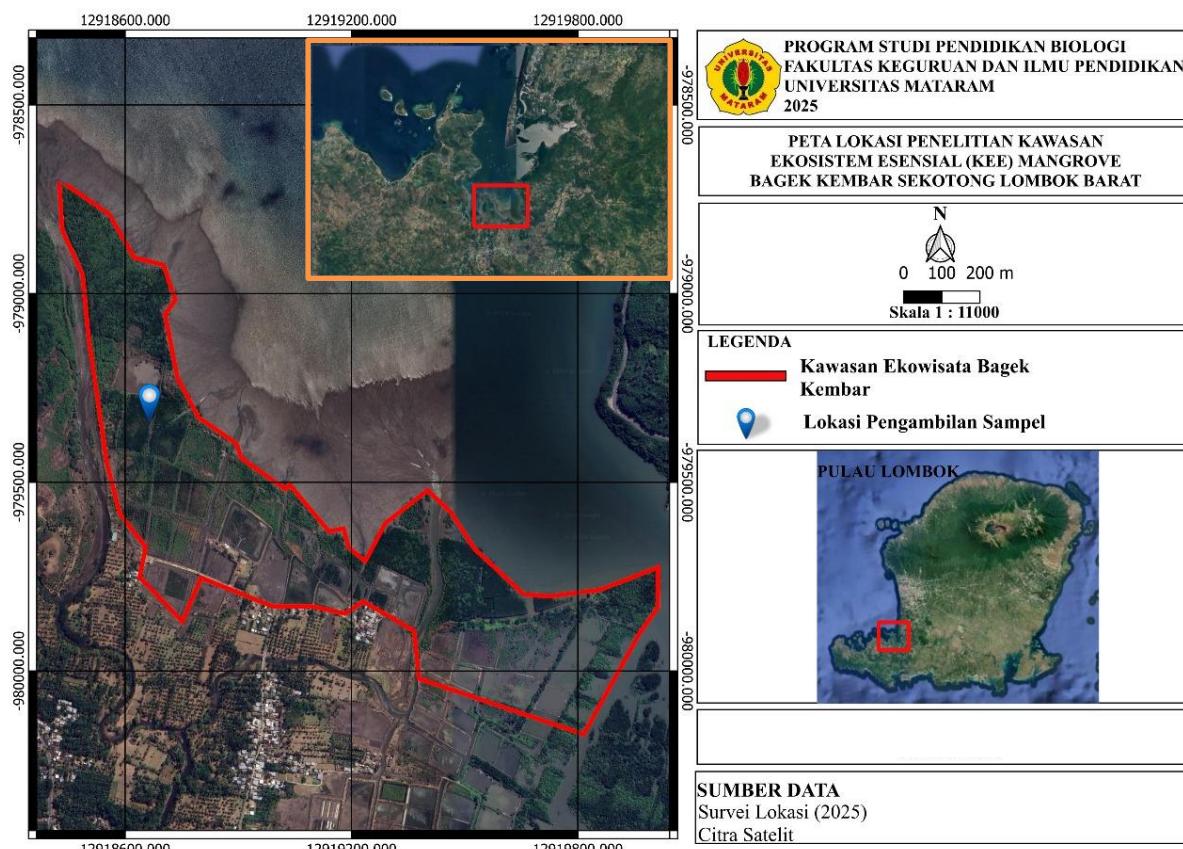


Figure 1. Rhizosphere soil sampling locations

Several dominant species found at the site were used as sampling points for rhizosphere soil, including *Rhizophora mucronata* and *Rhizophora apiculata*. These species were selected because they are widely distributed, have complex root systems, and contribute significantly to the formation of microbial rhizosphere habitats in the mangrove intertidal zone (Qudraty et al., 2023).

The vegetation at this location also shows a layered canopy structure, ranging from mature trees to saplings (Maulidah & Zakiyah, 2023). Plants with pneumatophore root systems provide microhabitats that are highly suitable for the growth and activity of microbes such as Actinomycetes (Pascale et al., 2020). The relatively dense vegetation structure and moist, organic-rich substrate conditions make the rhizosphere zone in these three locations a potential ecosystem for superior microbes. Research (Baskaran et al., 2023) shows that microbes found in the mangrove rhizosphere include Firmicutes, Proteobacteria, Actinobacteria, Bacteroidetes, and Halomonas, with 38 genera and 105 species. The various types of bacteria that grow in the mangrove rhizosphere are also adapted to various abiotic environmental factors.

This study also evaluated the environmental conditions of the mangrove rhizosphere soil at the sampling site in Bagek Kembar, Sekotong District. The environmental parameters measured included elevation, light intensity, pH, humidity, salinity, and soil temperature. These are important factors that can affect the growth and abundance of Actinobacteria.

Table 1. Environmental Parameters and Total Plate Count

Num	Environmental Factors	
1	Altitude (m)	1,02
2	Light intensity (lux)	2.116
3	Soil humidity (%)	69,12
4	Soil Temperature (°C)	30,2
5	Salinity (%)	11,9
6	Soil pH	6,4
7	TPC (Total Plate Count)	$3,2 \times 10^5$ cfu/g

Primary data sources processed in 2025

Measurements of environmental parameters at the sampling site indicate conditions that are relatively conducive to the growth of Actinobacteria. The soil pH value of 6.4 is classified as slightly acidic to neutral, in accordance with report (Sun et al., 2021), which states that most Actinobacteria grow optimally at a pH of 6–8. These conditions allow for the availability of essential nutrients and good microbial enzyme activity, thereby supporting primary metabolism and the production of secondary metabolites that are important in biotechnology applications.

Other environmental factors also have a significant influence. The soil temperature was recorded at 30.2 °C, which is within the optimum growth range for mesophilic Actinobacteria (25–37 °C). This temperature not only supports cell growth, but also affects the expression of extracellular enzymes and the biosynthesis of bioactive compounds (Sriragavi et al., 2023). Soil moisture reached 69.12%, which is quite high and has the potential to maintain microbial physiological activity. However, excessively high moisture levels can reduce soil aeration, so these conditions are likely to favor facultative aerobic Actinobacteria that are able to withstand moisture fluctuations (Zhu et al., 2019).

Soil salinity of 11.9% indicates relatively high conditions, approaching halophilic habitats. Several Actinobacteria species are known to be halotolerant or even halophilic, so these conditions may act as a selective factor that reduces competition with non-halotolerant microbes. The presence of halotolerant Actinobacteria has been widely reported to produce unique secondary metabolites, including antibiotics and salt-stable enzymes, which have high potential for industrial applications (R. Li et al., 2021).

The recorded light intensity of 2,116 lux basically has no direct effect on the growth of Actinobacteria, given that these microbes are heterotrophic. However, light can affect the soil microclimate, for example through the regulation of surface temperature and humidity, which indirectly impacts the dynamics of the microbial community.

The elevation of 1.02 m above sea level indicates that the sample was taken from a lowland area with tropical climate characteristics. Tropical conditions are generally rich in organic matter, warm temperatures, and high humidity, which are suitable for the growth of Actinobacteria. This factor is also associated with higher microbial biodiversity compared to subtropical or temperate regions.

The Total Plate Count (TPC) result of 3.2×10^5 cfu/g of soil indicates a fairly high microbial population. The high number of colonies indicates that the soil has good microbial fertility and is an indicator that environmental conditions support the growth of Actinobacteria. According to [Bhatti et al., \(2017\)](#), soil microbial density is greatly influenced by nutrient availability, aeration, humidity, and pH, all of which are within a relatively suitable range at this location.

Morphological Characteristics of Actinobacteria Isolates from the Mangrove Rhizosphere

Based on isolation from the rhizosphere of *Rhizophora* sp. mangroves, five Actinobacteria isolates (ACT R1, ACT 2, ACT R3, ACT R4, ACT R5) with varying colony morphologies were obtained. The colony shapes found included irregular (ACT R1) and circular (ACT R2, ACT R3, ACT R4, ACT R5). Colony size varied from small to medium, with undulate edges (ACT R1, ACT R2, ACT R4) or entire edges (ACT R3, ACT R5). Colony elevation was generally flat, while variations in aerial and substrate mycelium were also observed, predominantly white in color, except for ACT R2, which had cream-colored substrate mycelium. All isolates were Gram-positive, consistent with the characteristic features of Actinobacteria.

Table 2. Morphological Characteristics of Actinobacteria

Isolate	Shape	Size	Edge	Elevation	Mycelium Aerial	Mycelium Substrate	Gram Staining
ACT R1	Irregular	Small	Undulate	Flat	White	White	+
ACT R2	Circular	Medium	Undulate	Flat	White	Cream	+
ACT R3	Circular	Small	Entire	Flat	-	-	+
ACT R4	Circular	Medium	Undulate	Flat	White	White	+
ACT R5	Circular	Small	Entire	Flat	White	White	+

Colony morphology is one of the important parameters in the initial identification of Actinobacteria before physiological and molecular tests are performed. Variations in colony shape (irregular in ACT R1 and circular in ACT R2–R5) indicate differences in the growth strategies and adaptation of isolates to medium conditions. Circular colonies are generally associated with stable and homogeneous growth, while irregular shapes may reflect variations in filament growth or competition between microbes in the medium ([Golden et al., 2022](#)).

The varying colony sizes (small to medium) indicate that the isolates have different growth rates. Small colonies such as those in ACT R1 and R3 may grow more slowly than medium-sized colonies such as those in ACT R2 and R4. This is consistent with reports that the growth rate of Actinobacteria is greatly influenced by nutrient availability and environmental conditions ([Gaballa et al., 2025](#)). Undulate colony margins (ACT R1, R2, R4) show the characteristic filamentous growth of Actinobacteria, while entire margins (ACT R3, R5) tend to describe isolates with more regular growth. Flat elevation in all isolates indicates similarities in adaptation to Starch Casein Agar (SCA) media.

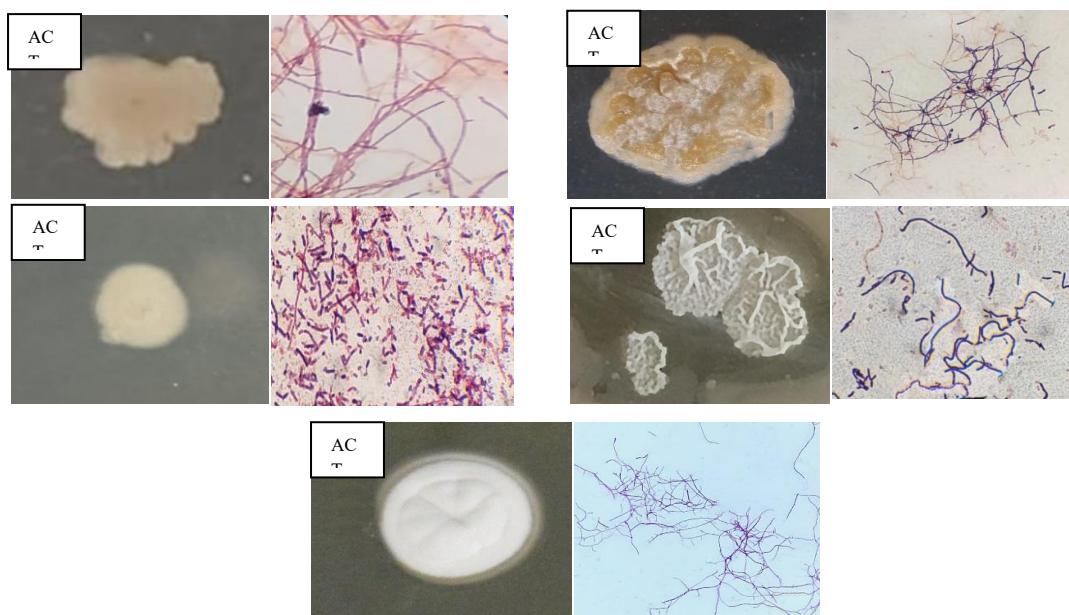


Figure 2. Macroscopic and Microscopic Characteristics of Actinobacteria Cells with 1000 \times magnification

The difference in color between aerial and substrate mycelium is also an important indicator. The dominance of white color in aerial mycelium (ACT R1–R5) is a common characteristic of *Streptomyces* sp. that produces light-colored spores (Kemung et al., 2020). However, the presence of cream color in the substrate mycelium of isolate ACT R2 indicates potential pigment variation that may be related to secondary metabolites. According to Govind et al., (2023), pigments in the substrate mycelium are often associated with the production of antibiotics or other bioactive secondary metabolites. All isolates exhibited Gram-positive reactions, confirming their affiliation with the Actinobacteria group, which is characterized by thick, peptidoglycan-rich cell walls containing diaminopimelic acid (DAP) as a cross-linking amino acid and high GC content in their genomic DNA. The presence of DAP in the peptidoglycan layer reflects a distinctive chemotaxonomic feature of many Actinobacteria genera, such as *Streptomyces*, *Nocardia*, and *Micromonospora*, distinguishing them from other Gram-positive bacteria that typically use lysine in their peptide bridges.

Based on Gram staining results, all Actinobacteria isolates (ACT R1–R5) showed a characteristic purple color, indicating that all isolates were Gram-positive. This is consistent with the general characteristics of Actinobacteria, which have cell walls rich in peptidoglycan, enabling them to retain crystal violet dye after the decolorization process. The cell walls of Actinobacteria are generally composed of thick peptidoglycan with teichoic acid content that plays a role in structural stability, adhesion, and protection against environmental stress (Rahlwes et al., 2019). In contrast, Gram-negative bacteria have a thin peptidoglycan layer surrounded by an outer membrane with lipopolysaccharides (LPS), making them prone to losing their primary color during the Gram staining process (Michael et al., 2017). These results reinforce the identification of the isolates as members of Actinobacteria.

Microscopic observations show that all isolates (ACT R1–R5) have a dominant cell structure in the form of filaments resembling hyphae. These filaments are characteristic of Actinobacteria, especially the genus *Streptomyces* sp., which grows by forming aerial mycelium and substrate mycelium. Aerial mycelium appears as a network of branching filaments that extend upward, while substrate mycelium grows attached to the culture medium. This structure plays an important role in the life cycle of Actinobacteria, from spore formation to the production of secondary metabolites, including antibiotics that are highly valuable in biotechnology. The filamentous growth pattern is the main distinguishing feature of Actinobacteria from non-filamentous bacteria, making them morphologically similar to fungi (Michael et al., 2017; Q. Li et al., 2016).

Furthermore, the results of the observation also showed morphological variations between isolates. ACT R2 and ACT R4 appeared to have denser filaments than other isolates, which could be associated with higher sporulation ability and greater potential for bioactive metabolite production. In addition, fragmentation was also found in several hyphae, indicating an asexual reproduction mechanism through the formation of conidia spores. This mechanism is very important for the survival of Actinobacteria in extreme mangrove ecosystems, as it allows microbes to survive and spread to new environments. These findings indicate morphological diversity that has the potential to influence the ecological function and biotechnological applications of each isolate.

The Antagonistic Potential of Actinobacteria Inhibits the Growth of *Ralstonia solanacearum*

Actinobacteria are known as a group of soil bacteria that have the ability to produce various secondary metabolites with antimicrobial activity, making them potential agents for biocontrol against plant pathogens (Ebrahimi-Zarandi et al., 2022). One important pathogen in solanaceous plants is *Ralstonia solanacearum*, which causes bacterial wilt disease that has a significant impact on agricultural productivity. Biological control efforts through the exploration of antagonistic microbes, particularly Actinobacteria, are an environmentally friendly alternative to the use of synthetic chemical pesticides. Therefore, this study assessed the antagonistic ability of five Actinobacteria isolates (ACT R1–R5) against *R. solanacearum* through an inhibition zone test.

The One-Way ANOVA test was used to determine whether there were significant differences between groups of Actinobacteria isolates in inhibiting the growth of *Ralstonia solanacearum*. The ANOVA results in Table 3 show a calculated F value of 81.444 with a significance value (Sig. < 0.001). This value is well below the significance threshold of 0.05, which means that there are very significant differences between isolates in producing inhibition zone indices. In other words, the antagonistic abilities of the five Actinobacteria isolates are not the same; rather, some isolates are clearly superior to others.

Table 3. One-Way ANOVA Test Results for the Inhibition Zone

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	105.847	4	26.462	81.444	<.001
Within Groups	3.249	10	.325		
Total	109.096	14			

The results of the ANOVA analysis showed a significant difference in antagonistic activity among the Actinobacteria isolates. The large variation observed between treatment groups compared to within-group variation indicates that the differences in inhibition ability are primarily due to the intrinsic biological characteristics of each isolate rather than random experimental error. This suggests that the observed antagonistic activity reflects true physiological differences in antimicrobial potential among isolates, emphasizing that the type of isolate plays a dominant role in determining inhibitory effectiveness against the pathogen.

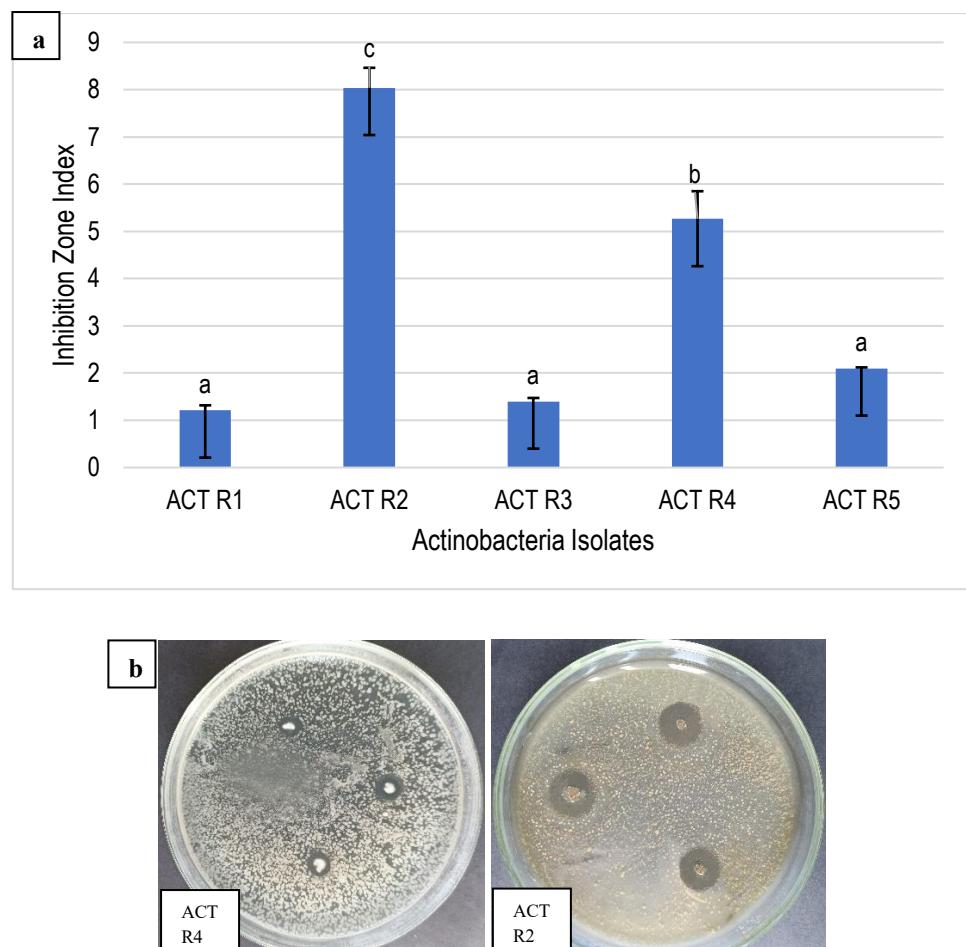


Figure 3. Antagonistic Actinobacteria Isolates against *R. solanacearum* (a: inhibition zone index of Actinobacteria isolates and b: halo zone formed)

In Figure 3, ACT R2 isolate has the highest inhibition zone index with an average of 8.04 and is significantly different from other isolates, marked with the letter notation “c”. ACT R4 isolate also shows moderate antagonistic potential with an average index of 5.26 (notation “b”). Meanwhile, isolates ACT R1, ACT R3, and ACT R5 only produced low inhibition zone indices (1.2–2.1) with the same notation (“a”), so they were not significantly different from each other. In general, isolate ACT R2 can be categorized in the moderate inhibition group, ACT R4 in the moderate category too, while ACT R1, R3, and R5 are classified as weak in inhibiting pathogen growth.

This difference in antagonistic potential can be explained by the ability of each Actinobacteria isolate to produce secondary metabolites such as antibiotics, siderophores, and hydrolytic enzymes. According to [Zaki et al., \(2022\)](#), Actinobacteria, especially those from the genus *Streptomyces*, can produce antibiotic compounds that directly inhibit the growth of *R. solanacearum*. In addition, siderophore production reduces the availability of Fe^{3+} in the environment, thereby inhibiting the growth of pathogenic bacteria that also require this ion for metabolic processes ([Ellermann & Arthur, 2017](#)). Enzymes such as protease, chitinase, and cellulase have also been reported to contribute to the degradation of the pathogen's cell wall ([Remijawa et al., 2020](#)).

These findings are in line with the research by [Zhao et al., \(2019\)](#) which reported that local Actinobacteria isolates were able to produce inhibition zones of more than 7 mm against *R. solanacearum* and were categorized as strong antagonistic agents. Another study by [Khaing et al., \(2021\)](#) also showed that Actinobacteria isolates with high siderophore and IAA production have better biocontrol efficacy against soil pathogens. Thus, the results of this study confirm that ACT R2 is a potential candidate for development as a biocontrol agent for *R. solanacearum*, while ACT R4 is still worth further exploration despite its lower activity.

CONCLUSION

This study successfully isolated five Actinobacteria isolates (ACT R1-R5) from the rhizosphere of Bagek Kembar mangroves, West Lombok, which showed Gram-positive characteristics with a distinctive morphological structure in the form of hyphae-like filaments. Analysis of environmental parameters at the study site showed that the soil pH was relatively neutral, and the temperature and salinity were suitable for supporting the growth of Actinobacteria. The Total Plate Count (3.2×10) calculation results showed that the abundance of Actinobacteria was in the high category, confirming the great potential of these microbes as a local biological resource, but the diversity of species successfully isolated was relatively low. Antagonistic tests against *Ralstonia solanacearum* showed variations in antimicrobial ability among isolates, with ACT R2 and ACT R4 being moderate, and ACT R1, ACT R3, and ACT R5 being weak. Overall, the results of this study confirm that mangrove ecosystems are a source of Actinobacteria with significant biotechnological potential, particularly as environmentally friendly biocontrol agents to support sustainable agriculture.

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