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Short Communication: Isolation of Actinomycetes from mangrove ecosystem in Torosiaje, Gorontalo, Indonesia

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Abstract. *Katili AS, Retnowati Y. 2017. Short Communication: Isolation of Actinomycetes from mangrove ecosystem in Torosiaje, Gorontalo, Indonesia. Biodiversitas 18: 826-833.* Actinomycetes is a group of positive gram microorganism known to produce secondary metabolic compounds that work as antibiotics, anti-fungus, anti-virus, anti-cancer, and other important enzymes for the industry. This research aimed at discovering the potentials of Actinomycetes of the mangrove ecosystem in Torosiaje, Gorontalo, Indonesia. It focused on finding out the diversity of Actinomycetes at mangrove ecosystem in Torosiaje. Hence, various types of Actinomycetes that have potentials to create secondary metabolic compounds/bioactive compounds for industrial purposes can be found. This purpose can only be attained through isolation of actinomycetes. This isolation was conducted through characterization of morphological characteristics of the colony and the spore, molecular characterization through isolation of DNA genome of the actinomycetes, amplification of 16S rRNA through PCR, sequencing, and reconstruction of the phylogenetic tree. We were able to obtain the actinomycetes isolate from the sediment in rhizosphere area of *Ceriops tagal, Bruguiera gymnorrhiza, Xylocarpus* sp, *Rhizophora apiculata*, *Avicennia* sp, and *Sonneratia alba* species of mangrove. Each isolated actinomycetes has specific morphological characteristics. The density of isolated actinomycetes in Torosiaje mangrove area was very low with an average of 1 x 10^5 CFU $gram^{-1}$ in each sediment.

Keywords: Actinomycetes, diversity, mangrove

INTRODUCTION

Mangrove ecosystem is a wetland in the coastal area with intertidal zone on the estuary, delta, sub-river system, lagoon, swamp, mud area especially in the tropical and sub-tropical areas. As one of the natural resources in the coastal area, mangrove forest plays important role in the ecosystem, economy, and the social aspects of life. In addition to its function as home to biodiversity, mangrove ecosystem also serves as genetic pool and supports the overall life cycle of those surrounding the area (Begen 2002). The roots of the mangrove provide nutrients for other shallow marine biota. The roots provide shelter for other biological community such as invertebrate algae, and other microorganisms (Giesen et al. 2006).

The microorganism is one of the crucial components in mangrove ecosystem, considering their role as decomposer and their involvement in biogeochemistry cycle. The mangrove area is influenced by the intertidal. This intertidal, in turn, influences the distribution and the diversity of the microorganism in this area. The extreme environmental condition is a limiting factor for the growth of the microorganism. Therefore, only groups of microorganism that are equipped with highly complex survival mechanism can grow and survive in this area. One of these is the Actinomycetes. Actinomycetes is a sub-group of Gram-positive bacteria group that is equipped with filament and has a high level of *G+C content* in its genetic materials. This organism is widely spread in both aquatic and terrestrial ecosystem, either natural or manmade ecosystem and plays an important role in degrading the organic materials (Naikpatil and Rathod 2011). This organism is known to have the ability to produce secondary metabolite with bioactivities such as; antibiotic, anti-fungus, anti-virus, anti-cancer, enzymes and other various compounds that are used within the industry sector (Suthindhiran and Kannabiran 2010; Mangamuri et al. 2014).

Most of the actinomycetes can be readily found in nature. Several reports on the ecology of actinomycetes revealed that this microorganism is also readily found in extreme environment like those of the mangrove ecosystem. The mangrove forest condition is vastly different from the terrestrial habitat. Thus, the distribution and biological characteristics of actinomycetes in mangrove area are expected to be different from those of the land ecosystem. This study is important not only for the sake of basic research but also for the exploration of the biotechnology of that particular microorganism (Mangamuri et al. 2012). Several types of research have been done to explore the actinomycetes in the mangrove ecosystem. These halophytic actinomycetes are potential to convert the bioactive compounds and enzymes (Sahoo and Dhal 2009; Amrita et al. 2012; Rajamanickam et al. 2014). The usage of molecular technique to find out the microbes' diversity has brought significant progress for the microbial ecology. Hence, it is possible to determine the diversity of natural

microbes especially underground microbes (Adegboye and Babalola 2012).

Exploration of actinomycetes in mangrove ecosystem is not commonly done in Indonesia, especially in Gorontalo Province. The southern coastal mangrove ecosystem of Gorontalo, in the area of Torosiaje village, has never been explored for its microbial diversity and the microbial metabolite. Hence, it is possible to explore and find out new types of actinomycetes from this mangrove ecosystem. Therefore, this research was designed to explore the diversity of actinomycetes of the mangrove ecosystem in Mangrove coastal area in the southern part of Gorontalo, and the ultimate objective of this research is to screen the bioactive compounds. This microbial screening is an important aspect because it serves as the source of secondary metabolite production that has high economic pharmacologic-biological activities. Previous researches have explored actinomycetes in several mangrove areas and have proven that those microbes were able to produce secondary metabolites that serve as anti-bacterial and even as anti-tumor compounds (Arifuzzaman et al. 2010). This research focused on finding out the diversity of actinomycetes from Torosiaje mangrove area. This is important as a data source for exploration of bioactive compounds that are important for the biotechnology; hence, this research is expected to serve as basic data for further researches in the pharmaceutical industry for the development of bioactive compounds production with biologic activities.

MATERIALS AND METHODS

Study area

The samples of sediment were taken from the mangrove forest area in Torosiaje Village, Popayato Subdistrict, Pohuwato District, Gorontalo Province, Indonesia, and the samples then were sent for further examination at the Laboratory of Microbiology, Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Negeri Gorontalo (State University of Gorontalo), Indonesia and the Laboratory of Microbiology, Universitas Gajah Mada, Yogyakarta, Indonesia, as well as the Assessment Institute for Agricultural Technology (BPTP) of Yogyakarta for physicochemical analysis of the sediment.

The sample locations are marked using the GPS coordinate. The lower zone comprises of four plots with the coordinate of each plots are: plot 1: N. 00°.28”.494’; E. 121°.27”.576’; plot 2: N. 00°.28”.505’; E. 121°.27”.568’; plot 3: N. 00°. 28”.4,82; E. 121°.27”.560’; and plot 4: N. 00°.28”.480’; E. 121°.27’.548’. The middle Zone consists of plot 1 N. 00°.28”.476’; E. 121°.27”.574’; plot 2: N. 00°.28”.505’; E. 121°.27”.568’; plot 3: N. 00°. 28”.4,82; E. 121°.27”.560’; and plot 4: N. 00°.28”.482’; E. 121°.27’.581’. Immage of these four plots is shown in Figure 1.

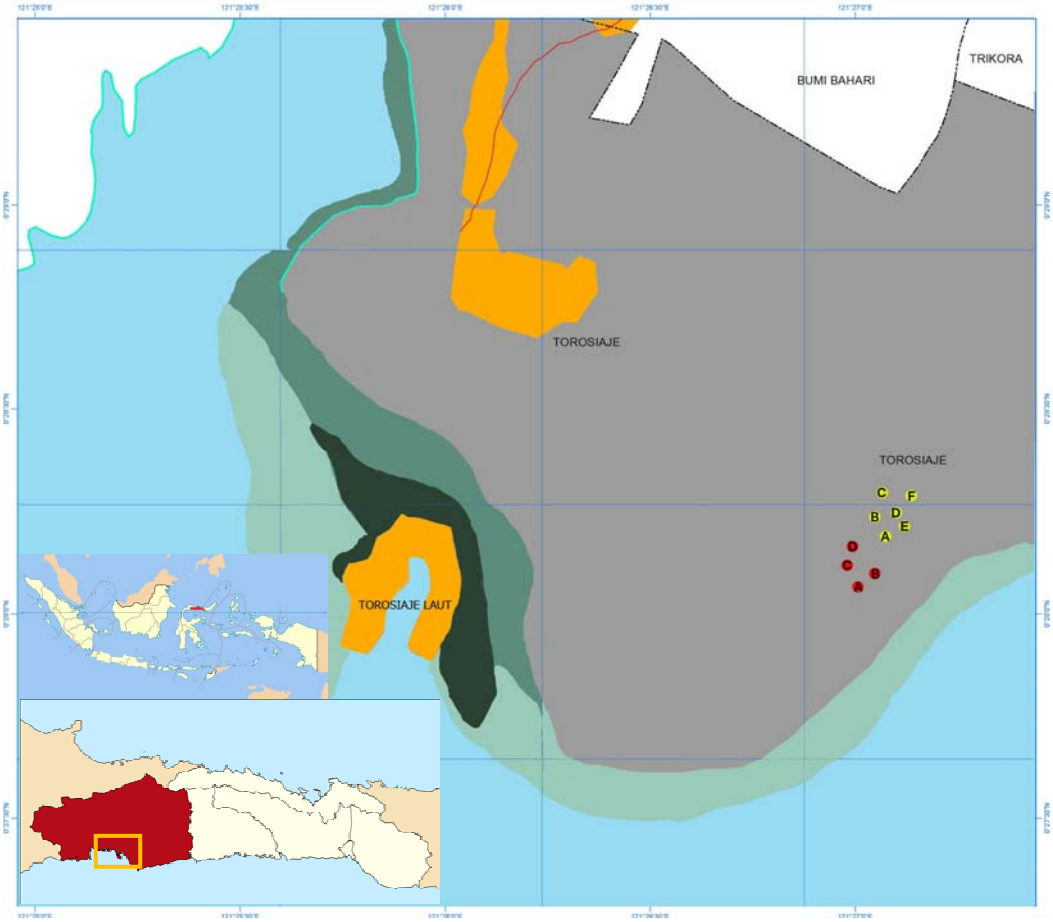

Figure 1. Map of sampling location in mangroves forest of Torosiaje, Pohuwato District, Gorontalo Province, Indonesia

The tools used in this research were: laminar air flow, autoclave, incubator, oven, water bath, analytic scale, incubator shaker, micropipette, light microscope, electron microscope, medium speed centrifuge, high-speed centrifuge, electrophoresis set, thermocycler, vortex, glass tubes, soil tester, Han clinometer, lux meter, thermometer, DO meter. The materials used in this research were: Starch Casein Agar medium, Ringer's solution, yeast malt dextrose agar medium, glass bead, lysozyme enzyme, RNAase, phenol, chloroform, buffer TE, absolute ethanol, ethanol 70%, proteinase K, CTAB, SDS, ethidium bromide, cyclohexamide, and nystatin.

Procedures

The data collected in this research were a diversity of actinomycetes isolated from the samples of mangrove rhizosphere sediment. The data was collected through the following procedures.

A collection of samples from mangrove forest sediment: The sampling techniques were based on the purposive sampling or stratified random sampling methods. The samples were collected from the upper zone area especially from *Xylocarpus sp, Ceriops tagal, Sonneratia alba* and *Avicennia marina* species of mangrove and were collected in five sampling points. The samples of sediments were collected using the modified soil core in the depth of 0-20 cm. Further, the five sampling points of each rhizosphere were then composited in the size of about 500 g. The composite sediment samples were then put into a sterilized sample bag and stored in a cooling box with an ice pack to preserve the sample quality.

Physicochemical analysis of the sediment: The chemical analysis of the soil that consisted of pH and salinity test were conducted directly in the field, and analysis of organic carbon level, nitrogen, phosphor, potassium, Zinc, iron, magnesium, cuprum, and soil texture were conducted in the laboratory.

Total genome analysis of the sediment: Total genome analysis on samples were conducted through isolation of DNA from the samples. Five g of sediment samples were taken and then added with 5 mL of the buffer lysis, 0.8 g of glass beads, 30 µL of proteinase K (20 mg mL^{-1}), 70 µL lysozyme, 750 µL of 10% CTAB, and 1.5 mL of 20% SDS. Then, we vortexed the mixture for about 5 minutes and incubated them for 2 hours in a 65 ^{0}C water bath. The mixture was mildly shaken in every 30 minutes. After that, we centrifuged the mixture in 3000 rpm speed for 20 minutes in 4 ^{0}C temperature room condition. The formed supernatant was then separated from the palate using the pipette and transferred into a 2 mL tube. Chloroform was added to the tube with the ratio of 1: 1 (supernatant: chloroform). The tube was shaken in high speed for 15 minutes, vortexed for a moment, and centrifuged in 12000 rpm for another five minutes. The supernatant was collected in a conical tube and added with absolute ethanol by the ratio of 2:1 of the supernatant. It was then stored overnight in-20 ^{0}C temperature and centrifuged at 12000 rpm for ten minutes in 1.5 mL of Eppendorf tube (the supernatant was disposed of in each centrifugation) or the pellet was taken directly. The DNA pellet was the added with 70% ethanol and centrifuged in 12000 rpm for 10 minutes. The supernatant was then disposed to let the DNA pellet stick on the bottom of the tube. The tube containing DNA pellet was then added with 30 µL of TE stored overnight at 4^{o}C temperature. The DNA quality was the checked through visualization using agarose gel electrophoresis.

Isolation of Actinomycetes: The samples were air-dried in room temperature and pretreated before isolation of the actinomycetes. Ten grams of sample were put into the ringer solution and heated at 55 ^{0}C water bath for 15 minutes. The pretreated soil suspension was then undergone series of dilution process in the dilution level of 10^{-1}-10^{-5}. A 100 µL of suspension was planted with surface plate technique in Starch Casein Agar medium (SCA) g/L: 10 g L^{-1} of starch, 1 g L^{-1} of casein powder, 15 g L^{-1} of agar, 50% of sea water and pH 7.2 ± 0.2 (Baskaran et al 2011) and supplemented with 25 µg mL^{-1} cycloheximide and 25 µg mL^{-1} of nystatin to minimize the growth of other bacteria and fungus. This sample was incubated at the temperature of ± 2 ^{o}C for 28 days. After the growth indicated by a morphologically different colony, this colony was then purified to obtain pure isolate by scratching it into *Yeast extract-malt extract dextrose agar* (ISP 2) medium. The purified colony was then further sub-cultured in an agar slope as a pure stock culture.

Determining the density of actinomycetes: Actinomycetes density in each rhizosphere of mangrove plants was determined by calculating a number of colonies in an isolation medium using the colony counter. The number of colonies was stated in Colony Forming Unit (CFU) and calculated using the following formula: $CFUgr^{-1}$ = number of actinomycetes colony x 1/dilution factor

Characterization of actinomycetes morphology: Morphological characteristics consisted of morphology of the colony (color, shape, structure of the colony edge), characteristic of culture and the aerial mycelium color), and substrate was observed by growing the actinomycetes isolate in *starch casein agar* (SCA) medium, *yeast extract-malt extract agar* (ISP 2), *oatmeal agar* (ISP 3), *Inorganic salt starch agar* (ISP 4), *glycerol asparagine agar* (ISP 5), *Tyrosine agar* (ISP 7) and agar clusters in the temperature of 30 ^{0}C for 14 days. The morphology of the spore was observed using SEM.

Molecular characteristics. Molecular characteristics of the actinomycetes isolate were derived from several stages, i.e. isolation of actinomycetes DNA, amplification of 16S RNA gene using the PCR, and sequencing the CPR product.

RESULTS AND DISCUSSION

Description of the sample sites in Mangrove forest of Torosiaje, Gorontalo

This research was conducted in Torosiaje mangrove forest area with a karst ecosystem. This mangrove forest was not influenced by the river flow. Hence, the fresh water needed for the growth of mangrove trees was supplied by the ground water. The type of mangrove in this

area was basin mangrove forest and overwash mangrove forest that composed of the species such as *Rhizophora mucronata, Rhizophora apiculata, Bruguiera gymnorrhiza, Xylocarpus sp, Avicennia marina, Ceriops tagal*, and *Sonneratia alba*. The survey revealed that the mangrove forest in Torosiaje was still considered natural and intact mangrove forest due to the population that consisted of trees, sapling, and seedlings. The basin mangrove forest as the main site from which the samples were taken was divided into three zone, upper, middle, and lower zone. The mangrove trees found in this location were *Rhizophora apiculata, Bruguiera gymnorrhiza, Xylocarpus, Avicennia marina, Sonneratia alba* and *Ceriops tagal*.

Torosiaje mangrove forest was influenced by the periodic tidal every four hours. The height of the water during high tide was 1.5 meter from the forest bed. This influences the physicochemistry of the forest bed, especially the sediment as the habitat of the actinomycetes, the focus in this research.

Measurement result of physical and chemical properties of the sediment

Middle and lower mangrove forest zone sediments have different physical and chemical conditions than those of the measurement results as shown in Table 1. These measurement results showed quite significant differences in physical and chemical properties of the sediment between two different sample locations. This condition may influence the distribution and diversity of the actinomycetes.

Result of DNA Analysis from the genome of the sediment

The analysis of the DNA genome within the soil sample showed that the sample from this upper zone mangrove plants rhizosphere contained the DNA genome. The isolation of DNA genome was followed by visualization of DNA in DNA agarose gel electrophoresis as shown in Figure 2. This figure indicates that based on the formed band position in the visualization using agarose gel electrophoresis, the four bands are in the same range position. The four sediment samples show the similarity of microbes population that inhabit the mangrove plants rhizosphere.

Density of Actinomycetes in rhizosphere sediment

Actinomycetes density in lower and middle zones of Torosiaje mangrove forest rhizosphere can be determined through stages of actinomycetes isolation. Actinomycetes isolation was conducted through Starch Casein Agar (SCA) medium in the temperature of 30 ^{0}C for 30 days to obtain several isolates, which are a combination of non-actinomycetes and actinomycetes bacteria. The growth of non-actinomycetes and actinomycetes bacteria in SCA is shown through different morphological characters as presented in Figure 3.

Non-actinomycetes and actinomycetes bacteria were grown in SCA medium after 30 days incubation at the temperature of 30 ^{0}C showed different colony morphology. The growing colony was then calculated to determine the density of the bacteria (Table 2). It is revealed in Table 2 that the population density of actinomycetes isolated from the sample sediment of mangrove plants rhizosphere is lower than that of the non-actinomycetes bacteria. This is, presumably, due to the environmental factor that was not favorable to the growth of general actinomycetes, hence, only several types of actinomycetes that are tolerance to the environment condition of mangrove ecosystem can grow here.

Table 2 shows five isolates of actinomycetes that were isolated from the rhizosphere of mangrove plants from the species of *Xylocarpus* sp, *Bruguiera gymnorrhiza* and *Ceriops tagal*. This indicates specific correlation among actinomycetes and certain types of mangrove plants.

Table 1. Analysis of physical and chemical properties of the sediment from the middle and the upper-level zones of mangrove forest

Parameter	Middle zone	Lower zone
Ph	5.33	5.76
Salinity	18.57	19.21
Texture:		
Sand	62.00	84.00
Dust	31.00	10.00
Clay	7.00	6.00
C-Organic	6.24	1.43
N-Total	0.25	0.06
Sulfur (S)	0.38	0.20
Nitrate (NO3)	0.07	0.01
Iron (Fe)	58.00	53.00
Mn	9.00	3.00
Zinc (Zn)	2.00	0.30
Coal (Cu)	200.00	1.00

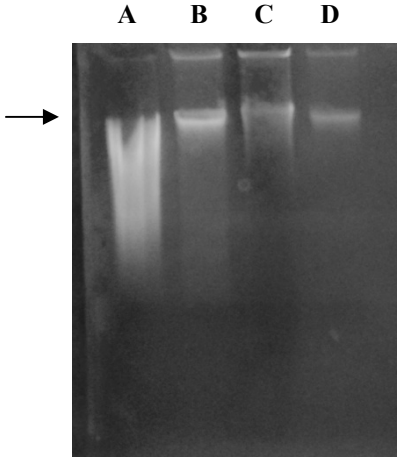

Figure 2. DNA Genome Visualization of sample sediments from the mangrove plants rhizosphere in Torosiaje, Gorontalo. The four sediment samples from all zones show the similarity of microbes population that inhabit the mangrove plants rhizosphere. A. The sediments sample 1; B. The sediments sample 2; C. The sediments sample 3; D. The sediments sample 4

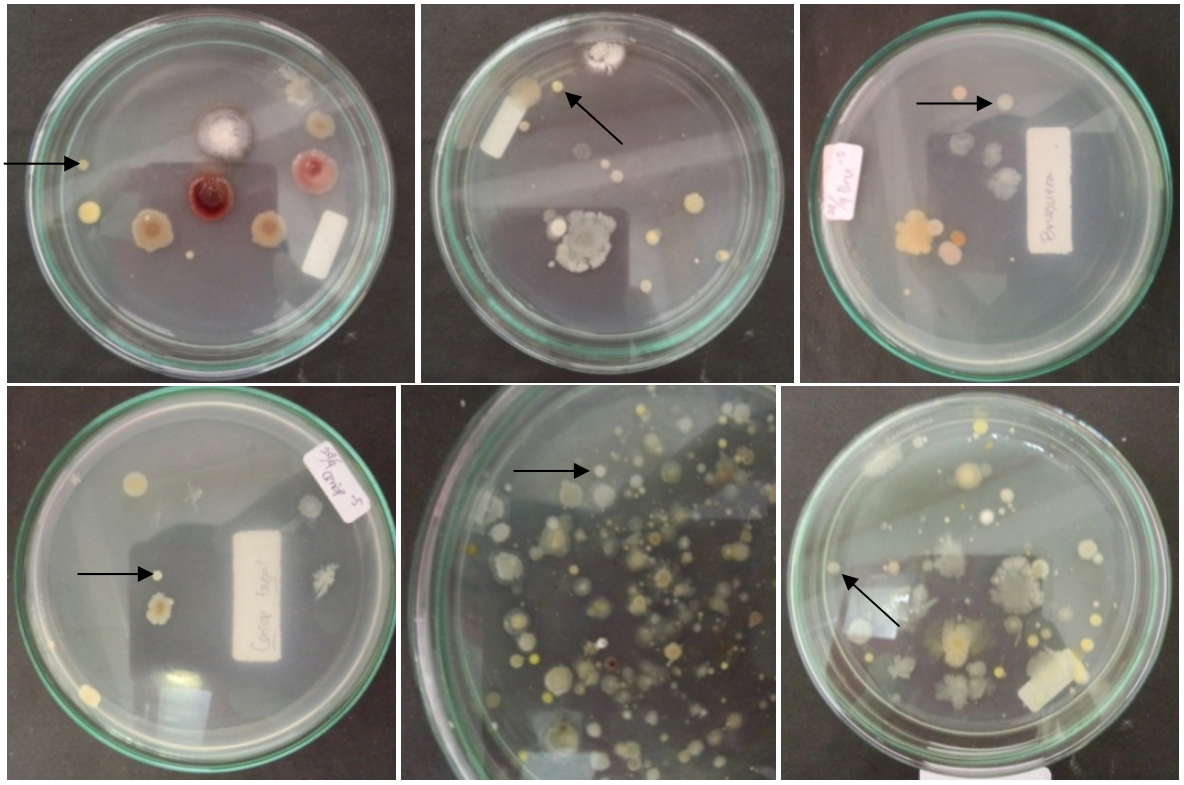

Figure 3. Development of non-actinomycetes and actinomycetes bacteria in SCA medium after 30 days incubation in 30^0C temperature. The arrow points the suspected actinomycetes isolates

Table 2. Number of bacteria and Actinomycetes that grow in SCA medium after 30 days of incubation

Sample origin	Number of total bacteria (cfu g^{-1})	Number of actinomycetes (cfu g^{-1})
Rhizosphere *Sonneratia alba*	25 x 10^3	0
Rhizosphere *Avicennia marina*	155 x 10^3	0
Rhizosphere *Xylocarpus* sp.	7.5 x 10^5	2 x 10^5
Rhizosphere *Ceriops tagal*	11 x 10^5	2 x 10^5
Rhizosphere *Bruguiera gymnorrhiza*	12 x 10^5	1 x 10^5
Rhizosphere *Rhizophora apiculata*	30 x 10^3	0

Morphological characteristics of actinomycetes isolates

Morphological characteristics of Pureactinomycetes isolate were observed from the color of their aerial mycelium color, substrate mycelium grown on several different media such as SCA, SCA, ISP 3, ISP 4, and Cluster's Agar. In addition, the observation was also conducted on the pattern on spores' ornamentation. The morphological characteristics of these actinomycetes in several media are shown in Table 3.

Actinomycetes have the ability to produce spores in their growth. The morphological characteristics of spores ornamentation is one of the important characters in revealing the identity of actinomycetes isolates. Observation result of the actinomycetes isolates using the SEM is shown in Figure 4.

The figure 4 show that the result of characterization based on color grouping on ISP 3 medium and spore morphology indicates that isolated actinomycetes isolated from the rhizosphere of some mangrove species in the mangrove ecosystem of Torosiaje show character as a member of the genus Streptomyces.

Molecular characterization of Actinomycetes

Pure actinomycetes isolate from the mangrove plants rhizosphere sediments in Torosiaje are was molecularly characterized. The initial stage of this molecular characterization was the isolation of actinomycetes genomic DNA which then followed by DNA visualization in 1% agarose gel electrophoresis as shown in Figure 4.

Figure 5 indicates that isolation of DNA has succeeded to obtain the genomic DNA, which is shown by the appearance of bands of DNA genome. These genomic DNA were then amplified using the 16S rRNA genes through PCR technique. The amplification result was visualized by agarose gel electrophoresis as shown in Figure 6. PCR amplification of 16S rRNA gene of isolates B1, C1, and C2 in Figure 15 shows that the amplicon has nucleotide length of more or less than 1500 bp.

Rhizosphere sediment is a habitat that supports the growth of microbes due to the aerobic condition provided

by the root system. In addition, exudate production of root cells also provides the nutrition needed for the microbes to grow. This is evident in the analysis of genomic DNA from the sample of mangrove plants rhizosphere sediments in Torosiaje, Gorontalo. DNA visualization using agarose gel electrophoresis shows the genomic DNA ribbons isolated from the sediments of mangrove plants rhizosphere. This indicates the presence of microbial community within the root system of mangrove plants. Isolation of genomic DNA directly from the sediment sample is one of the methods employed to study the community and diversity of the soil microbes. This method is classified as a unculturable method that does not need laboratory isolation of the microbes. The unculturable method is very effective because it can properly uncover the microbe community, either microbe groups that can or cannot be cultured in the laboratory. This will further become the basis for microbial isolation, especially actinomycetes (Bahnamiry et al. 2013).

Table 3. Culture characteristics of actinomycetes isolate on several growing medium.

No	Medium	Actinomycetes isolate				
		B-1	**C-1**	**C-2**	**X-1**	**X-2**
1.	Starch casein agar					
	Growth	Good	Good	Good	Good	Good
	Aerial mycelium	White	White	White	Gray	White
	Substrate mycelium	Brownish	White	Brownish	Black	Brownish
	Colony edge	Fibrous	Smooth	Smooth	Smooth	Smooth
2.	Cluster's agar					
	Growth	Less good	Not good	Less good	Good	Not good
	Aerial mycelium aerial	White	-	White	Gray	-
	Substrate mycelium	Brownish	-	Brownish	Black	-
3.	ISP4					
	Growth	Less good	Good	Good	Good	Good
	Aerial mycelium	White	White	White	Gray	White
	Substrate mycelium	Brownish	Brownish	Brownish	Black	Brownish
4.	Oatmeal Agar (ISP3)					
	Growth	Good	Less good	Good	Good	Good
	Aerial mycelium aerial	White	White	White	Gray	White
	Substrate mycelium	Brownish	White	Brownish	Black	Creamy White

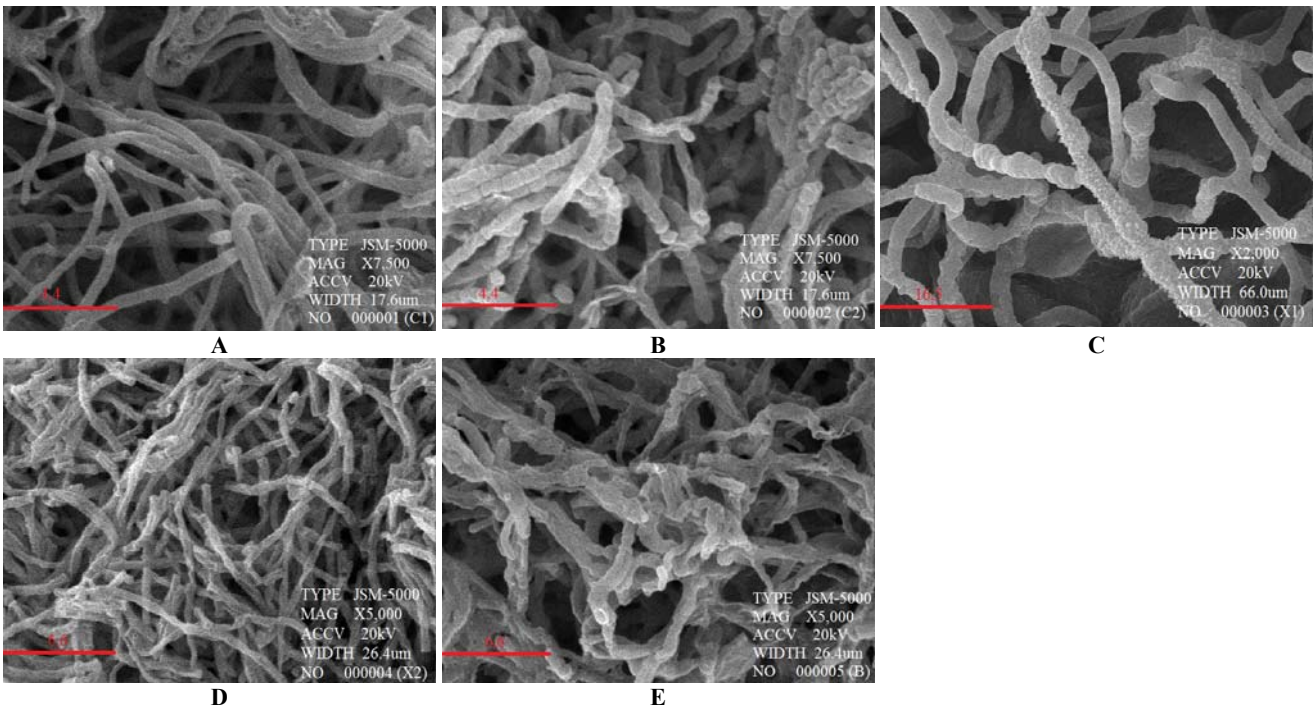

Figure 4. SEM spore morphology of Actinomycetes isolate rhizosphere mangrove plant. A. Isolate C1; B. Isolates C2; C. Isolate X1; D. Isolate X2; E. Isolate B.

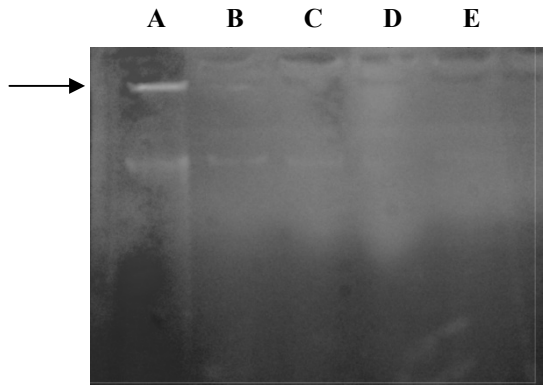

Figure 5. DNA profile of actinomycetes genome from the sediments of mangrove plants in Torosiaje, Gorontalo. A. Isolate B-1; B. Isolate C-1; C. Isolate C-2; D. Isolate X-1; E. isolate X-2. The arrow is pointing the position of DNA ribbon of actinomycetes genome

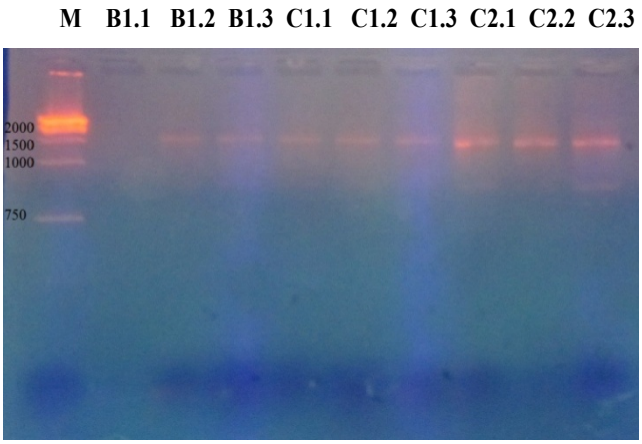

Figure 6. Profile of 16S rRNA gene from PCR in electrophoresis agarose gel with ethidium bromide as a fluorescent tag. M. Marker,; B1-1 to B1-3. Isolate B-1; C1-1 to C1-3. Isolate C-1; C2-1 to C2-3. isolate C-2; The Arrow is pointing the position of DNA ribbon of actinomycetes genome

The mangrove forest condition in Torosiaje Gorontalo is one of the key factors that influence actinomycetes' diversity. The availability of free fresh water from the river environment is one of the opportunities for the actinomycetes that grow in this area become an indigenous type. Actinomycetes types found in the mangrove area of Torosiaje is the one that can endure the extreme environment, especially high salinity. Nevertheless, the environmental factor is also one of the factors that limit the diversity of the actinomycetes in this area. This is evident of the number and types of actinomycetes obtained from this area that is less than those of non-actinomycetes bacteria. In addition, the physicochemical condition of the sediments, especially in this lower and middle zone of the fringe type mangrove forest largely determines actinomycetes diversity. This is proven by the findings of varying actinomycetes found in these two zones. In the middle zone where mangrove types are more varied, more types and number of actinomycetes were also found as compared to those in the lower zone. Four types of actinomycetes isolates were found in the sediment of mangrove plants rhizosphere in the middle zone and only one type isolate was found in the lower zone. This is probably due to the soil texture property. The more sandy soil texture of the middle zone, as compared to that of the lower zone, might have caused the sediment in this zone to be more porous. This porous soil texture provides a more oxygenic condition than the clay soil texture type of the sediment in the lower zone.

Fringe type vegetation conditions of the mangrove forest in Torosiaje were varied between the two zones. The lower zone was dominated by the *Rhizophora apiculata* and some *Bruguiera gymnorrhiza*. Whereas, in the middle zone, several mangroves types grew evenly such as *Xylocarpus sp, Avicennia marina, Sonneratia alba* and *Ceriops tagal* types were found. This varying vegetation condition plays an important role in determining the diversity of actinomycetes. In the present study, we found four types of actinomycetes isolates in the rhizosphere of mangrove sediments in the middle zone, two types in *Ceriops tagal* rhizosphere and two types in *Xylocarpus sp* rhizosphere. On the other hand, there was no actinomycete found in the rhizospheres of *Avicennia marina* and *Sonneratia alba.* In the lower zone, actinomycetes were only found in the rhizosphere of *Bruguiera gymnorrhiza,* and none was found in *Rhizophora apiculata*'s rhizosphere. These findings proved that the types of mangrove species determine the distribution, amount, and diversity of actinomycetes. The existence of mangrove vegetation in an ecosystem plays an important role in providing organic materials into the environment. The litter produced by mangrove vegetation is the main source of nutrient to nurture the microbial growth. Actinomycetes are one of the decomposers that play a role in decomposing complex organic materials into simple organic materials. Nevertheless, a microbe's ability in decomposing waste is also influenced by chemical compounds in that waste. The high level of tannin in a waste weakens the composition ability of a microbe. In other words, only certain types of microbes that are able to decompose tannin are able to carry out the decomposition process. This condition is likely to be a factor that influences the diversity of actinomycetes in mangrove plants' rhizosphere. This is evident in the isolation of actinomycetes from *Rhizophora apiculata's* rhizosphere. This type of mangrove is known to have a high level of tannin; hence, it is possible that there were no actinomycetes found in the rhizosphere of *Rhizophora apiculata*.

The root system or rhizosphere is a suitable environment for microbes to grow. Microbe population in the rhizosphere is far beyond the non-rhizosphere areas. The cell ability in the secretion of organic material exudates as important nutrients for microbes. Nevertheless, there is a specific correlation between exudate produced by the roots and the types of microbes in the rhizosphere. It influences the distribution, abundance, and diversity of

microbes, including actinomycetes. Therefore, this research shows that rhizosphere of certain mangrove plants contains actinomycetes with specific characters. Two actinomycetes isolate found in the rhizosphere of *Xylocarpus* sp. showed characters that were different from two actinomycetes isolates found in *Ceriops tagal*'s rhizosphere and one isolate found in *Bruguiera gymnorrhiza*'s rhizosphere. Characterization is a crucial part in revealing the identity of an organism, including unveiling the identity of actinomycetes isolate found in the rhizosphere of mangrove plants. The characters are basically composed of morphological, physiological, and biochemical characters. The characterization of the culture of actinomycetes isolates shows different growing capacity of each isolate in several media. In addition, the aerial and substrate mycelia produced by each isolate are also important characters. Microscopic observation using the SEM electron microscope showed that all five isolates had different morphology. Based on these data, it is suspected that the actinomycetes isolates found were of different types of isolates.

Advancement of molecular biology has made a tremendous breakthrough in unveiling the identity of certain microbe isolate based on the molecular characteristics of gene marker 16S rRNA. This activity was preceded by isolation of genomic DNA of actinomycetes. The isolated DNA was then qualitatively analyzed using agarose gel electrophoresis. In this research, we were able to find DNA ribbons, the genomic DNA of actinomycetes isolates. This isolated DNA was then subjected to PCR amplification of 16S rRNA gene region using RC17 and FC1492 universal primers with PCR amplicons of 1500 bp, which corresponded to the length of 16S rRNA gene. This result implies that the amplification process using the PCR successfully amplified the 16S rRNA gene.

To summarize, It can be concluded from the findings and the discussion of this research that the mangrove forest in Torosiaje has actinomycetes as its natural resources potentials stored within the sediment of mangrove plants' rhizosphere. There were five actinomycetes isolates isolated in this research with an average density of 1 x 10^5CFU g^{-1}. Of those five isolates, one isolate was found in *Bruguera gymnorrhiza'*s rhizosphere, two isolates were found in *Ceriops tagal's* rhizosphere, and two isolates were found in the rhizosphere of *Xylocarpus* sp. Each actinomycetes isolate showed unique morphological characteristics of aerial mycelium and mycelium substrate and different growth ability in the Starch Casein Agar, Oatmeal agar, Inorganic Salt Starch Agar and Cluster Agar media.

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