Isolation and Identification of Bacteria and Actinomycetes Isolated from Wilting Banana Plants (*Musa* Sp.)

IOP Conf. Series: Materials Science and Engineering **532** (2019) 012028 doi:10.1088/1757-899X/532/1/012028

Isolation and Identification of Bacteria and Actinomycetes Isolated from Wilting Banana Plants (*Musa* Sp.)

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Abstract. In Indonesia, wilting disease in banana due to *Fusarium* has spread in several areas. In addition, fungi, bacteria, and actinomycetes are also known to cause the disease. In this research, isolation and identification of bacteria and actinomysetes causing the wilting of rot and rhizosphere were done. Identification used several stages of biochemical tests. There were 40 bacterial isolates with 9 generas, which were *Pseudomonas* sp., *Xanthomonas* sp., *Caulobacter* sp., *Erwinia* sp., *Eschericia* sp., *Ralstonia* sp., *Neisseria* sp., *Staphylococcus* sp., and *Proteus* sp. Whilst 18 isolates of actinomycetes were obtained with 7 generas, including *Nocardia* sp., *Actinobiospora* sp., *Nocardiopsis* sp., *Streptomyces* sp., *Streptoverticillium* sp., *Streptosporangium* sp., and *Microbispora* sp.

Keywords: actinomycetes, bacteria, banana, isolation, wilt

1. Introduction

Banana (*Musa paradisiaca*) in Indonesia is widely used in everyday life as food. All of the banana plant can be utilized so that the availability of healthy banana plants is needed in the community. However, this is constrained because many banana plants are affected with the disease. The famous disease is the wilt caused by pathogen microbes. In Indonesia, *Fusarium* wilt disease has spread in several areas such as Jawa Barat, Jawa Timur, Sumatera Utara, and Riau (Nasir and Jumjunidang, 2003). Poerwanto (2013) proved from 2009-2012 the amount of banana production was decreased 24% due to the disease. This is affected to export the banana from Indonesia. The wilted on the banana is caused by microbes that takes nutrients from it. Previously, Saryono *et al*. (2018) have isolated fungi caused wilting banana plants and succesed to get 4 genera of fungi, including *Trichoderma*, *Fusarium*, *Aspergillus*, and *Penicillium*.

Microbes was attacked banana plants from out to inside of cells on the stump. This disease can also spread to other healthy or injured plants through rhizosphere soil or tools. Besides of the fungus, bacterial caused wilt on banana plants and rhizosphere soil identified as *Proteus* sp., *Erwinia* sp., *Klebsiella* sp., *Staphylococcus* sp., *Corynebacterium* sp., *Bacillus* sp., and *Cellulomonas* sp. [9, 15]. The pathogen bacterial

abilities in producing some hydrolytic enzymes such as cellulase, amylase, protease, and inulinase on cells of plants, which would be of great potential when applied to the biotechnology industry.

Actinomycetes are also found from rhizosphere soil of culture plant that can potentially produce antibiotics by inhibiting the growth of gram-positive bacterial [2]. Sudarma (2010) proved actinomycetes from the rhizosphere soil of wilting banana abilities to produced antibiotics. Actinomycetes produced bioactive compounds highly such as antivirus and anticancer, while in agriculture as herbicides, insecticides, and antiparasitic compounds [19]. Some bioactive compounds namely streptomycin, neomycin, chloramphenicol, and tetracycline could be produced from *Streptomyces* [20]. Therefore, isolation and identification of bacterial and actinomycetes from rhizosphere soil and stump of wilting banana in Panam, Pekanbaru. The results of isolated were then test using the biochemical test.

2. Material and methods

Samples was collected in jalan Kartama, Panam, Pekanbaru by purposive sampling method. Stump of banana plants samples showing the presence of disease were cut into small pieces. The surface of stump washed using flowed water, sterilized by soaking them in 70% ethanol for 3 minutes. And then, the stump were soaked in 0.2% $HgCl_2$ solution for few minutes. Samples were subsequently soaked back in 70% ethanol for 1 minute and rinsed with demineralized water twice. The stump were cut into smallest in blender into powder. Samples was added into 9 ml of 0.8% NaCl in test tube to dilution till 10^{-2}. Samples soil from rizhosphere wilting banana was taken from the plants grew. The samples taken were weighed for 1 g and put into a test tube containing 9 ml of 0.8% NaCl (10^{-1}), homogenized using a vortex, then repeated until 10^{-2} dilution. All dilution were micropippetted and inoculated into a petri dish containing NA and SCA with a spread method. Samples were incubated at 37°C for 2 days and 10 days, respectively. The growing colonies were separated based on different colonies, colors, and the shape of colonies. The single colony of actinomycetes and bacterial were subcultured on other NA and SCA media. And then, the isolates continued to be put under macroscopic and microscopic identification.

2.1. Morphological characterization of bacterial and actinomycetes

Characterization of growth colonies was such as: pinpoint/punctiform (point), small, moderate, large; shape: circular, irregular, spindle, filamentous, and rhizoid; elevation: flat, raised, convex, and umbonate. The growth of isolates were observed such as philliform, echinulate, beaded, arborescent, plumose, papilliate, and villose in straight NA and SCA media. The patterns of colony isolates were observed such as philliform, echinulate, beaded, effuse, spreading, plumose, and rhizoid in slant NA and SCA medium. Observations were also made on NB and SCB media to observe the growth of isolates based on O_2 requirements, with anaerobic facultative patterns (uniform turbidity and flocculent growth), aerobic (pellicle), microaerophilic (ring), and anaerob (sediment). Otherside, bacterial and actinomycetes isolates were also identified by gram-stain test for microscopic identification.

For acid-resistant coloration test, actinomycetes and bacterial isolates were added on glass preparations, spilled with carbolfuchsin and heated for 5 minutes. The preparate was washed and soaked in 96% alcohol for 15 minutes, then rinsed with flowing water. After that, the preparate was soaked with methylene blue for 2 minutes and rinsed back with flowing water. The observation was taken under a microscope. Positive test showed by red cells and negative test showed by blue or colorless.

2.2. Biochemical Test

2.2.1. Catalase test

The isolates was put into preparate test. The 3% H_2O_2 was dropped on the isolate. Positive test showed if gas formed on the preparate.

2.2.2. Starch hydrolysis test

The media was prepared by mixture 10 g soluble starch, beef extract 3 g, and 1 L demineralized water. The media was sterilized and put into a petri dish. The isolates were streaked into the media with a diffusion method [7] and incubated at 37°C for oberve the clear zone after being added with iodine solution.

2.2.3. Carbohydrate fermentation test

Phenol-red lactose/glucose/sucrose broth medium was consisted of lactose/glucose/sucrose each 10 g, trypton 10 g, NaCl 5 g, and phenol red 0.0180 g. The mixture was itaken put into test tube and durham tube was adjust carefully for observe gas formed during incubation at 37°C. The color change into yellow showed a positive fermentation reaction by producing acid. The test was purposed for observe the ability of bacteria in fermentation carbohydrate.

2.2.4. Casein hydrolysis test

Skim milk was weighed 28 g and put into demineralized water 1 L. The isolates were inoculated into the media with a diffusion method [7] during the incubation at 37°C. If a clear zone was formed then the test was positive.

2.2.5. Gelatin hydrolysis test

Gelatin media were made with the following composition of 15 g gelatin, 15 g agar, 4 g pepton, 1 g yeast extract in 1 L of demineralized water. The patterns of growth were observed, such as filiform, echinulate, beaded, arborescent, plumose, papilliate, and villose. The gelatin hydrolysis test was observed by incubating the media contained isolate into the freezer for 1 h. The test was positive if the media melted.

2.2.6. Methylene blue reduction test

The isolates was inoculated into NB media were added with 5 mL of 10% methylene blue solution. The homogenized mixture was stored for 60 min at 37°C and then observed the changes blue of color media. The change from blue into clear indicated that the isolates were abilities of reducing methylene blue [7].

2.2.7. Bromocresol Purple Milk Solids Glucose Agar (BCPM) Hydrolysis Test

The isolates were grown on media agar composed of mix solution A (1000 mL demineralized water, 80 g skim milk powder, 2% bromcresol purple (1.6% dissolved in 70% alcohol) mixed in Erlenmeyer and sterilized in autoclave), Solution B (40 g glucose and 200 ml demineralized water sterilized in autoclave), and solution C (30 g agar and 800 mL demineralized water, sterilized in autoclave). The isolates was inoculated with a diffusion method [7] during the incubation at 37°C. If the medium turned purple, it indicated the isolate hydrolyzed the skim milk, but if the medium color changed into yellow, it indicated the isolate hydrolyzed glucose.

3. Results and Discussion

In total isolates obtained were 40, which 20 isolates were isolated from the soil and 20 other isolates from the stump. Otherside, total isolates of actinomycetes obtained were 18, each 9 isolates from the soil and 9 other isolates from the stump. Bacterial isolates contained gram-positive and negative cells, however the cells of actinomycetes isolates were gram-positive. To determine the genera of bacterial and actinomycetes isolates, some of biochemical tests were performed, and matched on Bergeys manual book. There are results of morphology test and some of biochemical tests from one genus of bacterial and one genus of actinomycetes (Figure 1 and Figure 2).

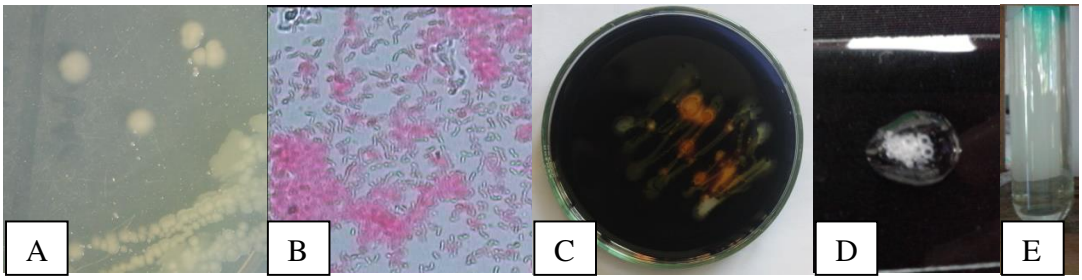

Figure 1. Genus *Pseudomonas* sp. (A) *Pseudomonas* colonies, (B) cell form (40x), (C) starch hydrolysis test, (D) catalase test, and (E) methylene blue reduction test .

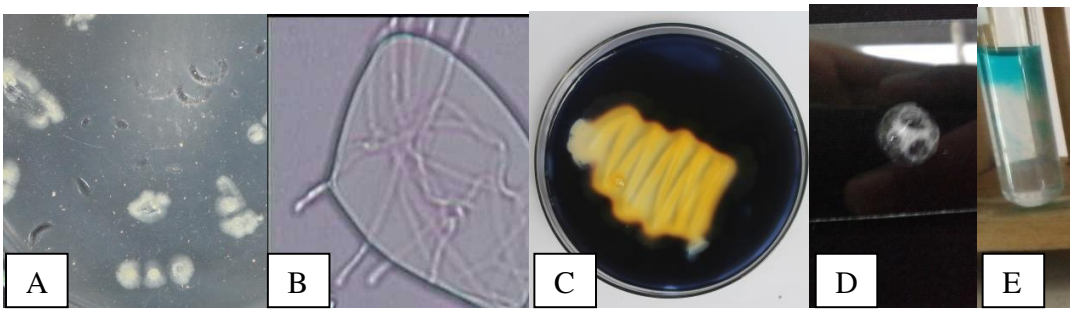

Figure 2. Genus *Streptomyces* sp. (A) *Streptomyces* colonies, (B) cell form (40x), (C) starch hydrolysis test, (D) catalase test, and (E) methylene blue reduction test.

Based on the results of morphological and biochemical tests, it could be determined the genera of isolates. All actinomycetes isolates were positive in gram-stain tests. Observations of bacterial and actinomycetes isolates can be seen in the following of tables.

Table 1. Morphological characterization of actinomycetes isolates

No.	Size	Morfology	Models	Elevation	Color of colony	Gram	Cell form	Genus (sp.)
1	Small	Rhizoid	Undulate	Raised	Brown	Positive	Filamentous	*Nocardia*
2	Small	Circular	Entire	Convex	Brown	Positive	Filamentous	*Actinobiospora*
3	Moderate	Rhizoid	Serrate	Umbonate	Brown	Positive	Filamentous	*Nocardiopsis*
4	Moderate	Circular	Entire	Convex	Beige	Positive	Filamentous	*Streptomyces*
5	Small	Circular	Undulate	umbonate	Beige	Positive	Filamentous	*Streptoverticillium*
6	Small	Rhizoid	Entire	Convex	Brown	Positive	Filamentous	*Streptosporangium*
7	Moderate	Circular	Undulate	Raised	Beige	Positive	Filamentous	*Microbispora*

Actinomysetes were indicated as rod-shaped, gram-positive, anaerobic or facultative aerobic. The actinomycetes structure was consisted of soft filaments often called hypha or mycelia, as found in the fungus, and had conidia [29]. Actinomycetes a group of gram-positive bacteria and is widely distributed in the soil. Actinomycetes could be divided into two groups, namely *Streptomyces* and *rare-actinomycetes. Rare-actinomycetes* used to refer to a genus other than *Streptomyses*. This group growth was slower and

relatively difficult with *Streptomyses* group. As in the results of this study, actinomycetes isolates have difficult form when grown in SCA media.

Other *Streptomyses*, there are genera namely *Nocardia*, *Micromonospora*, *Microbispora*, and *Streptosporangium* [17]. *Streptomyses* sp. in antagonistic test was unable against *Fusarium oxyporum* with highest value 82,59% [23]. Sunaryanto *et al*. (2010) isolated actinomycetes with genera *Streptomyces* and *Nocardia* from sediments. Actinomycetes in sediments were spreaded highly than in population in soil, while bacterial populations were more dominant in sediments. Sulistiyani and Nunuk (2011) revealed that *Staphylococcus* and *Streptococcus* were released antibiotic-resistant pathogens, and also produced antibiotics to inhibiting the growth of *S. aureus* and *C. albicans*. Based on previous research, it can be stated that the actinomycetes from wilting banana plants were not entirely a pathogen.

Actinomycetes has many varians of morphology, from the form of the circular/coccus (*Micrococcus*) and the rod-coccus (*Arthrobacter*), hyperfragment (*Nocardia*, *Rothia*), to the species with different branching mycelium (*Micromonospora* and *Streptomyces*). Other examples, *Actinobacteria*, *Actinoplanetes*, *Nocardioforms*, and *Streptomyces* were difficult phylogenics. Actinomysetes be able to saprophytes, but other of genus also parasite or symbiotic mutualism in plants and animals. Firstly, actinomysetes was classified in the fungus group, because the appearance of morphology and its development were similar to the fungus. However, with the advanced science, actinomisetes morphology was known to be closer to bacteria. From the size of the cell, its spores and miscellaneous, actinomisetes were categorized as bacteria that had the same characteristics as bacteria. Chitin and cellulose as constituents of fungi cell walls were not present in actinomycetes. Actinomycetes cell wall composed sugar polymers, amin-sugars, and some amino acids such as in cell of gram-positive bacteria. Actinomycetes was usually as a group of gram-positive bacteria that contained high levels of Guanin (G) and Citosin (C) in their DNA (> 55%) with the ability formed hypha branches during their optimized stages (Budiyanto and Farhan, 2012) . The color of the actinomycetes colonies were varied, because of the difference in compound of pigment in the cell, corresponding to each type of actinomycetes [26].

Table 2. Morphological characterization of bacterial isolates

No.	Size	Morfology	Models	Elevation	Color of colony	Gram	Cell form	Genus (sp.)
1	Small	Circular	Entire	Convex	Yellow	Negative	Bacil	*Pseudomonas*
2	Small	Circular	Entire	Convex	Yellow	Negative	Bacil	*Xanthomonas*
3	Moderate	Circular	Entire	Flat	Beige	Negative	Bacil	*Caulobacter*
4	Moderate	Circular	Entire	Convex	White-milk	Negative	Bacil	*Erwinia*
5	Small	Circular	Lobate	Flat	White	Negative	Bacil	*Eschericia*
6	Small	Circular	Entire	Convex	Yellow	Negative	Bacil	*Ralstonia*
7	Moderate	Circular	Entire	Convex	Yellow	Negative	Bacil	*Neisseria*
8	Moderate	Irregular	Lobate	Flat	White	Negative	Coccus	*Staphylococcus*
9	Small	Circular	Lobate	Flat	White	Negative	Bacil	*Proteus*

In this research, 9 genera of bacteria were obtained namely *Pseudomonas* sp., *Xanthomonas* sp., *Caulobacter* sp., *Erwinia* sp., *Eschericia* sp., *Ralstonia* sp., *Neisseria* sp., *Staphylococcus* sp., and *Proteus* sp. In addition, 7 genera actinomycetes were obtained namely *Nocardia* sp., *Actinobiospora* sp., *Nocardiopsi*s sp., *Streptomyces* sp., S*treptoverticillium* sp., *Streptosporangium* sp., *Microbispora* sp. Msogoya *et al*. (2012) and Habiba *et al*. (2002) also isolated *Proteus* sp., *Erwinia* sp*., Klebsiella* sp., *Staphylococcus* sp., *Corynebacterium* sp., *Bacillu*s sp., and *Cellulomona*s sp. from banana plants and its rhizosphere soil. Winarni (2013) successed to isolate *Pseudomonas* sp. from seeds of rice and soybean, *Pseudomonas* and *Xanthomonas* were pathogens in plants. *Pseudomonas* are cream-yellowish and circular.

Proteus is a group of Enterobacteriaceae which normal flora in humans and animals, also be found in the environment (Arisandi, 2016). *Ralstonia* sp. caused blood disease in banana plants [8]. On the other hand, Zhou *et al*. (2012) proved *Pseudomonas* as antagonistic bacteria against *Ralstonia*. Abidin *et al.*, (2015) proved a *Pseudomonas* sp. as antagonist against *Sclerotium rolfsii* Sacc. *Pseudomonas* sp. can released antibiotic, siderophores, and other secondary metabolites that inhibited fungal activity. In addition, according to Herdyastuti *et al*. (2010), *Pseudomonas* also produced chitinase highly.

Banana plants-wilting bacteria are capable of producing a variety of extracellular enzymes with different functions, including to degraded plant cell wall components and became to pathogens. Another virulent factor was acidic extrapolysaccharides from bacterial pathogen with high molecular weight caused blocked of the xylem and inhibited its function [8]. Susanna (2006) isolated bacteria from the rhizosphere of banana plants, which could be as antagonistic to *Fusarium oxysporum* f.sp. cubense. Based on this, the probability of isolates obtained from this study was not entirely of a pathogenic. According to Djatnika and Wakiah (1995), *Pseudomonas fluorescens* reduced the number of wilting disease by 85.7% in tissue culture of banana plants. Hedges and Messens (1990) stated that *Pseudomonas fluorescens* produced pyoverdine, pyrrolnitrin, and pyoluterin in inhibiting pathogens.

4. Conclusions

We have isolated actinomycetes and bacterial from wilting banana plants with 7 genera of actinomycetes and 9 genera of bacterial. Genera of actinomycetes are following: *Nocardia* sp., *Actinobiospora* sp., *Nocardiopsis* sp., *Streptomyces* sp., *Streptoverticillium* sp., *Streptosporangium* sp., and *Microbispora* sp. In the other hand, genera of bacterial are following: *Pseudomonas* sp., *Xanthomonas* sp., *Caulobacter* sp., *Erwinia* sp., *Eschericia* sp., *Ralstonia* sp., *Neisseria* sp., *Staphylococcus* sp., and *Proteus* sp.

Acknowledgement
This research was funded by Professor Grant of Universitas Riau on behalf of Prof. Dr. Saryono, M.Si.

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