

ISOLATION AND CHARACTERIZATION OF ACTINOMYCETES FROM PINEAPPLE PLANTATION POTENTIAL AS ANTAGONIST OF *DICKEYA* SP., THE PINEAPPLE SOFT ROT PATHOGEN

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Abstract: This study was aimed to isolate and characterize actinomycetes having antagonist activity to *Dickeya sp.*, the pathogen of pineapple soft rot. Soil samples were collected from four different pineapple plantation in Lampung. Samples were air-dried, serially diluted, and plated on actinomycetes isolation agar media; and potential colonies were selected and purified in malt agar medium. Isolates were grouped in different color series based on their aerial-mycelia color, and then morphologically characterized. Antagonist activity of the actinomycetes isolates on *Dickeya sp.* were evaluated using the same medium. From totally 51 actinomycetes isolates, only 34 isolates that grew well, then screened and tested for antagonistic activity. There were 35 tested isolates (including the control) exhibited a clear zone around the colonies indicating antagonistic activity; only 14 isolates showed clear zone higher than that of the positive control (chloramphenicol). From the smallholder pineapple plantation, isolate A-12 exhibited the highest antagonist activity against *Dickeya sp.* (diameter of clear zone = 13,69 mm) and from the private pineapple plantation isolate GGP-9 showed the best (diameter of clear zone = 19,44 mm). Based on their cultural and morphological characteristics, the isolated actinomycetes were suggested as genus of *Streptomyces*. Since many isolates showed inhibitory activity in vitro against *Dickeya sp.*, one of important pineapple soft rot pathogens, it is suggestive that pineapple plantation could be an interesting source to explore for actinomycetes potentially used as antagonist.

Keywords: actinomycetes; antagonist activity; *Dickeya sp.*; pineapple soft rot

I. INTRODUCTION

Actinomycetes are a group of gram-positive bacteria, aerobic, grow and have aerial hypha like fungi. They are easily found in the soil, especially in dry alkaline soil. Actinomycetes has been reported as the common producers of antibiotics, especially the genus *Streptomyces* [1], [2], [3]. So far, actinomycetes have been widely developed and utilized in many diferent fields such as in the pharmaceutical industry,

animal feed or poultry, food preservation as well as in agricultural practices applied as antibiotics antibacterial, antifungal, pesticides, and anti-parasites [3], [4], [5], [6].

Application of actinomycetes as biocontrol agents in the management of bacterial plant diseases have been reported. Reference [7] reported that actinomycetes isolated from agricultural soil inhibited *Erwinia amylovora* bacteria causing fireblight disease in apple plants and *Agrobacterium tumefaciens*, the crown gall pathogen. Actinomycetes isolated

from soil in Malaysia capable of inhibiting the growth of some types of plant pathogens [8], [9]. Actinomycetes has been used as biocontrol agents of onion bacterial rot diseases [10]. Some actinomycetes showed antagonistic effect on *Xanthomonas* pv. *oryzae*, the causal agent of bacterial leaf blight on rice [11]. There are still very limited information, however, concerning the use of actinomycetes as biocontrol agents of bacterial soft rot diseases in Indonesia.

This study was aimed to characterize actinomycetes isolates capable of inhibit the in vitro growth of *Dickeya* sp., the bacterial pathogen of pineapple soft rot disease in Lampung.

II. THE MATERIAL AND METHODS

2.1 Collection of Soil Samples and Isolation of Actinomycetes

The soil samples were taken from predetermined pineapple plantation sites located in Lampung during July – December 2016. The source of soil samples collection consisted of four pineapple plantations in different locations, two came from private companies: PT Great Giant Farm (GGF) located in Terbanggi Besar (Central Lampung) and PT Nusantara Tropical Farm (NTF) located in Way Jepara (East Lampung) and another two came from smallholder plantations located in Astomulyo (A) located in Punggur - Central Lampung and Mulyajaya (M) located in Tulang Bawang Tengah - West Tulang Bawang. From each of the plantation, soil samples were collected from five diagonal points in a depth of 15 cm below the soil surface, composited, kept in a plastic bag, and stored in a cooler during transportation.

Prior to isolation, the soil was air-dried for a week to reduce the growth of gram-negative bacteria. Furthermore, the isolation process followed a procedure described in [7]. Colonies of suspected actinomycetes having different appearance were subcultured in malt agar medium and incubated at room temperature for one week to obtain pure culture of each isolate.

2.2 Antagonistic Test

An antagonistic test was performed to investigate the potential of actinomycetes isolates as antagonistic agents or growth inhibitors of *Dickeya* sp. Antagonistic test was performed by agar diffusion method developed by [12] to determine the antagonistic effect of actinomycetes toward *Dickeya* sp. The tested pathogen, 24 hour *Dickeya* sp., was suspended in sterile water and mixed into culture medium. The actinomycetes isolates were taken from a 3-day culture using a 5 mm diameter sterile cork and placed on the surface of the medium mixed with the tested pathogen. Positive reactions are indicated by the formation of clear zones around the

actinomycetes. Observations and measurements of clear zones were conducted daily for 5 days.

2.3 Characterization

Collected isolates were characterized based on their cultural and morphological features of colonies grown on malt agar medium (colour of the aerial mycelia, substrate mycelia, and diffusible pigment). Visual observation of microscopic characteristics was done directly and indirectly under a compound light microscope and scanning electron microscope. SEM analysis was conducted at the Unit Pelaksana Teknis Laboratorium Terpadu dan Sentra Inovasi Teknologi (UPT LTSIT) University of Lampung. Important criteria observed included characteristics of the structures of aerial and substrate hyphae, spore chains, and ornamentation of spore surface .

III. RESULTS AND DISCUSSION

3.1 Isolates of Actinomycetes

The initial in vitro growth of actinomycetes colonies was relatively slow, it took about 3 days after incubation. The colonies that grew in malt agar media varied in their surface colors, surface structures, and color of diffusible pigment. Color series of colony surface included white, beige, gray, brown, yellow, and pink. Reference [13] described that the color of the aerial and substrate mycelia produced actinomycetes varied with different media. The colonies of actinomycete isolates were round-shaped with starchy surfaces, having clear edges and were firmly attached to the surface of the media. Those characters were in line with the description by

Reference [14] showed that colonies of actinomycetes show powdery consistency and stick firmly to agar surface; in culture media they produced hyphae and spora-like fungi. Any isolates suspected as actinomycetes were isolated and re-purified on the same medium for further characterization. From this step was obtained totally 51 isolates suspected as actinomycetes; however, only 45 isolates that grew well.

As many as 45 pure culture isolates that were successfully selected and collected from the previous process, 25 isolates obtained from soil samples taken from smallholder pineapple plantation and 20 isolates from private pineapple plantation. All of the isolates were confirmed as Gram Positive bacteria based on Ryu nonstaining KOH technique [15]. In general, the actinomycetes isolated from the pineapple plantations had variations in the colony shapes and colours (Figure 1) as well as the presence or absence of diffusible pigment produced in the growth medium.



Figure 1. Some variations of shapes and colours of actinomycetes colonies

3.2 Antagonistic test

In general, the result of antagonistic test showed clear zone formed around actinomycetes colonies started one day after incubation and increased up to following five days. The clear zone suggested that the growth of *Dickeya* sp. in agar medium was inhibited by actinomycetes (Figure 2). The clear zone implied that the pathogen did not grow well when exposed to actinomycetes, thus indicated that the actinomycetes may produced substance that acted as antibacterials [7]. References [16] and [17] showed that the antibacterial activity of actinomycetes inhibited the growth of *E. carotovora subsp. carotovora* and *E. chrysanthemi*, respectively. The presence of a growing clear zone also indicated that the more antibacterial compounds were produced, the stronger the antibacterial effect of the actinomycetes is.



Gambar 2. Tampilan zona bening di sekitar aktinomisetes

Figure 2. Formation of a clear zone around the actinomycete colonies

Some actinomycetes isolates did not form any clear zone, or in other words they were not be able to inhibit the growth of *Dickeya* sp. Based on samples source, out of 25 isolates from smallholder pineapple plantation tested for antagonistic only 19 isolates showed antagonistic effect (8 Astomulyo isolates and 11 Mulyajati isolates (Table 1). And out of 20 isolates from private pineapple plantation, only 10 GGF isolates and 5 NTF isolates that showed inhibition effect (Table 2). Various publications stated that the growth of actinomycetes is influenced by soil type and soil acidity. The population of actinomycetes will be especially high in dry and alkaline soils [18], [19]. However, it turned out that on pineapple plantations which is the soils tend to be wet and acid ($\text{pH} < 6.0$) still can be obtained various actinomycete isolates.

Table 1. Inhibition of in vitro growth of *Dickeya* sp. by actinomycetes isolates from smallholder pineapple plantation.

No	Isolate code	Clear zone (mm)	No	Isolate code	Clear zone (mm)
1.	A ₁	8,62	11.	MJ ₃	4,97
2.	A ₂	9,87	12.	MJ ₅	10,78
3.	A ₃	1,00	13.	MJ ₆	11,06
4.	A ₄	8,94	14.	MJ ₇	3,91
5.	A ₇	5,47	15.	MJ ₈	9,50
6.	A ₁₀	5,53	16.	MJ ₉	4,63
7.	A ₁₁	4,36	17.	MJ ₁₀	3,59
8.	A ₁₂	13,69	18.	MJ ₁₂	11,31
9.	MJ ₁	8,94	19.	MJ ₁₄	2,28
10.	MJ ₂	10,31	20.	Antibiotic	6,41

Table 2. Inhibition of in vitro growth of *Dickeya* sp. by actinomycetes isolates from private company's pineapple plantation

No	Isolate code	Clear zone (mm)	No	Isolate code	Clear zone (mm)
1.	GGP 1	0,47	9.	GGP 15	6,91
2.	GGP 2	16,00	10.	GGP 18	3,32
3.	GGP 4	3,91	11.	NTF 1	6,63
4.	GGP 7	1,19	12.	NTF 2	4,00
5.	GGP 9	19,44	13.	NTF 3	2,10
6.	GGP 10	2,88	14.	NTF 4	4,38
7.	GGP 11	3,32	15.	NTF 8	1,10
8.	GGP 14	10,85	16.	Antibiotik	6,41

3.3 Characterization of Selected Actinomycetes

The cultural characterization of selected actinomycetes showed that they were variable in colony appearance such as the colour of colony surface and pigment that diffuse and change the medium colour. The colour varied from bright white to dark blue. The surface were very similar with elevated starchy surface. The observation by scanning electron microscopy revealed various different branched hypha and aerial hypha containing spore chains [20]. The spore chains were variable in their number of spores, short chain fragments contained only a few spore compared to the longer fragments. The form of the spores varied from circular to ellipsoidal, and the spore surface was varied from smooth to rough (Figure 3). Based on their some typical characteristics, the selected actinomycete isolates from pineapple plantations were indicated as the genus

Streptomyces. Among actinomycetes genera, Streptomyces represented the largest genus [21]. The characters including the color of aerial and substrate mycelia, formation of diffusible pigments, structure of the substrate and aerial mycelium, branching of hyphae containing spores, and the spore surface. Observation under scanning electron microscope showed spore surface morphology that was very much alike Streptomyces [22].



Figure 3. SEM of actinomycetes of two different isolates indicated as *Streptomyces* sp.

IV. CONCLUSIONS

Actinomycetes were abundantly found in pineapple plantations. From several locations of pineapple plantations in Lampung can be collected as many as 34 isolates that potential as bacterial antagonist of *Dickeya* sp. Out of those 34 isolates, six isolates were considered as the best antagonist. From the smallholder pineapple plantation, isolate A-12 exhibited the highest antagonist activity against *Dickeya* sp. (diameter of clear zone = 13,69 mm) and from the private pineapple plantation isolate GGP-9 showed the best (diameter of clear zone = 19,44 mm). Based on the cultural and morphological characteristics, the actinomycete isolates were grouped as the genus of Streptomyces. Since many isolates showed inhibitory activity against *Dickeya* sp., one of the important pineapple soft rot pathogens, it is suggestive that pineapple plantation could be an interesting source to explore for actinomycetes potentially used as antagonist.

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