BIODIVERSITAS
Volume 22, Number 2, February 2021
Pages: 640-647

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d220216

The antagonistic activity of marine actinomycetes from mangrove ecosystem against phytopathogenic fungi *Colletotrichum* sp. KA

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Manuscript received: 18 November 2020. Revision accepted: 10 January 2021.

Abstract. *Fadhilah QG, Santoso I, Yasman. 2021. The antagonistic activity of marine actinomycetes from mangrove ecosystem against phytopathogenic fungi Colletotrichum sp. KA. Biodiversitas 22: 640-647.* Marine actinomycetes from mangrove ecosystems are known to be potential antifungal-producing isolates against phytopathogenic fungi. The aim of this research was to obtain potential marine actinomycetes isolates against the phytopathogenic fungi *Colletotrichum* sp. KA. Screening of 15 marine actinomycetes isolates using a dual culture method with a plug technique showed that 80% of isolates have antagonistic activity, represented as a percentage of growth inhibition range from 47.96% to 84.94%. Among 12 potential isolates, six isolates (SM4, SM11, SM14, SM15, SM18, and SM20) were evaluated for delayed antagonistic activity with incubation periods of 6, 9, and 12 days using the plug and streak techniques. The results showed that the percentage of growth inhibition of selected isolates inclined to increase along with the incubation period prior to inoculation of *Colletotrichum* sp. KA. Delayed antagonist assays using the streak technique resulted in higher inhibition results compared to the plug technique. Furthermore, the non-delayed assays of the two selected isolates, SM11 and SM15, also inhibited *Colletotrichum* sp. KA 57.99% and 59.88%, respectively. The delayed antagonist assay with a shorter incubation period of the two selected isolates also showed an increased percentage of growth inhibition of *Colletotrichum* sp. KA. According to our research, the delayed antagonistic assay of marine actinomycetes isolates with a 12-day incubation period using a plug technique was representative to evaluate the percentage of growth inhibition.

Keywords: Antagonistic activity, antifungal, *Colletotrichum*, dual culture, marine actinomycetes

INTRODUCTION

Actinomycetes are a group of bacteria known for producing bioactive compound (Janardhan et al. 2014). *Streptomyces*, one of the actinomycetes genera, has been reported to produce approximately 100,000 antimicrobial compounds (Bader et al. 2010). Continuous exploration of actinomycetes from marine ecosystems has revealed that marine actinomycetes are the potential source of diverse novel bioactive compounds (Solanki et al. 2008; Subramani and Aalbersberg 2012; Subramani and Sipkema 2019). Marine actinomycetes can produce compounds with various bioactivities, such as antiparasitic (Pimentel-Elardo et al. 2010), antifungal (Intra et al. 2011), antibacterial (Jiao et al. 2018), and anticancer (Davies-Bolorunduro et al. 2019). Therefore, actinomycetes play an important role in biotechnology and medicine, as well as agriculture (Barka et al. 2016).

Antifungal-producing marine actinomycetes can become biocontrol agents in the agriculture sector to help overcome fungal phytopathogens (Bressan 2003). Abdallah et al. (2013) stated that biocontrol agents can be a better alternative strategy to overcome plant disease rather than using pesticides or agrochemicals. Pesticides have negative impacts on the environment and on humans. In comparison, the biocontrol agents of fungal phytopathogens are more efficient, environmentally friendly, and can be used for broad-spectrum (Chen et al. 2018). Moreover, using actinomycetes as a biocontrol agent, they can also produce plant growth-promoting compounds, such as indole acetic acid and siderophores, which are important for plant growth and development (Viaene et al. 2016; Chitraselvi 2018).

The phytopathogenic fungi *Colletotrichum* spp. are one of the top fungal pathogens that can infect various crops (Dean et al. 2012; Gautam 2014a). *Colletotrichum* sp. infection on crop products causes anthracnose, which reduces marketable products (Intra et al. 2011; Gautam 2014b), consequently resulting in major economic losses of important crops (Dean et al. 2012). *Colletotrichum* sp. also infects coffee plants, causing coffee berry disease (Hindorf and Omondi 2011). Coffee is an important crop in the agriculture sector in Indonesia because it is one of the major export commodities (Martauli 2018). It is also well-known product used around the world, hence important export that impacts the world economy (Pandergras 2010).

Intra et al. (2011) reported the antifungal activity of *Streptomyces* from rhizospheric soils against *Colletotrichum* spp. In this research, several marine actinomycetes from a mangrove ecosystem were screened to obtain potential isolates that can be used against the fungal phytopathogen *Colletotrichum* sp. KA. Our previous

research showed that marine actinomycetes can produce bioactive compounds with antibacterial (Fadhilah et al. 2018) and antifungal activity (Alfisyahri et al. 2018; Zulfa et al. 2019). Therefore, marine actinomycetes isolated from mangrove ecosystems might be potential biocontrol agents against phytopathogenic fungi, such as *Colletotrichum* sp. KA.

MATERIALS AND METHODS

Microorganisms

Fifteen marine actinomycetes isolates and the fungal phytopathogen *Colletotrichum* sp. KA were obtained from the Laboratory of Microbiology, Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Indonesia. Marine actinomycetes were isolated from leaf litter (Fadhilah et al. 2018) and sediment (Alfisyahri et al. 2018) from a mangrove ecosystem, and were then labeled as SM and SD, respectively. Meanwhile, the fungal pathogen *Colletotrichum* sp. KA was isolated from infected coffee berries (Fadhilah et al. 2020). The isolates were purified using the quadrant streak method in cross streak media agar for marine actinomycetes isolates (Sengupta et al. 2015) and Potato Dextrose Agar (PDA) medium for *Colletotrichum* sp. KA.

Antagonistic activity screening of marine actinomycetes isolates

Antagonistic activity screening of marine actinomycetes against the fungal phytopathogen *Colletotrichum* sp. KA was performed using a dual culture method with a plug technique (Khamna et al. 2009; Qi et al. 2019). The marine actinomycetes isolates were grown on modified PDA medium which contained 50% PDA and 50% Starch Casein Agar (SCA) (Shrivastava et al. 2017). Starch Casein Agar contained 1% starch, 0.2% K_2HPO_4, 0.2% KNO_3, 0.03% casein, 0.005% $MgSO_4.7H_2O$, 0.002% $CaCO_3$, 0.001% $FeSO_4.7H_2O$, and 1.5% agar (Mohseni et al. 2013). The fungal phytopathogen *Colletotrichum* sp. KA was grown on PDA medium.

Antagonistic activity screening of marine actinomycetes was carried out with two marine actinomycetes isolates on the modified PDA medium. The marine actinomycetes isolate colonies were plugged using a straw (Ø 5 mm), then placed opposite each other at 4 cm. The plates were incubated at 30°C for 9 days prior to *Colletotrichum* sp articulation. KA, so called delayed antagonist assay. A plug of *Colletotrichum* sp. KA (Ø 5 mm) from a 5-day old colony was placed between the marine actinomycetes isolate colonies. All the experiments were performed in triplicates.

The result of the antagonist assay was observed after 5 days of incubation at 30°C. The inhibition of *Colletotrichum* sp showed a positive result of the antagonist assay. KA mycelium growth. The radius of *Colletotrichum* sp. KA colonies were measured using calipers. The percentage of growth inhibition was calculated based on Song et al. (2020), where R2 is the radius of the control *Colletotrichum* sp. KA colony and R1 is the radius of the treatment *Colletotrichum* sp. KA colony. The marine actinomycetes isolate with a higher percentage of growth inhibition were selected for further antagonist assays.

$$Percentage\ of\ growth\ inhibition\ (\%) = \frac{R2-R1}{R2} \times 100$$

Delayed antagonist assays of selected marine actinomycetes

Marine actinomycetes isolate selected based on antagonistic activity screening were used for further antagonist assays with various incubation periods prior to the inoculation of *Colletotrichum* sp. KA. The various incubation periods in the delayed antagonist assays were done to evaluate the effect of incubation on the antagonistic activity of marine actinomycetes isolates. The delayed antagonist assays were also carried out on modified PDA medium in triplicate. The colonies of selected marine actinomycetes isolates (5 days old) were plugged using a straw (Ø 5 mm), then the agar discs were placed on the modified PDA medium. The plates were incubated at 30°C for 6, 9, and 12 days before the inoculation of *Colletotrichum* sp. KA. After the various incubation periods, an agar disc of *Colletotrichum* sp. KA (Ø 5 mm) from a 5-day old colony was placed 1 cm opposite from the marine actinomycetes colony. The plates were observed after 5 days of incubation at 30°C. The radius of the *Colletotrichum* sp. KA colony opposite the selected marine actinomycetes isolates was measured using calipers and the percentage of growth inhibition of *Colletotrichum* sp. KA was assessed.

Delayed antagonist assays with a modified technique

Delayed antagonist assays of selected marine actinomycetes isolates were also carried out using the dual culture method with a streak technique (Khucharoenphaisan et al. 2013). The marine actinomycetes isolates were streaked along the middle of the agar plate. The plates were incubated at 30°C for 6, 9, and 12 days. After the various incubation periods, the agar discs of *Colletotrichum* sp. KA (Ø 5 mm) from a 5-day old colony were placed at a distance of 1 cm on both sides of the marine actinomycetes colony. The results of the delayed antagonist assays were observed after 5 days of incubation at 30°C. The percentage of growth inhibition was calculated after the radius of the *Colletotrichum* sp. KA colony was measured.

Non-delayed antagonistic activity assays of marine actinomycetes

The non-delayed antagonistic assays were done using two selected marine actinomycetes isolates based on the previous assays. The two selected isolates were subjected to a non-delayed antagonist assay along with a shorter (3 and 5 days) delayed antagonist assay period against *Colletotrichum* sp. KA. These assays were also performed in triplicate using the dual culture method with a modified PDA medium plug technique.

The marine actinomycetes isolate from a 5-day old colony and the *Colletotrichum* sp. KA colony was plugged using a straw (Ø 5 mm). The marine actinomycetes isolate

agar discs were placed on modified PDA medium. The agar disc of *Colletotrichum* sp. KA was inoculated 2 cm opposite from actinomycetes at the same time for a non-delayed antagonist assay. The *Colletotrichum* sp. KA agar disc inoculated on modified PDA medium without marine actinomycetes isolates served as the control.

The antagonist assay results were observed 5 days after inoculation of *Colletotrichum* sp. KA. The antagonistic activity of selected marine actinomycetes isolates was indicated by the percentage of *Colletotrichum* sp growth inhibition. KA. The radius of *Colletotrichum* sp. KA colonies opposite the marine actinomycetes isolates was measured using calipers, then used to calculate the percentage of growth inhibition.

RESULTS AND DISCUSSION

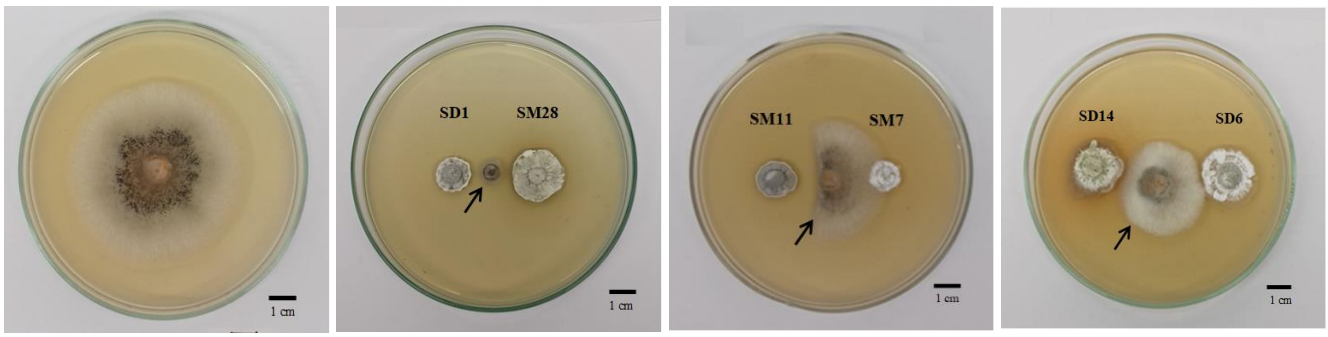
Antagonistic activity screening of marine actinomycetes isolates

The marine actinomycetes isolated from the mangrove ecosystem used in the present study showed antagonistic activity against fungal phytopathogen *Colletotrichum* sp. KA (Figure 1). Of the marine actinomycetes isolates, 80% have percentage of growth inhibition ranges from 47.96% to 84.94% (Table 1). Figure 1B very clearly shows strong inhibition of the two marine actinomycetes isolates (SD1 and SM28) against the growth of *Colletotrichum* sp. KA; whereas, Figure 1D shows moderate inhibition of marine actinomycetes isolates (SD14 and SD6). In contrast with the isolates SD1 and SM28, where the inhibition might be due to a mixture effect of the two isolates, it is obviously seen that isolate SM11 showed strong inhibition against the growth of the fungal phytopathogen *Colletotrichum* sp. KA (Figure 1C). Nevertheless, the radius of *Colletotrichum* sp. KA in all positive antagonistic activity screening results can still be obtained through detailed observation (Table 1). The negative results shown by the remaining isolates (SM2, SM7, and SM12) indicate that *Colletotrichum* sp. KA can grow over these three isolates as demonstratively represented by SM7 in Table 1 and Figure 1C. It is interesting to note that among the 12 marine actinomycetes isolates that showed a positive result, the higher percentage of growth inhibition (more than 80%) mostly belongs to the SM isolates from leaf litter (seven isolates); meanwhile, the SD isolate from sediment has only one isolate (SD1). On the contrary, it is also surprising to notice that all isolates that show a negative result came from the SM isolates. The present study results support the fact that marine actinomycetes constitute an ongoing and promising source of bioactive metabolites (Solanki et al. 2008; Subramani and Aalbersberg 2012; Subramani and Sipkema 2019). Among the 12 isolates that showed a positive result, six isolates with a higher inhibition percentage were selected for further delayed antagonistic assays.

Table 1. Results of antagonistic activity screening of marine actinomycetes against the fungal phytopathogen *Colletotrichum* sp. KA

Isolate	Radius of control (mm)	Radius of treatment (mm)	Percentage of growth inhibition (%)
SM2	34.91±2.30	-[a]	-
SM4		5.26±0.58	84.94±1.65
SM7		-	-
SM11		5.72±0.33	83.62±0.93
SM12		-	-
SM14		5.57±0.49	84.05±1.39
SM15		5.27±0.44	84.91±1.27
SM18		4.84±0.67	84.14±1.91
SM20		5.54±0.25	84.13±0.72
SM28		6.83±1.34	80.44±3.84
SD1		6.45±0.24	81.53±0.69
SD6		17.67±1.61	49.39±4.61
SD7		15.33±0.76	56.08±2.18
SD9		18.17±1.15	47.96±3.31
SD14		15.33±1.04	56.08±2.98

Note: [a]Indicates a negative value



A B C D

Figure 1. Representative results of antagonistic activity screening of marine actinomycetes isolates against the fungal phytopathogen *Colletotrichum* sp. KA: A. control; B. SD1 (*left*) and SM28 (*right*); C. SM11 (*left*) and SM7 (*right*); D. SD14 (*left*) and SD6 (*right*); (arrow: colony of *Colletotrichum* sp. KA)

Delayed antagonist assays of selected marine actinomycetes

The results of the delayed antagonist assays of six selected marine actinomycetes isolates using the plug technique showed that all of the selected isolates can inhibit the growth of *Colletotrichum* sp. KA. The percentage of growth inhibition ranged from 62.88% to 80.51%, 65.02% to 80.47%, and 61.18% to 84.16% for 6, 9, and 12 days, respectively (Table 2). The 12-day delayed incubation period resulted in the highest percentage of growth inhibition and was also significantly different as compared to the 6- and 9-day delayed incubation periods, except for in the case of the isolates SM11 and SM20 (Figure 2). Figure 3 provides a representative visualization of the antagonistic activity of the selected marine actinomycetes, SM15 isolate, toward *Colletotrichum* sp. KA, which had a percentage of growth inhibition of 78.02%, 76.02%, and 84.16% after 6-, 9-, and 12-day delayed incubation periods, respectively.

Table 2. Results of delayed antagonist assay of selected marine actinomycetes using the plug technique

Isolate	6 days			9 days			12 days		
	Radius of control (mm)	Radius of treatment (mm)	Percentage of growth inhibition (%)	Radius of control (mm)	Radius of treatment (mm)	Percentage of growth inhibition (%)	Radius of control (mm)	Radius of treatment (mm)	Percentage of growth inhibition (%)
SM4	33.15±1.84	7.21±0.27	78.25±0.80	32.21±0.45	8.10±0.53	74.84±1.65	31.71±0.53	6.12±0.13	80.69±0.42
SM11		6.46±0.30	80.51±0.90		6.41±0.22	80.10±0.70		6.54±0.12	79.37±0.38
SM14		6.54±0.38	80.27±1.14		6.29±0.37	80.47±1.13		5.60±0.08	82.34±0.24
SM15		7.29±0.27	78.02±0.82		7.72±0.29	76.02±0.92		5.02±0.20	84.16±0.63
SM18		7.00±0.27	78.89±0.81		6.77±0.14	78.99±0.44		5.62±0.61	82.27±1.94
SM20		12.31±0.68	62.88±2.06		11.27±0.49	65.02±1.53		12.31±0.09	61.18±0.28

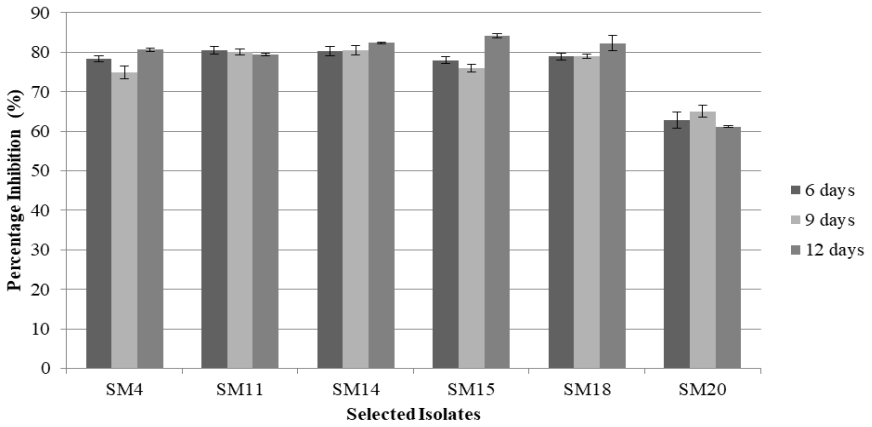


Figure 2. Diagram of percentage of growth inhibition of selected marine actinomycetes isolates using the plug technique

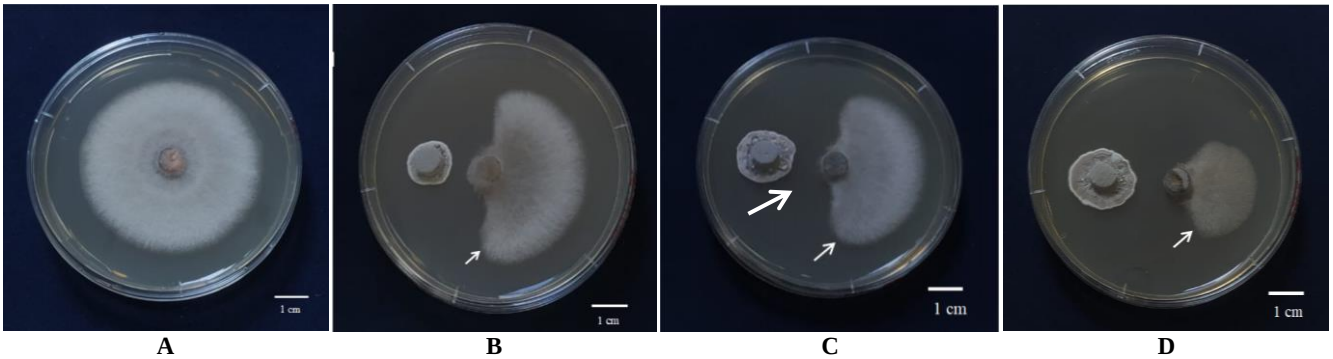


Figure 3. Representative results of antagonist assays using the plug technique on the SM15 isolate with various incubation periods: A. control; B. 6 days; C. 9 days; and D. 12 days; (arrow: colony of *Colletotrichum* sp. KA)

Delayed antagonist assay with modified technique

The results of the further delayed antagonistic assays of six selected marine actinomycetes isolate using the dual culture method with a streak technique are displayed in Table 3. The percentage of growth inhibition was slightly higher compared to when the plug technique was used, ranging from 76.75% to 86.51%, 73.47% to 84.88%, and 77.22% to 85.91% for 6, 9, and 12 days, respectively. In contrast with the plug technique results, the percentage of growth inhibition of various delayed antagonist assays with the streak technique showed insignificant differences as qualitatively indicated by the average and their standard deviation values overlapping with one another (Figure 4). There is also no trend indicating that a longer incubation period results in a higher percentage of growth inhibition of isolates, and vice versa. All isolates can inhibit the growth of *Colletotrichum* sp. KA with a percentage of growth inhibition of more than 81%, except the isolate SM20, which only inhibited *Colletotrichum* sp. KA up to 77%.

Figure 5 shows a representative visualization of antagonistic activity with the steak technique of the selected marine actinomycetes, SM15 isolate, against *Colletotrichum* sp. KA. Figure 4 represents the antagonistic activity assay of the SM15 isolate with a delayed culture of 6, 9, and 12 days, which show the values of percentage of growth inhibition of 86.51%, 84.88%, and 84.85%, respectively.

Table 3. Results of the delayed antagonist assay of selected marine actinomycetes using the streak technique

Isolate	6 days			9 days			12 days		
	Radius of control (mm)	Radius of treatment (mm)	Percentage of growth inhibition (%)	Radius of control (mm)	Radius of treatment (mm)	Percentage of growth inhibition (%)	Radius of control (mm)	Radius of treatment (mm)	Percentage of growth inhibition (%)
SM4	34.26±1.38	4.94±0.43	85.57±1.26	33.12±1.65	5.11±0.39	84.57±1.18	34.63±1.03	5.57±0.67	83.91±1.95
SM11		5.66±0.66	83.48±1.93		5.19±0.20	84.33±0.61		4.88±0.44	85.91±1.28
SM14		5.99±0.39	82.52±1.14		6.08±0.62	81.64±1.86		5.19±0.62	85.00±1.79
SM15		4.62±0.23	86.51±0.68		5.01±0.44	84.88±1.34		5.25±1.17	84.85±3.38
SM18		5.18±0.59	84.89±1.73		6.03±0.55	81.80±1.67		5.66±0.43	83.67±1.23
SM20		7.97±0.62	76.75±1.82		8.79±0.58	73.47±1.75		7.89±0.39	77.22±1.13

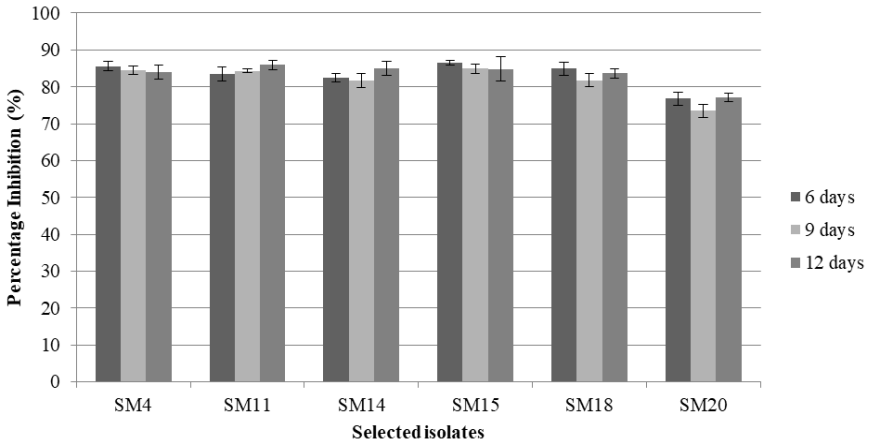
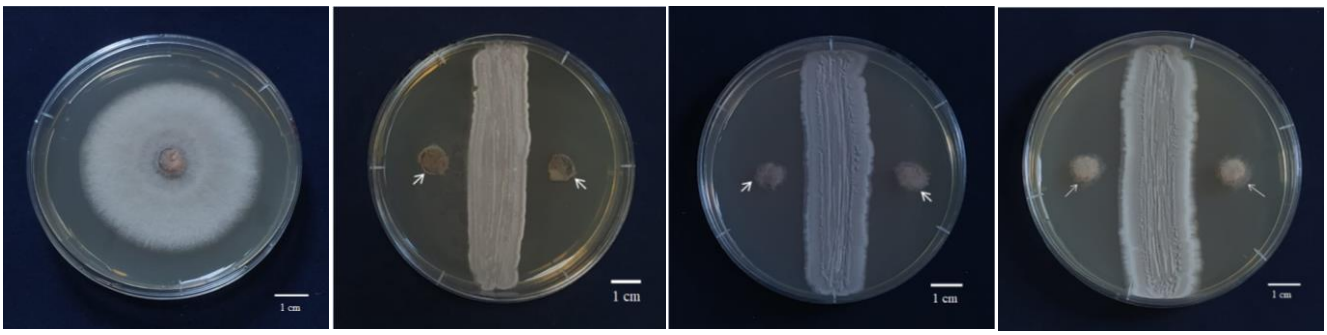


Figure 4. Diagram of the percentage of growth inhibition of selected marine actinomycetes isolates

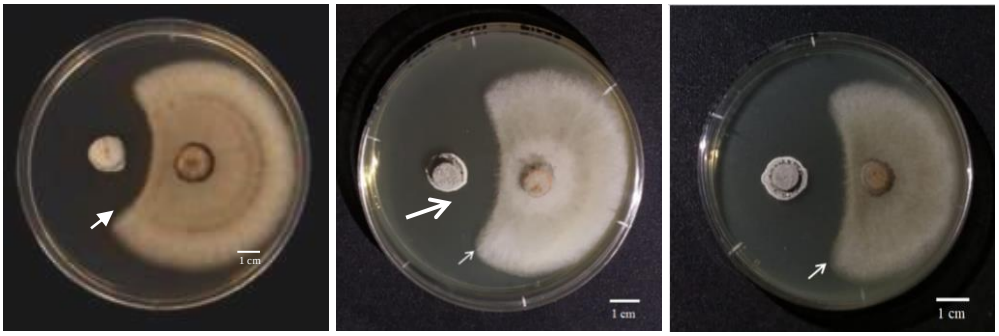


A **B** **C** **D**

Figure 5. Representative results of antagonist assays using the streak technique of SM15 isolate with various incubation periods: A. control; B. 6 days; C. 9 days; and D. 12 days; (arrow: colony of *Colletotrichum* sp. KA)

Table 4. Results of antagonist assays of SM11 and SM15 isolates

Isolate	Non-delayed antagonist assay			Delayed antagonist assay					
				3 days			5 days		
	Radius of control (mm)	Radius of treatment (mm)	Percentage of growth inhibition (%)	Radius of control (mm)	Radius of treatment (mm)	Percentage of growth inhibition (%)	Radius of control (mm)	Radius of treatment (mm)	Percentage of growth inhibition (%)
SM11	32.09±0.45	13.48±0.78	57.99±2.42	32.84±0.28	13.61±0.36	58.56±1.11	32.90±0.34	11.93±0.11	63.74±0.32
SM15		12.88±0.41	59.88±1.27		12.70±0.29	61.34±0.89		11.16±0.73	66.08±2.23



A **B** **C**

Figure 6. Representative results of antagonistic activity of marine actinomycetes SM11 isolate: A. non-delayed antagonistic assay; B. delayed antagonistic assay for 3 days; C. delayed antagonistic assay for 5 days; (arrow shows the colony of *Colletotrichum* sp. KA)

Non-delayed antagonistic activity assay of marine actinomycetes

The percentage of growth inhibition in the non-delayed antagonist assays for SM11 and SM15 was 57.99% and 59.88%, respectively (Table 4). The results of the delayed antagonist assays exhibited a percentage of growth inhibition of SM11 and SM15 isolates to be increased slightly from 58.56% to 63.74% and 61.35% to 66.08%, respectively. It was shown that the longer incubation period of marine actinomycetes isolates prior to inoculation of *Colletotrichum* sp. KA can increase antagonistic activity. Nevertheless, it is interesting to note that the percentage of growth inhibition in non-delayed assays was not significantly different from the percentage of growth inhibition at 3 days of delayed incubation, but just starts to be significantly different at 5 days of delayed incubation (Table 4 and Figure 6).

Discussion

Our study showed that the potential marine actinomycetes isolate against phytopathogenic fungi, *Colletotrichum* sp. KA was isolated mostly from the leaf litter rather than the sediment of the mangrove ecosystem (Table 1). Azman et al. (2015) stated that mangrove ecosystems have unique characteristics, such as high salinity, extreme tides, muddiness, and high temperatures. These conditions make mangrove ecosystems good supporting habitats for actinomycetes, which are a potential source of bioactive compounds (Xu et al. 2014). In the mangrove ecosystem, leaf litter was present on the surface of the sediment, which was exposed to the tides; therefore, nutrients were accumulated. According to Srisunont et al. (2017), nutrients in mangrove ecosystems are provided from tidal inundation, fauna, and microbial activities. Activities such as decomposition is a source of nutrients from litterfall in the mangrove area. Since the nutrients are carried by tides and litterfall, various organisms, including microorganisms, can live on leaf litter. Various microorganisms in mangrove ecosystem might cause competition among microorganism populations, including marine actinomycetes. In their natural habitats, microorganisms live within communities and may produce bioactive compounds. The bioactive compounds used for nutrition or competition for space may inhibit other microorganisms (Yu et al. 2019).

Sediment in mangrove ecosystems also reported can contain many nutrients. The sediment becomes a storage place for the nutrients that accumulate on the surface. About 50% of nutrients in sediment are obtained from the litter (Srisunont et al. 2017). Nevertheless, microorganism diversity in sediment might be lower than in the leaf litter. Since only certain populations of microorganisms can live in sediment, less competition might occur among microorganisms, including marine actinomycetes. Consequently, marine actinomycetes might produce limited bioactive compounds. Therefore, the probability of obtained potential bioactive compound–producing actinomycetes from the leaf litter was higher than from the sediment.

Marine actinomycetes are known for their potential to produce various novel bioactive compounds (Subramani and Aalsbersberg 2012). Agreeing with that statement, the screening of antagonistic marine actinomycetes in this

study exhibited that 80% of isolates were potential antifungal-producing actinomycetes. Among the 12 active actinomycetes, eight isolates (67%) showed antagonism of 80.44% to 84.94%, whereas only four isolates (33%) showed antagonistic activity in a range of 47.96% to 56.08%. According to Khucharoenphaisan et al. (2013), the percentage of growth inhibition in a range of 61% to 100% is categorized as highly efficient in significantly inhibiting growth activity; meanwhile, the percentage of growth inhibition in a range of 41% to 60% is categorized as having the ability to inhibit the growth of fungal mycelium.

Actinomycetes having the potential to inhibit fungal phytopathogen growth were also reported by some other studies. Crawford et al. (1993) reported actinomycetes antagonist activity against the fungal root pathogen *Pythium ultimum*. Khamna et al. (2009) assessed the antagonistic activity of actinomycete isolates, of which, 27 were active against at least one of the six plant pathogenic fungi: *Alternaria brassicicola*, *A. porri*, *Colletotrichum gloeosporioides*, *Fusarium oxysporum*, *Penicillium digitatum*, and *Sclerotium rolfsii*. Haidar et al. (2016) also reported that actinomycetes isolates were active towards the fungal phytopathogen *Phaeomoniella chlamydospora*. These findings, again, confirm that actinomycetes constitute a promising and ongoing source of bioactive metabolites that can act against phytopathogenic fungi (Solanki et al. 2008; Subramani and Aalbersberg 2012; Subramani and Sipkema 2019).

Delayed antagonistic assays using both the plug and streak techniques provided an opportunity for actinomycetes to grow and secrete their bioactive compounds into the medium. In the present study, various incubation periods in delayed antagonistic assays before the inoculation of *Colletotrichum* sp. KA were used to find out the optimal period for antifungal activity effect. Actinomycetes were found to produce bioactive compounds in the stationary phase (Barka et al. 2016). This study confirmed actinomycete species' stationary phase varied. Even though most of the selected actinomycetes isolates showed their highest antagonistic activity at 12 days of delayed incubation, the SM11 and SM20 isolates showed their highest antagonistic activity at 6 and 9 days of delayed incubation, respectively. To the best of our knowledge, using various delayed antagonist assays to find the optimal period for screening antifungal activity are reported here for the first time.

The percentage of growth inhibition in the delayed antagonist assay when using the streak technique (Table 3) was slightly higher than that of plug technique (Table 4). This is likely because the colony of marine actinomycetes isolates used in the streak technique was wider and more populated than in the plug technique, so the actinomycetes were able to produce more bioactive compounds to inhibit the growth of *Colletotrichum* sp. KA. This could also be the reason why the various delayed antagonist assays using the streak technique gave results that were not significantly different. It is assumed that the remaining medium at both sides of the actinomycetes isolates colony was saturated with the bioactive compounds produced by the actinomycetes.

A non-delayed antagonistic assay using the plug technique proved that actinomycetes secrete bioactive compounds into the medium. The percentage of growth inhibition in the non-delayed antagonist assays was not significantly different in comparison with the percentage of growth inhibition in the assay with 3 days of delayed incubation. It is assumed that the actinomycetes took some time to grow and produce bioactive compounds. The remaining bioactive compounds secreted during the sporulation stage into the isolates' plug medium took the responsibility for the antagonistic activity, in addition to those secreted during the 5 days of incubation of the antagonist assay itself. Khamna et al. (2009) and Khucharoenphaisan et al. (2013) reported that antagonistic activity might be due to the production of bioactive compounds by the actinomycetes. This study also recommended the use of a delayed antagonist assay with the plug technique because of both microorganisms – marine actinomycetes isolates and *Colletotrichum* sp. KA – have a similar plug agar dimension, therefore, an equal antagonism.

Further study of marine actinomycetes isolates from mangrove ecosystems have to be assessed using antibiosis assays, which will ensure that isolates produce antifungal compounds rather than food or space competition for the fungal phytopathogen *Colletotrichum* sp. KA. In conclusion, the isolates of marine actinomycetes from mangrove ecosystems are potential antagonistic isolates. The antagonist assay using the plug technique was the representative technique to obtain percentage of growth inhibition. The delayed antagonist assay with a 12-day incubation period prior to inoculation of *Colletotrichum* sp. KA was optimal to evaluate the antagonistic activity of marine actinomycetes isolates.

ACKNOWLEDGEMENTS

This research was financially supported by Grant *Publikasi Terindeks Internasional* (PUTI) *Saintekes* 2020 from the Universitas Indonesia to Dr. rer. nat. Yasman (No. NKB-2409/UN2.RST/HKP.05.00/2020).

REFERENCES

Abdallah ME, Haroun SA, Gomah AA, El-Naggar NE, Badr HH. 2013. Application of actinomycetes as biocontrol agents in the management of onion bacterial rot diseases. Arch Phytopathol Pflanzenschutz 46: 1-12.

Alfisyahri R, Santoso I, Yasman Y. 2018. Isolation and screening antimicrobial activity of actinomycetes from sediment's coastal Pramuka Island, Kepulauan Seribu, Jakarta, Indonesia. AIP Conf Proc 2023: 020126. DOI: 10.1063/1.5064123.

Azman A, Othman I, Velu SS, Chan K, Lee L. 2015. Mangrove rare actinobacteria: taxonomy, natural compound, and discovery of bioactivity. Front Microbiol 6: 856. DOI: 10.3389/fmicb.2015.00856.

Bader J, Mast-Gerlach E, Popovic MK, Bajpai R, Stahl U. 2010. Relevance of microbial co-culture fermentation in biotechnology. J Appl Microbiol 109: 371-387.

Barka EA, Vasta P, Sanchez L, Gaveau-Vaillant N, Jacquard C, Klenk H, Clément C, Ouhdouch Y, van Wezel GP. 2016. Taxonomy, physiology, and natural products of actinobacteria. Microbiol Mol Biol Rev 80: 1-44.

Bressan W. 2003. Biological control of maize seed pathogenic fungi by use of actinomycetes. Biol Control 48: 233-240.
Chen Y, Zhou D, Qi D, Gao Z, Xie J, Luo Y. 2018. Growth promotion and disease suppression ability of a *Streptomyces* sp. CB-75 from banana rhizosphere soil. Front Microbiol 8: 2704. DOI: 10.3389/fmicb.2017.02704.
Chitraselvi RPE. 2018. Actinomycetes: dependable tool for sustainable agriculture. Curr Investig Agric Curr Res 1: 128-130.
Crawford DL, Lynch JM, Whipps JM, Ousley MA. 1993. Isolation and characterization of actinomycetes antagonists of a fungal root pathogen. Appl Environ Microbiol 59: 3899-3905.
Davies-Bolorunduro OF, Adeleye IA, Akinleye MO, Wang PG. 2019. Anticancer potential of metabolic compounds from marine actinomycetes isolated from Lagos Lagoon sediment. J Pharm Anal 9: 201-208.
Dean R, Van Kan JAL, Pretorius ZA, Hammond-Kosack KE, Pietro AD, Spanu PD, Rudd JJ, Dickman M, Kahmann R, Ellis J, Foster GD. 2012. The top 10 fungal pathogens in molecular plant pathology. Mol Plant Pathol 13: 414-430.
Fadhilah QG, Santoso I, Maryanto AE, Yasman. 2020. Potential antifungal from Bacillus sp. against phytopathogenic fungi *Colletotrichum* sp. Proceeding of International Symposium on Current Progress in Mathematics and Sciences (ISCPMS). Universitas Indonesia, Depok, 27-28 October 2020.
Fadhilah QG, Santoso I, Yasman Y. 2018. Isolation and screening antibacterial activity of actinomycetes from mangrove ecosystem, Pramuka Island, Kepulauan Seribu, Jakarta, Indonesia. AIP Conf Proc 2023: 020119. DOI: 10.1063/1.5064116.
Haidar R, Roudet J, Bonnard O, Dufour MC, Corio-Costet MF, Fert M, Gautier T, Deschamps A, Fermaud M. 2016. Screening and modes of action of antagonistic bacteria to control the fungal pathogen *Phaeomoniella chlamydospora* involved in grapevine trunk disease. Microbiol Res 192: 172-184.
Hindorf H, Omondi CO. 2011. A review of three major fungal diseases of *Coffee arabica* L. in the rainforests of Ethiopia and progress in breeding for resistance in Kenya. J Adv Res 2: 109-120.
Intra B, Mungsuntisuk I, Nihira T, Igarashi Y, Panbangred W. 2011. Identification of actinomycetes from plant rhizospheric soils with inhibitory activity against *Colletotrichum* spp., the causative agent of anthracnose disease. BMC Res Notes 4: 98. DOI: 10.1186/1756-0500-4-98.
Janardhan A, Kumar AP, Viswanath B, Saigopal DVR, Narasimha G. 2014. Production of bioactive compounds by actinomycetes and their antioxidant properties. Biotechnol Res Intl 2014: 217030. DOI: 10.1155/2014/217030.
Jiao W-H, Yuan W, Li Z-Y, Li J, Lei L, Sun J-B, Gui Y-H, Wang J, Ye B-P, Lin H-W. 2018. Anti-MRSA actinomycins D1-D4 from the marine sponge-associated *Streptomyces* sp. LHW52447. Tetrahedron 74: 5914-5919.
Khamna S, Yokota A, Peberdy JF, Lumyong S. 2009. Antifungal activity of *Streptomyces* spp. isolated from rhizosphere of Thai medicinal plants. Int J Integr Biol 6: 143-147.
Khucharoenphaisan K, Sinma K, Lorrungruang C. 2013. Efficiency of actinomycetes against phytopathogenic fungus of chilli anthracnose. J Appl Sci 13: 472-478.
Martauli ED. 2018. Analisis produksi kopi di Indonesia. J Agribisnis Sci 10: 112-120. [Indonesian]
Mohseni M, Norouzi H, Hamedi J, Roohi A. 2013. Screening of antibacterial producing actinomycetes from sediments of the Caspian Sea. Intl J Mol Cell Med Spring 2: 64-71.
Pendergrast M. 2010. Uncommon Grounds: The History of Coffee and How It Transformed Our World. Basic Books, New York.
Pimentel-Elardo SM, Kozytska S, Bugni TS, Ireland CM, Moll H, Hentschel U. 2010. Anti-parasitic compounds from *Streptomyces* sp. strains isolated from Mediterranean sponges. Mar Drugs 8: 373-380.
Qi D, Zou L, Zhou D, Chen Y, Gao Z, Feng R, Zhang M, Li K, Xie J, Wang W. 2019. Taxonomy and broad-spectrum antifungal activity of *Streptomyces* sp. SCA3-4 isolated from rhizosphere soil of *Opuntia stricta*. Front Microbiol 10:1390. DOI: 10.3389/fmicb.2019.01390.
Sengupta S, Pramanik A, Ghosh A, Bhattacharyya M. 2015. Antimicrobial activities of actinomycetes isolated from unexplored regions of Sundarbans mangrove ecosystem. BMC Microbiol 15: 170-185.
Shrivastava P, Kumar R, Yandigeri MS. 2017. In vitro biocontrol activity of halotolerant *Streptomyces* aureofaciens K20: a potent antagonist against *Macrophomina phaseolina* (Tassi) Goid. Saudi J Biol Sci 24: 192-199.
Solanki R, Khanna M, Lal R. 2008. Bioactive compounds from marine actinomycetes. Indian J Microbiol 48: 410-431.
Song L, Jiang N, Wei S, Lan S, Pan L. 2020. Isolation, screening, and identification of actinomycetes with antifungal and enzyme activity assays against *Colletotrichum* dematium of *Sarcandra glabra*. Mycology 28: 37-43.
Srisunont C, Jaiyen T, Tenrung M, Likitchaikul M, Srisunont T. 2017. Nutrient accumulation by litterfall in mangrove forest at Klong Khone, Thailand. Thammasat Int J Sci Tech 22: 9-18.
Subramani R, Aalbersberg W. 2012. Marine actinomycetes: an ongoing source of novel bioactive metabolites. Microbiol Res 167: 571-580.
Subramani R, Sipkema D. 2019. Marine rare actinomycetes: a promising source of structurally diverse and unique novel natural products. Mar Drugs 17: 1-40.
Viaene T, Langendries S, Beirincks S, Maes M, Goormachtig S. 2016. *Streptomyces* as a plant's best friend? FEMS Microbiol Ecol 92: (8): fiw119. DOI: 10.1093/femsec/fiw119.
Xu D-B, Ye W-W, Han Y, Deng Z-X, Hong K. 2014. Natural products from mangrove actinomycetes. Mar Drugs 12: 2590-2613.
Yu M, Li Y, Banakar SP, Liu L, Shao C, Li Z, Wang C. 2019. New metabolites from the co-culture of marine-derived actinomycetes *Streptomyces rochei* MB037 and fungus *Rhinocladiella similis* 35. Front Microbiol 10: 915. DOI: 10.3389/fmicb.2019.00915.
Zulfa NV, Fitroh M, Santoso I, Maryanto AE, Yasman. 2019. Antagonistic potential of *Streptomyces cellulosae* SM12 against *Ganoderma* sp. TB3 and *Ganoderma* sp. TB4. Proceeding of International Science and Mathematics in Academic Research Talks (ISMART). Universitas Indonesia, Depok, 20-21 August 2019.