Potential Antibacterial Co-culture Streptomyces flavalbus from Indonesian mangroves induces the production of pyocyanin by Pseudomonas aeruginosa

Nuril Azhar
Diponegoro University, Indonesia
Ervia Yudiati (eyudiati@gmail.com)
Diponegoro University, Indonesia

Research Article

Keywords: Actinobacteria, antibacterial, co-culture, Mangrove, Streptomyces spp.

Posted Date: September 12th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-2019447/v1

Abstract

The potential Actinobacteria from the sediment and root *Soneratia alba* with co-culture as an antibacterial agent. 28 strains of Actinobacteria were found, composed of 17 strains from the sediment and 11 strains from the endophyte root of *Sonneratia alba*. Out of 580 fermentation, 540 fermenter co-cultures and 34 single fermenter cultures were assessed. Twenty-one pairs of co-culture were resulted positively against *Staphylococcus aureus*, and 16 pairs were positive against *Pseudomonas aeruginosa*. The highest clearance zone against *S. aureus* was obtained from 10 (Actino)-*Aspergillus fumigatus* (dead body) extract (21.57 ± 0.90 mm) while against *P. aeruginosa* was resulted from co-culture strains 3/37 extract (19.18 ± 0.80 mm). Strain 41 was determined to be *Streptomyces flavalbus* (98.07%) with the accession number MZ540332 when co-cultured with *Pseudomonas aeruginosa* to produce pyocyanin. Other Actinobacteria (6, 10, and 37) strains were identified as *Streptomyces albogriseolus* (99.79%), *Streptomyces labedae* (99%), and *Streptomyces rochei* (93.52%). It is concluded that co-culture is a valuable strategy to develop the new Actinobacteria product as a powerful antibacterial agent.

Introduction

Actinobacteria is a Gram-positive, constructed as rigid cell wall, containing murammate acid and teichoic acid (Becker et al. 1965; Kamisango et al. 1982; Glaser et al. 2004). Actinobacteria possesses several unique phenotypes indicated by the various coccus morphology up to differentiate mycelia. Dominated by *guanine-plus-cytosine* (G + C) DNA material which encloses 50 mol% − 70 mol% (Goodfellow 2012). Moreover, these bacteria are anaerobic (von Wintzingerode et al. 2001), anaerobic facultative (Roth et al. 2011) or aerobic (Becker et al. 1965). Mostly are chemoorganotrophe, grown well in neutral pH (Ludwig et al. 2012). Some of them are acidophilic (Johnson et al. 2009) or alkaliphilic (Thumar et al. 2010). Actinobacteria is also halophilic (Sharma 2015) and thermophilic (Hugenholtz and Stackebrandt 2004; Johnson et al. 2009). In addition, most Actinobacteria are saprophytic. Nevertheless, some are pathogenic (Raoult et al. 2003) and symbionts (Solans et al. 2015; Riyanti et al. 2016). These bacteria is distributed in a wide range whether in the river (Cottrell et al. 2005), estuary (Sharma 2015), terrestrial (Reimmann et al. 1997) dan seawater (Maldonado et al. 2005). Actinobacteria is predominantly mesophilic that growth optimally in 25 and 30°C. Some grow well at 50–60°C (Goodfellow 2012).

Up to 50 years, there are more than 10.100 natural compounds of antibiotics produced from filamentous Actinomycetales. *Streptomyces* sp. are dominated and cover 7.600 compounds, while 2.500 are discobered from rare Actinomycetes. Actinobacteria have represented the most significant part of microbial bioactive metabolite (45%) from the known 22.500 antibiotics from microbes. Amongst those, only 1% (150 compounds) have been developed into drugs. This antibiotic has been used for human, animal, and agricultural purposes (Berdy 2005).

The trend of using the silent gene has been developed in order to identify the new natural antibiotic. Moreover, research on the silent gene has been done to identify the new antibiotic natural product (Pettit 2011; Moody 2014; Abdelmohsen et al. 2015). Alexander Fleming introduced the co-culture strategy in

1928 by discovering β-lactam antibiotic penicillin (Fleming 1929). This was then followed by several similar inventions. Some research in terms of Actinobacteria pointed to the strategy of activating the silent gene of antibacterial by elicitation chemically (Kawai et al. 2007), biologically (Schäberle et al. 2014) and molecularly (Hosaka et al. 2009). Recently, the study in Actinobacteria silent gene as an antibacterial by Actino-Actino (Dashti et al. 2014), Actino-other bacteria (Luti and Mavituna 2011c) and Actino-Fungi (König et al. 2013) remarkably promising and worthy. This research aims to discover and identify the potential Actinobacteria from the mangrove sediment and root with co-culture as an antibacterial agent.

Material And Methods

The mangrove sampling

The mangrove sediment and root sample were collected from Jepara, Central Java, Indonesia (S6°30'40,8952"; E110°40'11,9470") (Fig. S1). The sampling methods were referred to Sangkanu et al. (2017) and Jiang et al. (2018) with some modification. The sediment sample is taken at 0–5 m in depth and kept into the sterile plastic pack. The root of the mangrove was cut off, the tip of the root was then burned. Soon as fire sterilization, the tips of the root are wrapped off with parafilm. The other parts of the root were then cleaned and rinsed with 70% alcohol and sterile aquadest. The root sample was then collected into sterile polyethylene. Lastly, all the samples were taken into a coolbox and ready to be further analyzed, no more than 48 hrs.

Isolation and purification Actinobacteria.

Actinobacteria isolation used spread plate methods (Jett et al. 1997; Herigstad et al. 2001) with some modification. ISP 2 media, contained 4.0 g Bacto-Yeast Extract, 10.0 g Bacto-Malt Extract, 4.0 g Bacto-Dextrose dan 20.0 g Bacto agar were administered (Shirling and Gottlieb 1966). Gauze's Medium No. 1 was also conducted. This media contained 20.0g Soluble starch, 1.0g KNO_3, 0.5g K_2HPO_4, 0.5g $MgSO_4$, 0.5g NaCl, 0.01g $FeSO_4$ dan Agar 30.0g (Atlas 2010) with cycloheximide (10mg/ml) and nalidixic acid (10mg/ml) addition (Rajagopal and Kannan 2017). There were five steps of root isolation procedures. It was started with surface sterilization by rinsing in 5% NaOCl (4–10 minutes), followed by 2.5% $Na_2S_2O_3$ (10 minutes), and 5 minutes rinsing in 75% ethanol and sterile water and cleansed in 10% $NaHCO_3$ (10 mins) (Qin et al. 2009). All the root samples were then blended into powder and diluted at 10^{-3}. One hundred µL of this final solution was then plated in solid media. Leave these for 2–5 weeks to incubate (Liu et al. 2016; Jiang et al. 2017; Jiang et al. 2018).

The sediment sample was dried in the air for 7 days at room temperature. The 10 g of sediments was heated up to 55 ^oC (6 minutes) for bacterial selection, suspended to 100 mL sterile seawater and stirred well for 30 mins (Huang et al. 2015; Huang et al. 2018). The sediment suspension was then diluted serially until 10^{-3} by sterile seawater. The 100 µL of the diluted solution was plated into media and

incubated at 28 ± 2^{o}C for 10 to 28 days (Retnowati et al. 2017). The different colony was then poured in agar media Gauze no.1 (Retnowati et al. 2017; Jiang et al. 2018). The glycerol stock was made by preparing the liquid culture media. Freeze and stored the mycelia glycerol stock media in − 80^{o}C (Shepherd et al. 2010).

Morphological and microscopic characterization of Actinobacteria strains

The colony morphology of the isolates was observed for colour, aerial and substrate mycelium, as well branching. The isolates grown were then observed for spore chain morphology. The microscope with adequate magnification (400x) was used to examine the presence of spore chains and observe the sporophores' nature (Priyanka et al. 2019).

Microbial Strain and Susceptibility

The microbes strain used were bacteria and fungus. *Staphylococcus aureus* FNCC-0047 and *Pseudomonas aeruginosa* ATC-9027 as bacteria and *Aspergillus fumigatus* FNCC-6127 as fungus. Those isolates were purchased from Microbiology Laboratory, Center of Food Study, Gadjah Mada University.

The assessment of antibiotic resistance was only done on bacterial isolates. The bacterial susceptibility to antibiotics was carried out by Kirby-Bauer disc diffusion test using the 6 mm diameter of Whatman no. 3 disc paper (Bauer et al. 1966). The type of antibiotics and inhibition interpretation (resistance, intermediate and sensitive) was specific in each bacteria, according to CLSI m100. The commercial antibiotics and assessed concentration against *Staphylococcus aureus* were Tetracyline (30 µg), Doxycline (30 µg), Rifamycin (5 µg), Erythromycin (15 µg), Azhytromycin (15 µg), Gentamycin (10 µg), Penicilin (10 units), Ofloxacin (5 µg), Minocycline (30 µg), Chloramfenicol (30 µg), Ciprofloxacin (5 µg), Levofloxacin (5 µg), and Clindamycin (2 µg), respectively. Ceftadazime (30 µg), Gatifloxacin (5 µg), Gentamycin (10 µg), Levofloxacin (5 µg), Ciprofloxacin (5 µg), Tobramycin (10 µg), Doripenem (10 µg) and Meropenem (10 µg) were tested against *Pseudomonas aeruginosa*. Nutrient broth (Merck) was applied as culture media, and Muller-Hinton Agar (Merck) was used as testing media. The bacteria were cultured at liquid media for 18 hrs to get the 0.5 McFarland standard (Andrews 2001). The inhibition zone was then be categorized as resistance, intermediate and sensitive based on Clinical and Laboratory Standards Institute (CLSI) m100 (CLSI 2018).

Co-Culture Fermentation, Extraction and Screening for Antibacterial agents

The fermentation process was administered with a total of 574 fermenters and six fermenters (as control). These included 28 single culture fermenters and 546 co-culture fermenters and were divided into three co-cultures: the Actino-Actino (378 fermenters), Actino-other bacteria (112 fermenters) and Actino-Fungus (56 fermenters). The control fermenter used one strain live cell and dead body (killed by bolling water), ie. two bacteria species (*S. aureus* and *P. aeruginosa*) and one fungus species (*A. fumigatus*).

Methods used in this study is referred to König et al. (König et al. 2013), Ding et al. (2015), Luti and Mavituna (2011a), Luti and Mavituna (2011b), and Kawai et al. (Schäberle et al. 2014) with slight modification. All media used, namely ISP 2 (Actinobacteria), Nutrient Broth (Merck) for other bacteria and Potato Dextrose Broth (Merck) as Fungi seed media and media 27 as fermentation media. One ose bacterial culture were inoculated in seed media (1×10 mL/vial) and then followed by 24 hrs (*S. aureus* and *P. aeruginosa*) and 48 hrs (Actinobacteria and *A. fumigatus*) incubation at 28°C. We measured 500 µL from single strain fermentation culture and 250 µL for co-culture fermentation, then we moved into 20 mL vial. These were then added with media 27 until reached 4.5 mL and continued with incubation at 28°C for 14 days. After incubation, the supernatant on each vial was centrifuged and extracted using ethyl acetate 2:1 (v:v). The ethyl acetate fraction was separated, taken out at 1 mL and transferred into the vial. The vial was evaporated in a hotplate (40°C). As it dried, the paper disk was ready to use and screened for the antibacterial activity.

Antibacterial screening of the extracts was determined by disc diffusion assay (Bauer et al. 1966). The test bacteria applied were *S. aureus* and *P. aeruginosa* (Govindarajan et al. 2014).

The bacteria were cultured in NB media until the density reached at 0.5 McFarland standard. These were then added with 20 mL of NA media, waited until get hardened, and cultured the bacteria in a small sterile cotton swab (onemed) on the surface of the agar media. This then continued by leaving for the bacterial inoculum to dry (3–5 minutes). We put the disc paper into a petri dish that has been inoculated with the test bacteria. Petri dishes were incubated at 37°C for 24 hours (Jiang et al. 2018).

Antagonist test against S. aureus *and* P. aeruginosa

The antagonist test was carried out using the reference overlay method by Shetty et al. (2014) with modifications using Gauze medium no 1. The test bacteria *S. aureus* and *P. aeruginosa* were incubated for 24 hours. We cultured the bacteria in a small sterile cotton swab (onemed) on the surface of the agar media and leave to dry (Bauer et al. 1966). Pure Actinobacteria strains were scrapped in the middle of the agar using an ose needle. Petri dishes were incubated at 30°C for 72 hours. The visible zone of inhibition will be recorded as positive results and indicated by light-strong production.

Co-Culture Fermentation, Extraction and Antibacterial test

All fermented products were extracted with ethyl acetate, referring to Thirumurugan et al. (2018) with slight modifications except Actinobacteria-*P. aerugenosa* using pyocyanin extraction and purification method. The supernatant (except for Actinobacteria-*P. aerugenose*) was extracted with an equal volume of ethyl acetate (2:1). Ethyl acetate supernatan was separated from the mycelia using centrifugation at 5000 rpm for 15 minutes and was then filtered using Whatman No.1. The mixture was collected and concentrated with a rotary evaporator at 50°C.

Actinobacteria-*P. aerugenose* culture cells and supernatant were separated, and extracted using chloroform 3:5 (v/v). Chloroform partition was reextracted using 1 ml of 0.2 N HCl. The colour was turned from pink to deep red solution and then reseparated (Essar et al. 1990). The extract was neutralized using

0.1 M NaOH and turned into blue colour. The extract was repeated 2–3 times (El-Shouny et al. 2011; El-Zawawy and Ali 2016). Prior to neutralization using NaOH, we were measured the concentration of pyocyanin quantity (mg/ml) using 520 nm spectrophotometer. In this study, the intervals of 20 nm were recorded in a range of 420–600 nm The supernatant was calculated with an absorbance coefficient of 520 (17.072) and calculated based on this following equation (Watson et al. 1986; Essar et al. 1990).

Pyocyanin Concentration (µg/ml) = O.D520 * 17.072

The antibacterial activity of the extract was determined by disc diffusion assay. The compound will show the activity by producing the inhibition zone on Muller–Hinton (MHA) agar (Merck) (Bauer et al. 1966). The filter paper disc used was Whatman, no. 3 (Valgas et al. 2007). Mueller-Hinton Agar (Merck) was used as a test medium with a composition of 300.0 g beef infusion, 17.5 g acid hydrolysate of casein, 1.5 g starch and 17.0 g agar (Mueller and Hinton 1941; Atlas 2010). Bacterial density was adjusted to the standard 0,5 McFarland (1–2 x 10^8 CFU). The test bacteria used were *S. aureus* and *P. aeruginosa* (Govindarajan et al. 2014). The crude extract was concentrated to 500 mg/mL and added 20 µL crude extract/ paper disc, and dried. The final concentration of the extract on the paper disc was 10 mg/disc.

The bacteria were cultured in NB media until the density reached to the standard of 0.5 McFarland. These were then continued by added 20 mL of MHA media, waited until hardened, and the bacterial culture was swabbed on the MHA surface in a petri dish. Bacterial inoculum was then allowed to dry for 3–5 minutes (Bauer et al. 1966). We put three paper discs as repetitions into a petri dish (Radjasa et al. 2007), incubated at 37°C for 24 hours (Jiang et al. 2018) and 48 hours (Sabdono et al. 2019). These results were observed by measuring the inhibition zone surrounding the paper disc area using a digital calliper.

Co-cultivation in solid media

Co-cultivation in solid media carried out using the reference by Traxler et al. (2013) with some modification. All experiments were done on 1:10 (v/v) ISP2 (Shirling and Gottlieb 1966), media 27 (Ding et al. 2015) and 20 g agar. For all experiments, 10 ml of agar was added to standard 100-mm petri plates with 2 mm of thickness. This is suitable to the preparation for IMS. One ose strains were inoculated onto agar and was spotted to the single as well as to the co-cultures. Petri plates were incubated at 28 ± 2 ^{o}C for about 1–2 weeks.

Identification Actinobacteria

Identification was carried out molecularly to determine the type of Actinobacteria and phylogenetically defined (Jiang et al. 2018).

Result

Sample collection

Mangroves were taken from Ujung Piring, Central Java, (Fig. 1). We have collected mangrove roots, and sediment. This root characteristically by brownish-greenish black colour, with an inverted peg with a pointed tip at the top in shape (Fig. 1b). The sediment was physical characterized by granular sand with a small amount of fine sand on 1–3 cm at the top and a bit of silt subsequently (Fig. 1c). The flower has 6 to 8 petals with the green colour in outer skin and reddish inside with a bell shape (Fig. 1d). The leaves are light green, shaped like an inverted ovoid and rounded ends (Fig. 1e). Based on the characteristics described, this mangrove has identified as *Sonneratia alba* (Kusmana and Onrizal 2003; Giesen et al. 2007). *S. alba* is a pioneer species in mangrove habitat, intolerant to freshwater exposure. The preferred substrate is consolidating silt and sand, occasionally rock, coral and gravel. It is often found in coastal locations protected from intense wave action and in estuary areas and around offshore islands. The distribution of *Sonneratia alba* grows from East Africa, the Seychelles and Madagascar, throughout Southeast Asia, to tropical Australia, New Caledonia, the west Pacific islands and Southwest Oceania. Southeast Asia includes various countries such as Brunei, Cambodia, Indonesia, Malaysia, Myanmar, PNG, Philippines, Singapore, Thailand and Viet Nam (Giesen et al. 2007).

Isolation and Purification Actinobacteria

Isolation and Purification Actinobacteria (Fig. S2) resulted in some various types of Actinobacteria.

The purification results are presented in Table 1. We have purified 28 isolates from the total of 106 isolates. The 28 pure isolates consist of 18 from sediment and 10 from mangrove roots. Sediment isolate codes were 3, 6, 10, 14, 17, 31, 32, 34, 35, 37, 39, 41, 45, 46, 51, 52, 71 and 87. While root isolates were encoded as 13, 16, 22, 26, 42, 62, 63, 64, 72 and 90, respectively.

Actinobacteria Characterization

The pure Actinobacteria isolates were characterized macroscopic and microscopically. The visible results are presented macroscopically in Table S1 and microscopically (Table S2). Actinobacteria from sediments have aerial hypha with cream white, white, dark grey, bluish-grey, brown-grey, yellowish ash and white ash in colour. In other hand, roots isolates have dark grey, dark brown, white-grey, middle white brown and middle white grey colours. Actinobacteria mycelium substrates from sediment were black, white, dark grey, brown, dark brown, blackish brown, cream blackish, brownish-yellow, yellow, cream, dark orange and grey. Main while, Actinobacteria from roots were black grey, grey-brown, dark brown, reddish-brown, cream, white, cream brown and red-black. Based on Table S2, all the Actinobacteria microscopy isolates predominantly by the spiral shape. Other observed forms were Verticillatispore and Rectiflexibilesspore chains. Based on this, all the Actinobacterias are characterized to the genus of *Streptomyces* sp. (Nomi 1960; Williams and Davies 1967; Tashiro et al. 1990; Nomi 1992).

Susceptibility of S. aureus and P. aerugenosa

The *S. aureus* and *P. aerugenosa* bacteria were tested against several antibiotics. The mixed results obtained are presented in Table S3. *P. aerugenous* bacteria were sensitive to all types of antibiotics tested, including Ceftadazime, Gatifloxacin, Gentamycin, Levofloxacin, Ciprofloxacin, Tobramycin, Doripenem and Meropenem. Different results were shown in *S. aureus* bacteria which have Resistance, Intermediate and Sensitive categorized results. The resistance of *S. aureus* was found in Penicillin and Rifamycin antibiotics. Erythromycin, Azithromycin and Clindamycin antibiotics had no inhibition zones detected. The intermediate categorized were found in the antibiotics Minocycline and Chloramphenicol. Sensitive interpretation of *S. aureus* was found in several types of antibiotics including Tetracycline, Doxycycline, Gentamycin, Ofloxacin Ciprofloxacin, and Levofloxacin.

Fermentation and Screening Antibacterial agents

The fermentation process with a total of 574 fermenters and six fermenters as controls are presented in Figure S2. Before Fermentation (Fig. 2a), After 14 days of Fermentation (Fig. 2b), Extraction with Ethyl Acetate: culture (2:1) (Fig. 2c) and Antibacterial Screening against *S. aureus* (Fig. 2d) and *P. aeruginous* (Fig. 2e). All antibacterial screening resulted positively against the bacteria test (1 bacteria or both) were found in the co-culture method (Table S4). Positive results on screening were 21 pairs co-culture fermenters, including 8 actino-actino, 3 Actino-*S. aureus* (2 life cultures and 1 dead body), 1 Actino-*P. aerugenosa* (Life culture) and 9 Actino- *A. fumigatus* (5 life cultures and 4 dead cultures). Eight fementers actino-actino were co-culture strains 64/90, 10/46, 6/37, 14/13, 14/31, 45/62, 41/46 and 3/41. Three fermenters Actino-*S. aureus* with life cultures covering strains 6 and 10 and with dead cells, namely 63. Actino-*P, aerugenosa* (life culture) fermenter is strains 41. The isolate code for Actino-Aspergillus fumigatus 9 fermenters in life culture includes strains 52, 35, 37, 63 and 62, and dead culture include strains 62, 64, 63 and 10, respectively.

Antagonist test against S. aureus and P. aeruginosa

Actinobacteria antagonist test against *S. aureus* and *P. aeruginosa* has positive and negative results. The results are presented in Fig. S3. Antagonist tests have 3 categories in Fig. S4. Antagonist tests against *S. aureus* with strong positives were resulted from 6, 35, and 90 isolates, and 46 isolate against *P. aeruginosa*, respectively. Light positive against both bacteria were found in strains 45, 62, 63, and 64, while 10 and 46 isolates against *S. aureus*. Negative results for both bacteria were from 3, 13, 14, 31, 37, 41, and 52 isolates, respectively. Other negative results for *P. aeruginosa* were developed from 6, 10, 35 and 90 isolates, respectively.

Co-culture Fermentation, Extraction dan Antibacterial test

The 100 mL co-culture fermentation, extraction and testing were carried out for the screening, except for Actino-*P. aeruginosa* (500 mL). The extraction flow chart and purification pyocyanin are presented in Fig. 3. Test results of crude extract from co-culture fermentation are presented in Fig. 4. The weight of the co-culture extract has an average of 0.16 grams. The highest extract weight was obtained from co-culture strains 45/62, 63 (dead body *S. aureus*), and 63 (dead body *A. fumigatus*) in different co-cultures with Actinobacteria, other bacteria, and fungi. The antibacterial activity of the extract against *S. aureus* and *P.*

aerugenosa resulted in 21 positive tests and 16 positive tests, respectively. Clear zones from co-culture extracts had an average of 13.02 ± 0.64 (*S. aureus*) and 12.12 ± 0.54 (*P. aeruginosa*). The highest zone of inhibition against *S. aureus* was found in extract 10 (Actino)-*Aspergillus fumigatus* (dead body) and for *P. aerugenosa* was found in extract strain 6/37 (Actino-actino). Strain 10 had the lowest extract weight in the three co-cultures (Actino, *S. aureus* and *A. fumigatus*). Strain 10 had the highest zone of inhibition against both test bacteria (except against *P. aeruginosa*, the 2nd highest) with three types of co-culture. Extract weight of 6/37 was the second-lowest after 10/46, but the extract was increased as the strains 6 and 37 were co-cultured separately with *S. aureus* (life culture) and *A. fumigatus* (life culture). This co-culture also resulted in decreased effectiveness against *P. aeruginosa*. Co-culture strain 45/62 had high extract weight but low activity and contrast in strain 63 (dead body *S.aureus*). Even though strain 63 was decreased when co-cultured with *A. fumigatus* (live and dead). On the other hand, strain 41 activated pyocyanin on *P. aeruginosa* even though the extract was small because it was susceptible and degraded fastly. Pyocyanin with a concentration of 0.1806 µg/disc has a clear zone of 10.69 ± 0.13 mm. In addition, strain 41 can be co-cultured with other Actinobacteria, such as strains 3 and 46.

Co-cultivation in solid media

Based on Table 2, there were five combinations developed the extracellular compounds. The co-cultured strains 13/14 had a red compound. The color was obtained from both lines when one colony was predominant. Strain 14 was also co-cultured with strains 31, producing a dark brown color shared by strains 31. Strain 41 could induce *P. aeruginous* to produce pyocyanin (green-blue pigment). Strains 35 and 62 can induce yellow compounds in *A. fumigatus*. Co-culture of Strain 41 with strains 3 and 46 did not produce colored compounds but showed antibacterial activity after being covered with the bacteria test. This results were also occured in other strains.

Molecular Identification of Actinobacteria

Identification of Actinobacteria was carried out for only a few strongest strains. The identification results are presented in Table S5 and the phylogenetic tree is depicted in Fig. S5. Based on Table S5, identification was carried out on the isolate codes 6, 10, 37, and 41. All of the strains are *Streptomyces* spp. Strain isolate 6 was identified as *Streptomyces albogriseolus* with accession number MZ701854, sequence length 1408 Bp, and have 99.79% of similarity. Strain coded isolate 10 was identified as *Streptomyces labedae* with accession number MZ701855, sequence length 1396 Bp, noted 99% of similarity. Strain coded isolate 37 was identified as *Streptomyces rochei* with accession number MZ558503, sequence length 1427 Bp, and observed at 93.52% of similarity. The isolate coded strain 41 was identified as *Streptomyces flavalbus* with accession number MZ540332, sequence length 1399 Bp with 98.07% similarity.

Discussion

Streptomyces flavalbus was first isolated in 2018 by Cao et al. (2018) from the rhizosphere of corn (*Zea mays* L.). In fact, *Streptomyces flavalbus* strains 41 from mangrove sediments co-culture with *P.*

aeruginosa was able to produce pyocyanin. Pyocyanin is a blue redox-active secondary metabolite produced by *Pseudomonas aeruginosa*. In *Pseudomonas aeruginosa*, endogenous pyocyanin antibiotics activate SoxR, a transcription factor stored in Proteo- and Actinobacteria. *P. aeruginosa* and *Streptomyces coelicolor* revealed that most SoxR regulation in bacteria lacks the genes required for the stress response, despite many of these organisms still producing redox-active small molecules, which suggests that redox-active pigments play an independent role of oxidative stress (Dietrich et al. 2008). Through mutation analysis, Dietrich et al. (2006) demonstrated that pyocyanin is a physiological signal for the upregulation of this quorum-sensing-controlled gene during the stationary phase and that the response is mediated by the transcription factor SoxR. This compound has antibacterial activity against *Staphylococcus aureus* (Baron and Rowe 1981; Rahman et al. 2009; Abdul-Hussein and Atia 2016), *Escherichia coli* (Baron and Rowe 1981; Abdul-Hussein and Atia 2016), *Micrococcus luteus*, *Bacillus subtilis* (Baron and Rowe 1981; Rahman et al. 2009), *Proteus vulgaris*, *Bacillus licheniformnis*, Acinetobacter, *Paracoccus denitrificans* (Baron and Rowe 1981) P. aeruginosa (Rahman et al. 2009; Abdul-Hussein and Atia 2016) *Pseudomonas teessidea*, and *P. clemancea* (Rahman et al. 2009).

Neomycin was isolated from *Streptomyces albogriseolus* in 1954 by Benedict et al. (1954). These compounds can be developed as an antibacterial against Streptomycin-Resistant Bacteria (Waksman and Lechevalier 1949). Furthermore, *Streptomyces albogriseolus* isolates K36C5 from soil samples (depth 10–15 cm) in the northwest Iran has antibacterial against *E. coli*, *S. aureus*, *Y. enterocolitica*, and *B. cereus* (Maleki et al. 2013). While *Streptomyces albogriseolus* from marine environment has an activity against *Bacillus cereus* ATCC 14579 and *Escherichia coli* ATCC 13706 (Gong et al. 2018). It was explained that Methyl-4,8-dimethylundecanate from the marine actinobacterium *Streptomyces albogriseolus* ECR64 was active against *Pseudomonas fluorescens, Vibrio cholerae, V. parahaemolyticus, V. alginolyticus*, and *Aeromonas hydrophila* (Thirumurugan et al. 2018). In another study, *Streptomyces albogriseolus* A2002 led to the isolation of echinosporin and 7-deoxyechinosporin. Flow cytometric analysis suggested that these two compounds exerted their anti-proliferative effects on those cells through inhibiting the cell cycle at the G2/M phase and inducing apoptosis (Cui et al. 2007). Additionally, *Streptomyces albogriseolus* was produced Fungichromin B (6'-methyl-fungichromin). These Actinobacteria was effective against *Saccharomyces cerevisiae, Fusarium oxysporum*, and *Aspergillus niger* (Zeng et al. 2013). Benzo[f][1,7]Naphthyridine that isolated from *Streptomyces albogriseolus* originating from mangrove sediments from *Avicennia alba* (Li et al. 2010). One of the improvements in developing the bacterium *Streptomyces albogriseolus* was also successful using Biosynthesized with AgNPs which inhibit the growth of food pathogens such as *Bacillus cereus, Escherichia coli*, and *Staphylococcus aureus* (Samundeeswari et al. 2012). Some research in relation to the co-culture was also conducted on *Streptomyces albogriseolus* with *Brevibacillus borstelensis* from the field soils in the northwestern region of India. The Actinobacteria was managed to reduce the concentration of sulfosulfuron from 10 g/ml in an initial hour from 8.34 g/ml in 12 h to 6.66 g/ml in 20 h (Arya et al. 2016). However, there has been no research on co-culture with *Streptomyces albogriseolus* as an antibacterial.

Streptomyces labedae isolated from the sediments of the east coast (Bay of Bengal) region of Tamilnadu, India have antibacterial against pathogenic fish bacteria such as *Vibrio cholerae, V. parahaemolyticus, V. alginolyticus, Pseudomonas fluorescens*, and *Aeromonas hydrophila* (Thirumurugan and Vijayakumar 2015). Moreover, *Streptomyces labedae* from specimens of the marine sponge *Mycale* sp. exhibited the most potent antimicrobial activity against *Staphylococcus aureus* MTCC 1430, *Bacillus subtilis* MTCC 44, and *Vibrio species* (*Vibrio diabolicus* and *Vibrio parahaemolyticus*) (Su et al. 2014). *S. labedae* (soil samples salted area) was able to inhibit *S. aureus, B. subtilis*, and *S. viridochromogenes* (Sajid et al. 2008) and biofilm-forming ability of Acinetobacter and Moraxella (Saleem et al. 2015). In addition, *Streptomyces labedae* ASU-03 has the degrading ability of Ficus elastica rubber latex, isolated from Egyptian soil (Hesham et al. 2015). However, there has been no research on co-culture with *Streptomyces labedae* as an antibacterial agent.

In this study, *Streptomyces rochei* co-cultured with *Streptomyces labedae* (Lacey 1987) and *A. fumigatus* can factually increase the antibacterial production by activating silent genes, postulately. *Streptomyces* sp. 7434-AN4 with linear plasmid-like DNA (pSLA2) produce lankacidin (Hayakawa et al. 1979). *Streptomyces rochei* 7434-AN4 has three large linear plasmids, pSLA2-L, M and S. The involvement of pSLA2-L had an important role in producing lankacidin and lankamycin in *S. rochei* 7434-AN4 (Kinashi et al. 1994). Futhermore, *Streptomyces rochei* strain 7434AN4 was found to span 31 kb of the giant linear plasmid pSLA2-L and contains a polyketide synthase (PKS)/nonribosomal peptide synthetase (NRPS) hybrid gene (lkcA), type I PKS genes, and pyrroloquinoline quinone (PQQ) biosynthetic genes (lkcK-lkcO) (Arakawa et al. 2005). Additionally, Bundlin A (lankacidin) and bundlin B (Uramoto et al. 1969) have antibacterial properties (Sakamoto et al. 1962; Tsuchiya et al. 1971). Research by Arakawa et al. (2012) reported that the presence of two active components SRB1 (2-(1'-hydroxy-6'-oxo-8'-methylnonyl)-3-methyl-4-hydroxybut-2-en- 1,4-olide) and SRB2 (2-(1'-hydroxy-6'-oxo-8'-methyldecyl)-3-methyl-4-hydroxybut-2-en-1,4-olide) can increase Lankacidin and Lankamycin Production in *Streptomyces rochei*. Moreover, research co-culture of *Streptomyces rochei* MB037 with a gorgonian-derived fungus *Rhinocladiella similis* 35 was carried out to stimulate the production of new, including two new fattymacids with rare nitrile group, borrelidins J and K (1 and 2), one chromone derivativemas a new natural product, 7-methoxy-2,3-dimethylchromone-4-one (3), together with two known 18-membered macrolides, borrelidin (4) and borrelidin F (5). The five compounds have the ability as antibacterial (Yu et al. 2019). Lately, in his study, Hashimoto et al. (2020) discovered a novel cryptic biosynthetic gene cluster in *Streptomyces rochei* IFO12908, a new polyene macrolactam compound from the JBIR-156 biosynthetic gene cluster. The biosynthetic gene uses the SUKA strain host derived from *Streptomyces avermitilis*.

Several studies have shown that Actino-actino can produce and increase the production of antibiotics. Co-culture between *Streptomyces tanashiensis* IAM0016 and *Streptomyces griseus* st-21-2 produced compounds that could inhibit the growth of *Bacillus subtilis* bacteria (Yamanaka et al. 2005). Rhodostreptomycin A and B were produced from Co-cultures of *Rhodococcus fascians* and *Streptomyces padanus*. These compounds have antibacterial abilities, including *Streptomyces padanus*, *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, and *Helicobacter pylori* (Kurosawa et al. 2008). Another study revelead that *Streptomyces lividans* TK23, was induced by co-culture with *Tsukamurella pulmonis*

TP-B0596 produced a new antibiotic, Ichivemycin A (Onaka et al. 2011). Moreover, *Actinokineospore* sp. EG49 and *Nocardiopsis* sp. RV163 succeeded in inducing biosynthesis by producing 3 natural products that were not produced by a single culture, namely N-(2-hydroxyphenyl)-acetamide, 1,6-dihydroxyphenazine and 5a,6,11a,12-tetrahydro-5a,11a-dimethyl[1, 4] benzoxazino[3,2-b][1,4]benzoxazine. Only compound 1,6-dihydroxyphenazine has antibacterial activity against *Bacillus* sp. P25 (Dashti et al. 2014). *Streptomyces coelicolor* interacting with Amycolatopsis sp. AA4 produces antibiotic compounds, namely acyl-desferrioxamine (acyl-DFO), -Actinorhodin and prodiginine (Traxler et al. 2013).

Co-culture research that produced and pointed to increase antibacterial production is also carried out on Actino-other bacteria with live cells and dead bodies. *Streptomyces griseorubiginosus* 43708 was stimulated by a mixed culture with *Pseudomonas maltophilia* 1928 produced the antibiotic biphenomycin A (Ezaki et al. 1992; Ezaki et al. 1993). Another study reported that *Streptomyces tenjimariensis* produces the antibiotics istamycin. Twelve of the 53 bacterial species tested induce Istamycin production and inhibit competing colonies' growth (Slattery et al. 2001). Moreover, *Myxococcus xanthus* cells prey on *S. coelicolor*. The interaction increases the antibiotic compound actinorhodin production by *S. coelicolor* up to 20-fold and triggers aerial mycelium production (Pérez et al. 2011). Elicitation with dead body bacteria *B. subtilis* increased the maximum undecylprodigiosin concentration by threefold and *S. aureus* by fivefold compared with the pure culture of *S. coelicolor* (Luti and Mavituna 2011c). In addition, interacted with live or heat-killed cells of *Bacillus subtilis*, *Streptomyces coelicolor* A3 (2), increased undecylprodigiosin production up to 175–211% in the bioreactor, 256% in comparison to the pure cultures of *S. coelicolor* (Luti and Mavituna 2011b). *Streptomyces coelicolor* was also produced bioactive compounds, namely undecylprodigiosin (Red) with the corallopyronin A producer, *Corallococcus coralloides*, which could be enhanced by a myxobacterial competitor and increased production of Red a 60-fold of the intracellular concentration (Schäberle et al. 2014). Proteobacteria (Burkholderia vietnamiensis ATCC BAA-248, *Brucella neotomae* ATCC 23459, *Yersinia pestis* A1122, and *Xanthomonas axonopodis* ATCC 8718) induced the production of resistomycin in *Streptomyces* sp. B033 at significantly higher rates (65%) (Carlson et al. 2015).

Co-culture study of Actinomycetes–Fungi resulted in new product which able to increase the production of antibacterial agents. Marine-derived fungus *Emericella* sp., induced the production of emericellamides A and B was observed during co-culture with the marine actinomycete *Salinispora arenicola* (Oh et al. 2007). *Streptomyces rapamycinicus* was able activate a silent biosynthetic gene cluster in the human pathogenic fungus *A. fumigatus* produces fumicyclines A and B (König et al. 2013). Lastly, *Fusarium tricinctum* was co-cultivated with *Streptomyces lividans* can increase enniatin A1 and B1 against *S. aureus, S. pneumoniae*, and *E. faecalis* (Ola et al. 2013).

Conclusion

Conclusion in this work, we co-cultured several combination of Actino-Actino, Actino-other bacteria and Actino-fungi. There were 28 strains Actinobacteria from *Sonneratia alba* sediment and root foundly active to pathogenic *Staphyoococcus aureus* and *Pseudomonas aeruginosa.* All 28 strains were identified as

Streptomyces spp. Besides the potential *Streptomyces albogriseolus, Streptomyces labedae* and *Streptomyces rochei, Streptomyces flavabus* is a promising firstly reported Actinobacteria from Indonesian tropical waters.

Declarations

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Authorship contribution statement

Nuril Azhar.: Conceptualization, methodology, validation, formal analysis, investigation, Ervia Yudiati.: resources, writing—original draft preparation, writing—review and editing, visualization, supervision, project administration, funding acquisition. Both authors have read and agreed to the published version of the manuscript.

Funding

The authors gratefully acknowledge funding from the Indonesian Government's Ministry of Research and Higher Education (contract no: 187-64/UN7.6.1/PP/2021).

Availability of data and materials

The study's original contributions are included in the journal material; further questions can be directed to the corresponding author/s.

Acknowledgements

We would like to state my special thanks of gratefulness to Diponegoro University and the Indonesian Government's Ministry of Research and Higher Education.

References

1. Abdelmohsen UR, Grkovic T, Balasubramanian S, Kamel MS, Quinn RJ, Hentschel U (2015) Elicitation of secondary metabolism in actinomycetes. J Biotechnol Adv 33:798-811 doi: https://doi.org/10.1016/j.biotechadv.2015.06.003
2. Abdul-Hussein Z, Atia S (2016) Antimicrobial effect of pyocyanin extracted from *Pseudomonas aeroginosa*. Eur J Exp Biol 6:1-4
3. Andrews JM (2001) Determination of minimum inhibitory concentrations. J Antimicrob Chemother 48:5-16 doi: https://doi.org/10.1093/jac/48.suppl_1.5

4. Arakawa K, Sugino F, Kodama K, Ishii T, Kinashi H (2005) Cyclization mechanism for the synthesis of macrocyclic antibiotic lankacidin in *Streptomyces rochei*. Chem. Biol. 12:249-256 doi: https://doi.org/10.1016/j.chembiol.2005.01.009
5. Arakawa K, Tsuda N, Taniguchi A, Kinashi H (2012) The butenolide signaling molecules SRB1 and SRB2 induce lankacidin and lankamycin production in *Streptomyces rochei*. Chembiochem 13:1447-1457 doi: https://doi.org/10.1002/cbic.201200149
6. Arya R, Mishra NK, Sharma AK (2016) *Brevibacillus borstelensis* and *Streptomyces albogriseolus* have roles to play in degradation of herbicide, sulfosulfuron. 3 Biotech 6:246 doi: https://doi.org/10.1007/s13205-016-0562-z
7. Atlas RM (2010) Handbook of microbiological media 4th Edition. CRC press
8. Baron SS, Rowe JJ (1981) Antibiotic action of pyocyanin. Antimicrob. Agents. Chemother. 20:814-820 doi: https://doi.org/10.1128/aac.20.6.814
9. Bauer A, Kirby W, Sherris JC, Turck M (1966) Antibiotic susceptibility testing by a standardized single disk method. Am J Clin Pathol 45:493 doi: https://doi.org/10.1093/ajcp/45.4_ts.493
10. Becker B, Lechevalier M, Lechevalier H (1965) Chemical composition of cell-wall preparations from strains of various form-genera of aerobic actinomycetes. Appl Microbiol. 13:236-243 doi: https://doi.org/10.1128/am.13.2.236-243.1965
11. Benedict RG, Shotwell OL, Pridham TG, Lindenfelser LA, Haynes WC (1954) The production of the neomycin complex by *Streptomyces albogriseolus*, nov. sp. Antibiot. Chemother. 4:653-656
12. Berdy J (2005) Bioactive microbial metabolites. J Antibiot 58:1 doi: https://doi.org/10.1038/ja.2005.1
13. Cao T et al. (2018) *Streptomyces flavalbus* sp. nov., an actinobacterium isolated from rhizosphere of maize (Zea mays L.). Antonie Van Leeuwenhoek 111:1047-1054 doi: https://doi.org/10.1007/s10482-017-1004-6
14. Carlson S, Tanouye U, Omarsdottir S, Murphy BT (2015) Phylum-specific regulation of resistomycin production in a *Streptomyces* sp. via microbial coculture. J Nat Prod 78:381-387 doi: https://doi.org/10.1021/np500767u
15. CLSI (2018) Performance Standards for Antimicrobial Susceptibility Testing, 28th Edn. Wayne, PA: Clinical and Laboratory Standards Institute. CLSI supplement M100. 38
16. Cottrell MT, Waidner LA, Yu L, Kirchman DL (2005) Bacterial diversity of metagenomic and PCR libraries from the Delaware River. Environ Microbiol 7:1883-1895 doi: https://doi.org/10.1111/j.1462-2920.2005.00762.x
17. Cui CB et al. (2007) Echinosporins as new cell cycle inhibitors and apoptosis inducers from marine-derived *Streptomyces albogriseolus*. Fitoterapia 78:238-240 doi: https://doi.org/10.1016/j.fitote.2006.11.017
18. Dashti Y, Grkovic T, Abdelmohsen UR, Hentschel U, Quinn R (2014) Production of induced secondary metabolites by a co-culture of sponge-associated actinomycetes, *Actinokineospora* sp. EG49 and *Nocardiopsis* sp. RV163. Mar Drugs 12:3046-3059 doi: https://doi.org/10.3390/md12053046

19. Dietrich LE, Teal TK, Price-Whelan A, Newman DK (2008) Redox-active antibiotics control gene expression and community behavior in divergent bacteria. Science 321:1203-1206 doi: https://doi.org/10.1126/science.1160619
20. Ding L et al. (2015) Bacaryolanes A–C, rare bacterial caryolanes from a mangrove endophyte. J Nat Prod 78:2963 doi: https://doi.org/10.1021/acs.jnatprod.5b00674
21. El-Shouny W, Al-Baidani A, Hamza W (2011) Antimicrobial activity of pyocyanin produced by *Pseudomonas aeruginosa* isolated from surgical wound-infections. J Int J Pharm Med Sci 1:1-7 doi: https://doi.org/10.1128/AAC.03069-14
22. El-Zawawy NA, Ali SS (2016) Pyocyanin as anti-tyrosinase and anti tinea corporis: A novel treatment study. Microb Pathog 100:213-220 doi: https://doi.org/10.1016/j.micpath.2016.09.013
23. Essar DW, Eberly L, Hadero A, Crawford I (1990) Identification and characterization of genes for a second anthranilate synthase in *Pseudomonas aeruginosa*: interchangeability of the two anthranilate synthases and evolutionary implications. J Bacteriol 172:884-900 doi: https://doi.org/10.1128/jb.172.2.884-900.1990
24. Ezaki M, Iwami M, Yamashita M, Komori T, Umehara K, Imanaka H (1992) Biphenomycin A production by a mixed culture. Appl Environ Microbiol 58:3879-3882 doi: https://doi.org/10.1128/aem.58.12.3879-3882.1992
25. Ezaki M, Shigematsu N, Yamashita M, Komori T, Umehara K, Imanaka H (1993) Biphenomycin C, a precursor of biphenomycin A in mixed culture. J Antibiot (Tokyo) 46:135-140 doi: https://doi.org/10.7164/antibiotics.46.135
26. Fleming A (1929) On the Antibacterial Action of Cultures of a Penicillium, with Special Reference to their Use in the Isolation of *B. influenzæ*. Br J Exp Pathol 10:226-236
27. Giesen W, Wulffraat S, Zieren M, Scholten L (2007) Mangrove guidebook for Southeast Asia. Mangrove guidebook for Southeast Asia.
28. Glaser B, Turrión Ma-B, Alef K (2004) Amino sugars and muramic acid—biomarkers for soil microbial community structure analysis. Soil Biol Biochem 36:399-407 doi: https://doi.org/10.1016/j.soilbio.2003.10.013
29. Gong B, Chen S, Lan W, Huang Y, Zhu X (2018) Antibacterial and Antitumor Potential of Actinomycetes Isolated from Mangrove Soil in the Maowei Sea of the Southern Coast of China. Iran J Pharm Res 17:1339-1346
30. Goodfellow M (2012) Phylum XXVI. Actinobacteria phyl. nov. In: Bergey's Manual® of Systematic Bacteriology. Springer, New York, pp 33-2028
31. Govindarajan G, Santhi VS, Jebakumar SRD (2014) Antimicrobial potential of phylogenetically unique actinomycete, *Streptomyces* sp. JRG-04 from marine origin. Biologicals 42:305-311 doi: https://doi.org/10.1016/j.biologicals.2014.08.003
32. Hashimoto T et al. (2020) Novel macrolactam compound produced by the heterologous expression of a large cryptic biosynthetic gene cluster of *Streptomyces rochei* IFO12908. J Antibiot (Tokyo) 73:171-174 doi: https://doi.org/10.1038/s41429-019-0265-x

33. Hayakawa T, Tanaka T, Sakaguchi K, Otake N, Yonehara H (1979) A linear plasmid-like DNA in *Streptomyces* sp. Producing lankacidin group antibiotics. J Gen Appl Microbiol 25:255-260 doi: https://doi.org/10.2323/jgam.25.255
34. Herigstad B, Hamilton M, Heersink J (2001) How to optimize the drop plate method for enumerating bacteria. J Microbiol Methods 44:121-129 doi: https://doi.org/10.1016/s0167-7012(00)00241-4
35. Hesham AE-L, Mohamed NH, Ismail MA, Shoreit AAM (2015) Degradation of natural rubber latex by new *Streptomyces labedae* strain ASU-03 isolated from Egyptian soil. Microbiology 84:351-358 doi: https://doi.org/10.1134/S0026261715030078
36. Hosaka T et al. (2009) Antibacterial discovery in actinomycetes strains with mutations in RNA polymerase or ribosomal protein S12. Nat. Biotechnol. 27:462-464 doi: https://doi.org/10.1038/nbt.1538
37. Huang H-q et al. (2015) *Nocardiopsis mangrovei* sp. nov., isolated from mangrove sediment. Antonie van Leeuwenhoek 107:1541-1546 doi: https://doi.org/10.1007/s10482-015-0447-x
38. Huang H et al. (2018) *Nonomuraea mangrovi* sp. nov., an actinomycete isolated from mangrove soil. Int J Syst Evol Microbiol 68:3144-3148 doi: https://doi.org/10.1099/ijsem.0.002954
39. Hugenholtz P, Stackebrandt E (2004) Reclassification of *Sphaerobacter thermophilus* from the subclass Sphaerobacteridae in the phylum Actinobacteria to the class Thermomicrobia (emended description) in the phylum Chloroflexi (emended description). Int J Syst Evol Microbiol 54:2049-2051 doi: https://doi.org/10.1099/ijs.0.03028-0
40. Jett BD, Hatter KL, Huycke MM, Gilmore MS (1997) Simplified agar plate method for quantifying viable bacteria. Biotechniques 23:648-650 doi: https://doi.org/10.2144/97234bm22
41. Jiang Z-K et al. (2017) *Marmoricola endophyticus* sp. nov., an endophytic actinobacterium isolated from *Thespesia populnea*. Int J Syst Evol Microbiol 67:4379-4384 doi: https://doi.org/10.1099/ijsem.0.002297
42. Jiang Z-k et al. (2018) Diversity, novelty, and antimicrobial activity of endophytic actinobacteria from mangrove plants in Beilun Estuary National Nature Reserve of Guangxi, China. Front Microbiol 9:868 doi: https://doi.org/10.3389/fmicb.2018.00868
43. Johnson DB, Bacelar-Nicolau P, Okibe N, Thomas A, Hallberg KB (2009) *Ferrimicrobium acidiphilum* gen. nov., sp. nov. and *Ferrithrix thermotolerans* gen. nov., sp. nov.: heterotrophic, iron-oxidizing, extremely acidophilic actinobacteria. Int J Syst Evol Microbiol 59:1082-1089 doi: https://doi.org/10.1099/ijs.0.65409-0
44. Kamisango K-I et al. (1982) Structures and biological activities of peptidoglycans of *Listeria monocytogenes* and *Propionibacterium acnes*. J Biochem 92:23-33 doi: https://doi.org/10.1093/oxfordjournals.jbchem.a133918
45. Kawai K, Wang G, Okamoto S, Ochi K (2007) The rare earth, scandium, causes antibiotic overproduction in *Streptomyces* spp. FEMS Microbiol Lett 274:311-315 doi: https://doi.org/10.1111/j.1574-6968.2007.00846.x

46. Kinashi H, Mori E, Hatani A, Nimi O (1994) Isolation and characterization of linear plasmids from lankacidin-producing *Streptomyces* species. J Antibiot (Tokyo) 47:1447-1455 doi: https://doi.org/10.7164/antibiotics.47.1447
47. König CC et al. (2013) Bacterium induces cryptic meroterpenoid pathway in the pathogenic fungus *Aspergillus fumigatus*. Chembiochem 14:938-942 doi: https://doi.org/10.1002/cbic.201300070
48. Kurosawa K et al. (2008) Rhodostreptomycins, antibiotics biosynthesized following horizontal gene transfer from *Streptomyces padanus* to *Rhodococcus fascians*. J. Am. Chem. Soc. 130:1126-1127 doi: https://doi.org/10.1021/ja077821p
49. Kusmana C, Onrizal S (2003) Jenis-jenis pohon mangrove di teluk Bintuni, Papua. Fakultas Kehutanan, Institut Pertanian Bogor dan PT. Bintuni Utama Murni Wood Industries, Bogor
50. Lacey J (1987) Nomenclature of *Saccharopolyspora erythraea* Labeda 1987 and *Streptomyces erythraeus* (Waksman 1923) Waksman and Henrici 1948, and Proposals for the Alternative Epithet *Streptomyces labedae* sp. nov. Int J Syst Evol Microbiol 37:458-458 doi: https://doi.org/10.1099/00207713-37-4-458
51. Li XL, Xu MJ, Zhao YL, Xu J (2010) A novel benzo[f][1,7]naphthyridine produced by *Streptomyces albogriseolus* from mangrove sediments. Molecules 15:9298-9307 doi: 10.3390/molecules15129298
52. Liu S-W et al. (2016) *Phycicoccus endophyticus* sp. nov., an endophytic actinobacterium isolated from Bruguiera gymnorhiza. Int J Syst Evol Microbiol 66:1105-1111 doi: https://doi.org/10.1099/ijsem.0.000842
53. Ludwig W et al. (2012) Road map of the phylum Actinobacteria. In: Bergey's Manual® of Systematic Bacteriology: Volume Five The Actinobacteria, Part A and B. Springer, New York, pp 1-28
54. Luti KJ, Mavituna F (2011a) Elicitation of *Streptomyces coelicolor* with dead cells of *Bacillus subtilis* and *Staphylococcus aureus* in a bioreactor increases production of undecylprodigiosin. Appl Microbiol Biotechnol 90:461-466 doi: https://doi.org/10.1007/s00253-010-3032-2
55. Luti KJ, Mavituna F (2011b) *Streptomyces coelicolor* increases the production of undecylprodigiosin when interacted with *Bacillus subtilis*. Biotechnol Lett 33:113-118 doi: https://doi.org/10.1007/s10529-010-0401-y
56. Luti KJK, Mavituna F (2011c) Elicitation of *Streptomyces coelicolor* with dead cells of *Bacillus subtilis* and *Staphylococcus aureus* in a bioreactor increases production of undecylprodigiosin. Appl Microbiol Biotechnol 90:461-466 doi: https://doi.org/10.1007/s00253-010-3032-2
57. Maldonado LA, Stach JE, Pathom-aree W, Ward AC, Bull AT, Goodfellow M (2005) Diversity of cultivable actinobacteria in geographically widespread marine sediments. Antonie Van Leeuwenhoek 87:11-18 doi: https://doi.org/10.1007/s10482-004-6525-0
58. Maleki H, Dehnad A, Hanifian S, Khani S (2013) Isolation and Molecular Identification of *Streptomyces* spp. with Antibacterial Activity from Northwest of Iran. BioImpacts : BI 3:129-134 doi: https://doi.org/10.5681/bi.2013.017
59. Moody SC (2014) Microbial co-culture: harnessing intermicrobial signaling for the production of novel antimicrobials. Future Microbiol 9:575-578 doi: https://doi.org/10.2217/fmb.14.25

60. Mueller JH, Hinton J (1941) A protein-free medium for primary isolation of the *Gonococcus* and *Meningococcus*. Proc Soc Exp Biol Med 48:330 doi: https://doi.org/10.3181%2F00379727-48-13311
61. Nomi R (1960) On the Classification of *Streptomyces*. J Antibiot 13:236 doi: https://doi.org/10.11554/antibioticsa.13.4_236
62. Nomi R (1992) Together with *Streptomyces* for Thirty-five Years. Nippon Hosenkin Gakkaishi 6:113 doi: https://doi.org/10.3209/saj.6_113
63. Oh DC, Kauffman CA, Jensen PR, Fenical W (2007) Induced production of emericellamides A and B from the marine-derived fungus *Emericella* sp. in competing co-culture. J Nat Prod 70:515-520 doi: 10.1021/np060381f
64. Ola AR, Thomy D, Lai D, Brötz-Oesterhelt H, Proksch P (2013) Inducing secondary metabolite production by the endophytic fungus *Fusarium tricinctum* through coculture with *Bacillus subtilis*. J Nat Prod 76:2094-2099 doi: https://doi.org/10.1021/np400589h
65. Onaka H, Mori Y, Igarashi Y, Furumai T (2011) Mycolic acid-containing bacteria induce natural-product biosynthesis in *Streptomyces* species. Appl Environ Microbiol 77:400-406 doi: https://doi.org/10.1128/aem.01337-10
66. Pérez J, Muñoz-Dorado J, Braña AF, Shimkets LJ, Sevillano L, Santamaría RI (2011) *Myxococcus xanthus* induces actinorhodin overproduction and aerial mycelium formation by *Streptomyces coelicolor*. Microb Biotechnol 4:175-183 doi: https://doi.org/10.1111/j.1751-7915.2010.00208.x
67. Pettit RK (2011) Small-molecule elicitation of microbial secondary metabolites. Microb Biotechnol 4:471-478 doi: https://doi.org/10.1111/j.1751-7915.2010.00196.x
68. Priyanka S, Jayashree M, Shivani R, Anwesha S, Rao KB (2019) Characterisation and identification of antibacterial compound from marine Actinobacteria: In vitro and in silico analysis. J Infect Public Health 12:83 doi: https://doi.org/10.1016/j.jiph.2018.09.005
69. Qin S et al. (2009) Isolation, diversity, and antimicrobial activity of rare Actinobacteria from medicinal plants of tropical rain forests in Xishuangbanna, China. Appl Environ Microbiol 75:6176-6186 doi: https://doi.org/10.1128/AEM.01034-09
70. Radjasa ok et al. (2007) Antibacterial Activity of Marine Bacterium *Pseudomonas* sp. Associated with Soft Coral *Sinularia polydactyla* against *Streptococcus equi* Subsp. zooepidemicus. Int J Pharmacol 3:170 doi: https://doi.org/10.3923/ijp.2007.170.174
71. Rahman PK, Pasirayi G, Auger V, Ali Z (2009) Development of a simple and low cost microbioreactor for high-throughput bioprocessing. Biotechnol Lett 31:209-214 doi: https://doi.org/10.1007/s10529-008-9853-8
72. Rajagopal G, Kannan S (2017) Systematic characterization of potential cellulolytic marine actinobacteria *Actinoalloteichus* sp. MHA15. Biotechnol Rep (Amst) 13:30-36 doi: https://doi.org/10.1016/j.btre.2016.12.003
73. Raoult D et al. (2003) Tropheryma whipplei Twist: a human pathogenic Actinobacteria with a reduced genome. Genome Res 13:1800-1809 doi: 10.1101/gr.1474603

74. Reimmann C et al. (1997) The global activator GacA of *Pseudomonas aeruginosa* PAO positively controls the production of the autoinducer N-butyryl-homoserine lactone and the formation of the virulence factors pyocyanin, cyanide, and lipase. Mol Microbiol 24:309-319 doi: https://doi.org/10.1046/j.1365-2958.1997.3291701.x
75. Retnowati Y, Sembiring L, Moeljopawiro S, Djohan TS, Soetarto ES (2017) Diversity of antibiotic-producing Actinomycetes in mangrove forest of Torosiaje, Gorontalo, Indonesia. Biodiversitas 18:1453-1461 doi: https://doi.org/10.13057/biodiv/d18032
76. Riyanti R, Nurkhasanah W, Radjasa OK (2016) Diversity and Antifungal Activity of *Actinomycetes* Symbiont Hard Coral Mucus of Genera *Goniopora* and *Porites*. Makara J Sci:193-198 doi: https://doi.org/10.7454/mss.v20i4.6707
77. Roth E, Schwenninger SM, Eugster-Meier E, Lacroix C (2011) Facultative anaerobic halophilic and alkaliphilic bacteria isolated from a natural smear ecosystem inhibit *Listeria* growth in early ripening stages. Int J Food Microbiol 147:26-32 doi: https://doi.org/10.1016/j.ijfoodmicro.2011.02.032
78. Sabdono A, Trianto A, Radjasa OK, Wijayanti DP (2019) Antagonistic activity of bacteria isolated from coral *Acropora* sp of Karimunjawa Islands, Indonesia against Acroporid White Syndrome. Biodiversitas 20 doi: doi.org/10.13057/biodiv/d200526
79. Sajid I, Fotso Fondja Yao CB, Shaaban KA, Hasnain S, Laatsch H (2008) Antifungal and antibacterial activities of indigenous *Streptomyces* isolates from saline farmlands: prescreening, ribotyping and metabolic diversity. World J Microbiol Biotechnol 25:601 doi: https://doi.org/10.1007/s11274-008-9928-7
80. Sakamoto JMJ, Kondō S-I, Yumoto H, Arishima M (1962) Bundlins A and B, Two Antibiotics Produced by *Streptomyces griseofuscus* Nov. sp. The Journal of Antibiotics, Series A 15:98-102 doi: https://doi.org/10.11554/antibioticsa.15.2_98
81. Saleem HG, Aftab U, Sajid I, Abbas Z, Sabri AN (2015) Effect of crude extracts of selected actinomycetes on biofilm formation of *A. schindleri, M. aci,* and *B. cereus*. J. Basic. Microbiol. 55:645-651 doi: https://doi.org/10.1002/jobm.201400358
82. Samundeeswari A, Dhas SP, Nirmala J, John SP, Mukherjee A, Chandrasekaran N (2012) Biosynthesis of silver nanoparticles using actinobacterium *Streptomyces albogriseolus* and its antibacterial activity. Biotechnol. Appl. Biochem. 59:503-507 doi: https://doi.org/10.1002/bab.1054
83. Sangkanu S, Rukachaisirikul V, Suriyachadkun C, Phongpaichit S (2017) Evaluation of antibacterial potential of mangrove sediment-derived Actinomycetes. Microb Pathog 112:303-312 doi: https://doi.org/10.1016/j.micpath.2017.10.010
84. Schäberle TF, Orland A, König GM (2014) Enhanced production of undecylprodigiosin in *Streptomyces coelicolor* by co-cultivation with the corallopyronin A-producing myxobacterium, *Corallococcus coralloides*. Biotechnol Lett 36:641-648 doi: https://doi.org/10.1007/s10529-013-1406-0
85. Sharma SCSSC (2015) Antioxidant Efficacy in Selected Estuarine Actinomycetes. J Life Sci 1

86. Shepherd MD, Kharel MK, Bosserman MA, Rohr J (2010) Laboratory maintenance of *Streptomyces* species. Curr Protoc Microbiol 18:10E. 11.11-10E. 11.18 doi: https://doi.org/10.1002/9780471729259.mc10e01s18
87. Shetty PR, Buddana SK, Tatipamula VB, Naga YV, Ahmad J (2014) Production of polypeptide antibiotic from *Streptomyces parvulus* and its antibacterial activity. Braz J Microbiol 45:303 doi: https://doi.org/10.1590/s1517-83822014005000022
88. Shirling ET, Gottlieb D (1966) Methods for characterization of *Streptomyces* species. Int J Syst Bacteriol 16:313-340 doi: https://doi.org/10.1099/00207713-16-3-313
89. Slattery M, Rajbhandari I, Wesson K (2001) Competition-Mediated Antibiotic Induction in the Marine Bacterium *Streptomyces tenjimariensis*. Microb. Ecol. 41:90-96 doi: https://doi.org/10.1007/s002480000084
90. Solans M, Ruiz OA, Wall LG (2015) Effect of actinobacteria on *Lotus tenuis–Mesorhizobium loti* symbiosis: preliminary study. Symbiosis 65:33-37 doi: https://doi.org/10.1007/s13199-015-0315-5
91. Su P, Wang DX, Ding SX, Zhao J (2014) Isolation and diversity of natural product biosynthetic genes of cultivable bacteria associated with marine sponge *Mycale* sp. from the coast of Fujian, China. Can. J. Microbiol. 60:217 doi: https://doi.org/10.1139/cjm-2013-0785
92. Tashiro N, Miyashita K, Suzui T (1990) Taxonomic studies on the *Streptomyces* species, isolated as causal organisms of potato common scab. Japanese Journal of Phytopathology 56:73 doi: https://doi.org/10.3186/jjphytopath.56.73
93. Thirumurugan D, Vijayakumar R (2015) Characterization and structure elucidation of antibacterial compound of *Streptomyces* sp. ECR77 isolated from east coast of India. Curr. Microbiol. 70:745-755 doi: https://doi.org/10.1007/s00284-015-0780-3
94. Thirumurugan D, Vijayakumar R, Vadivalagan C, Karthika P, Khan MKA (2018) Isolation, structure elucidation and antibacterial activity of methyl-4, 8-dimethylundecanate from the marine actinobacterium *Streptomyces albogriseolus* ECR64. Microb Pathog 121:166-172 doi: https://doi.org/10.1016/j.micpath.2018.05.025
95. Thumar JT, Dhulia K, Singh SP (2010) Isolation and partial purification of an antimicrobial agent from halotolerant alkaliphilic *Streptomyces aburaviensis* strain Kut-8. World J Microbiol Biotechnol 26:2081-2087 doi: https://doi.org/10.1007/s11274-010-0394-7
96. Traxler MF, Watrous JD, Alexandrov T, Dorrestein PC, Kolter R (2013) Interspecies interactions stimulate diversification of the *Streptomyces coelicolor* secreted metabolome. mBio 4 doi: https://doi.org/10.1128/mBio.00459-13
97. Tsuchiya K, Yamazaki T, Takeuchi Y, Oishi T (1971) Studies on T-2636 antibiotics. IV. In vitro and in vivo antibacterial activity of T-2636 antibiotics. J Antibiot (Tokyo) 24:29-41 doi: https://doi.org/10.7164/antibiotics.24.29
98. Uramoto M, Otake N, Ogawa Y, Yonehara H (1969) The structures of Bundlin A (lankacidin) and Bundlin B. Tetrahedron Lett:2249-2254 doi: https://doi.org/10.1016/s0040-4039(01)88133-8

99. Valgas C, Souza SMd, Smânia EF, Smânia Jr A (2007) Screening methods to determine antibacterial activity of natural products. Braz J Microbiol 38:369 doi: https://doi.org/10.1590/S1517-83822007000200034
100. von Wintzingerode F et al. (2001) *Salana multivorans* gen. nov., sp. nov., a novel actinobacterium isolated from an anaerobic bioreactor and capable of selenate reduction. Int J Syst Evol Microbiol 51:1653-1661 doi: https://doi.org/10.1099/00207713-51-5-1653
101. Waksman SA, Lechevalier HA (1949) Neomycin, a New Antibiotic Active against Streptomycin-Resistant Bacteria, including Tuberculosis Organisms. Science 109:305-307 doi: https://doi.org/10.1126/science.109.2830.305
102. Watson D, MacDermot J, Wilson R, Cole PJ, Taylor GW (1986) Purification and structural analysis of pyocyanin and 1-hydroxyphenazine. Eur J Biochem 159:309-313 doi: https://doi.org/10.1111/j.1432-1033.1986.tb09869.x
103. Williams ST, Davies FL (1967) Use of scanning electron microscope for the examination of actinomycetes. J. Gen. Microbiol. 48:171 doi: https://doi.org/10.1099/00221287-48-2-171
104. Yamanaka K et al. (2005) Desferrioxamine E produced by *Streptomyces griseus* stimulates growth and development of *Streptomyces tanashiensis*. Microbiology 151:2899-2905 doi: https://doi.org/10.1099/mic.0.28139-0
105. Yu M et al. (2019) New Metabolites From the Co-culture of Marine-Derived Actinomycete *Streptomyces rochei* MB037 and Fungus *Rhinocladiella similis* 35. Front Microbiol 10:915 doi: https://doi.org/10.3389/fmicb.2019.00915
106. Zeng Q, Huang H, Zhu J, Fang Z, Sun Q, Bao S (2013) A new nematicidal compound produced by *Streptomyces albogriseolus* HA10002. Antonie Van Leeuwenhoek 103:1107-1111 doi: https://doi.org/10.1007/s10482-013-9890-8

Tables

Tables 1 and 2 available in the Supplementary Files section.

Figures

Figure 1

Mangroves in Ujung Piring, Central Java. a). Mangroves; b). Root; c). Root Sediment; d). Flower; e). Leave.

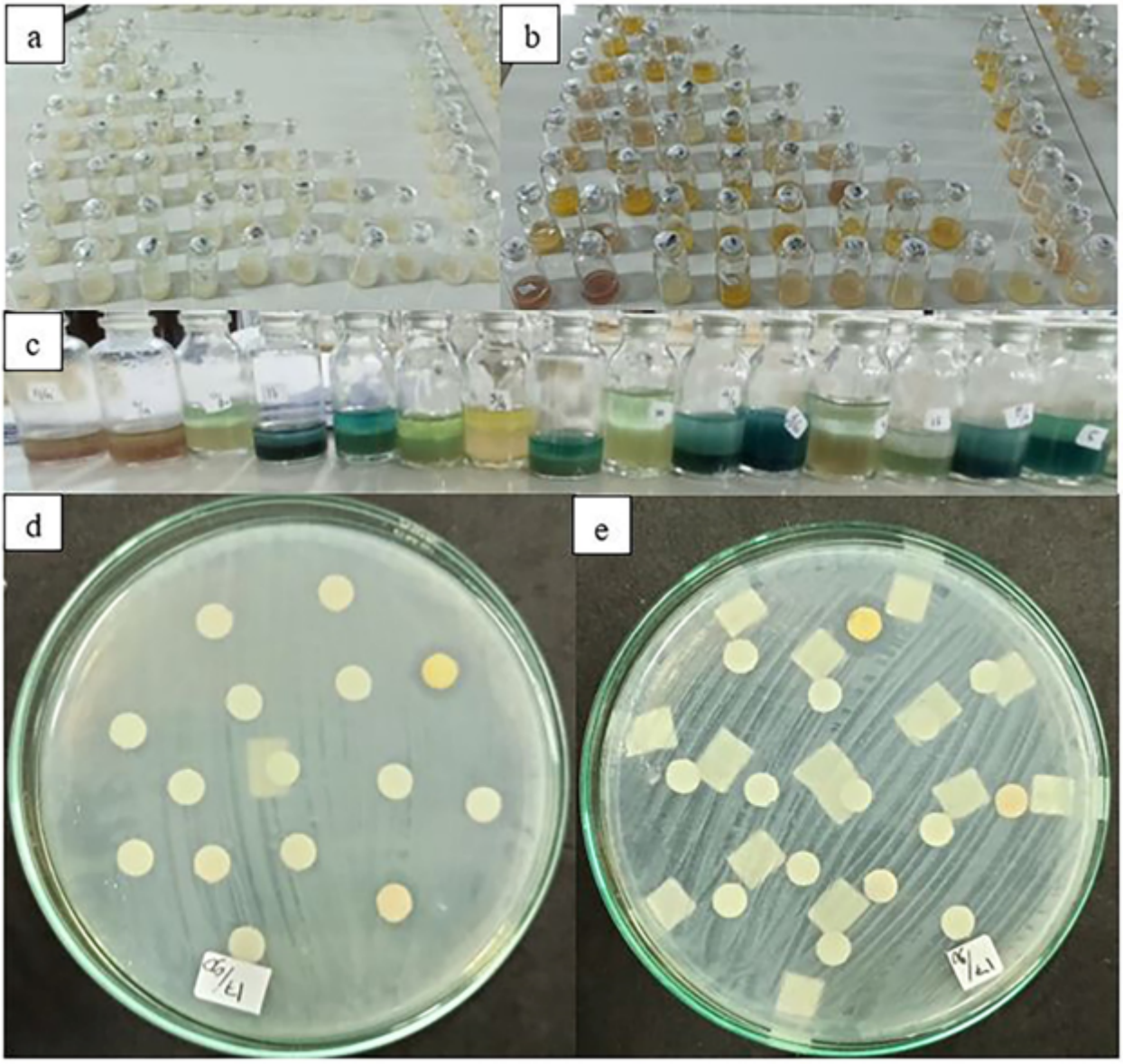

Figure 2

Antibacterial screening with the single colony and Co-culture Fermentation method. a). Before Fermentation; b). After 14 days of Fermentation, c). Extraction with Ethyl Acetate:culture (2:1); Antibacterial Screening against *S. aureus* (d) and *P. aeruginous* (e)

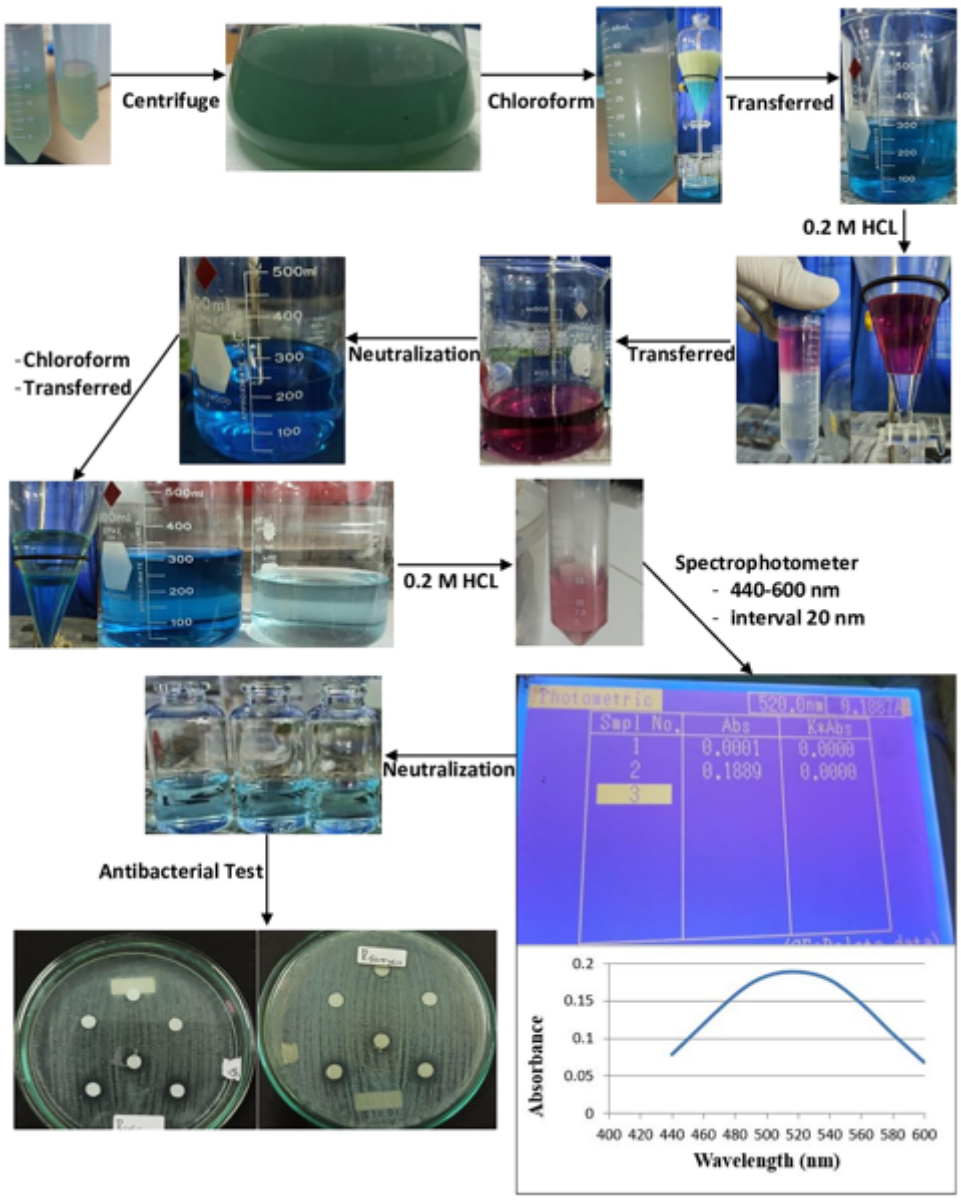

Figure 3

Flow chart extraction and purification of pyocyanin from Actinobacteria isolate code 41 with *P. aerugenosa* co-culture and antibacterial assay.

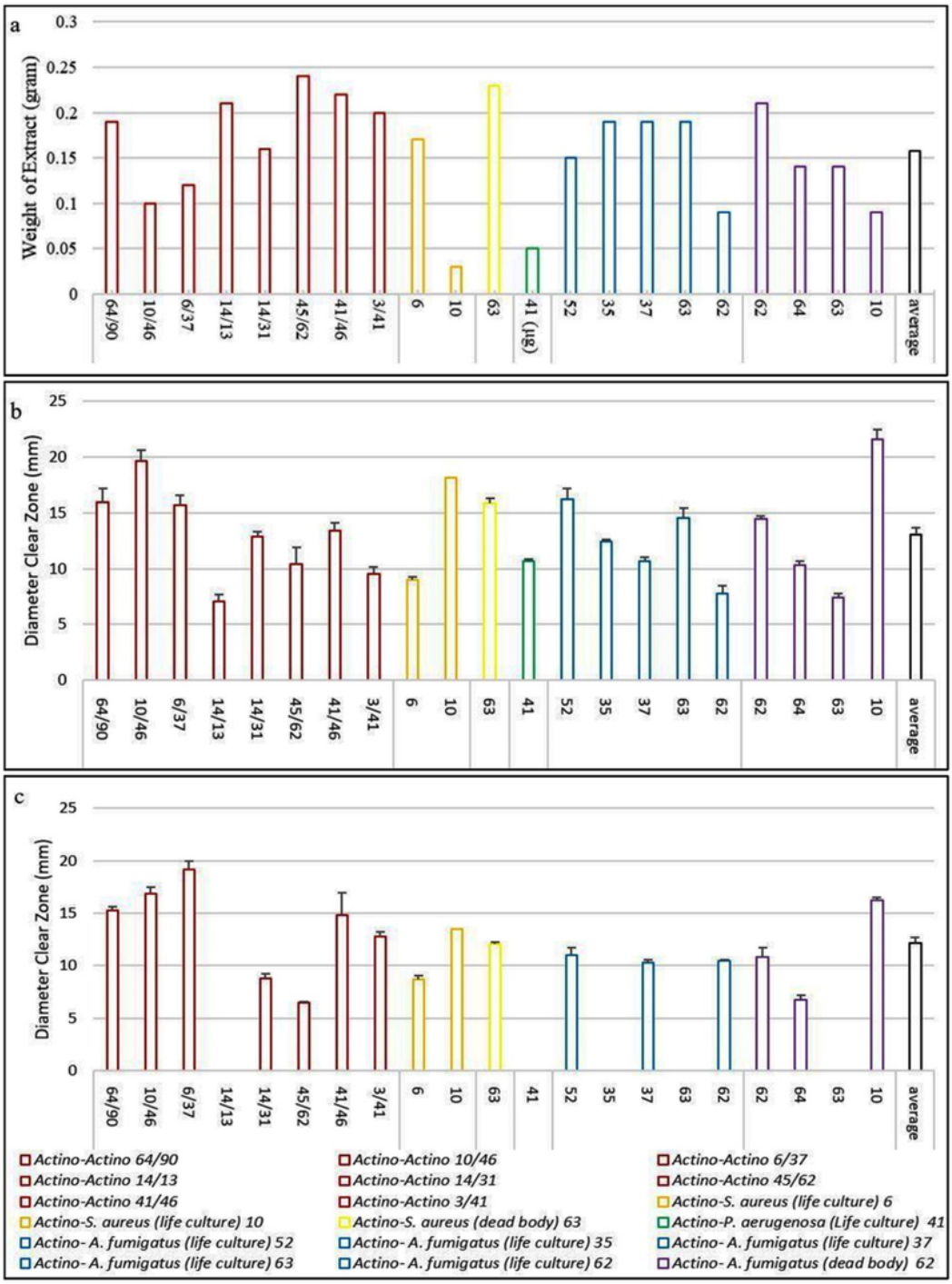

Figure 4

Weight (a) and Clear zone of extract Co-culture Actinobacteria with Actinobacteria, *S. aureus*, *P. aerugenosa*, and *A. fumigatus* against *S. aureus* (b) and *P. aerugenosa*

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- suplementationdata.docx
- Tables.docx