

ACTINOMYCETES AS A SOURCE OF POTENTIAL ANTIMICROBIAL AND ANTIBIOFILM AGENTS

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ABSTRACT

Actinomycetes are Gram-positive bacteria, anaerobic or facultative with a fungal-like morphology, widely distributed in both waters and land. Actinomycetes are bacteria with the largest taxonomic unit with diverse phyla diversity related to morphology, physiology, and metabolic ability. Actinomycetes have been known to have many benefits in medicine, including antibiotics, antifungals, antivirals, and anticancer. The content of secondary metabolites produced by actinomycetes varies widely. Actinomycetes contain active compounds from the polyene, terpenoid, phenolic, polyketide, phenazine, piperazine, and non-polyene groups, which have been shown to have antibacterial, antifungal, and antibiofilm activities. *Streptomyces* known produce most of the antibiotics. Many compounds have been isolated from actinomycetes have antimicrobial and antibiofilm activities. It can be developed for further research because there are no antibiofilm candidate drugs from actinomycetes that the FDA approved until now. This paper discusses the active compounds isolated from actinomycetes which have antibacterial, antifungal, and antibiofilm activities, and their mechanism of action.

Keywords: actinomycetes, antibiotic, antifungal, antibiofilm

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INTRODUCTION

Antimicrobial resistance has become a global issue and has a severe impact cause increased morbidity and mortality of patients (Akova, 2016). The formation of biofilms is one of the factors causing various antimicrobial resistance (Akers *et al.*, 2014). Microbes that have formed biofilm colonies are tough to treat with conventional systemic antimicrobials, causing permanent injury, mainly when patients use medical implant devices (Weigel *et al.*, 2007; Stoodley *et al.*, 2008; Römling and Balsalobre, 2012; Kostakioti, Hadjifrangiskou and Hultgren, 2013). In addition, killing bacteria in a biofilm requires 1000 times the dose of antibiotics needed to achieve the same results as planktonic cells (Hetrick *et al.*, 2010). The finding for active compounds from actinomycetes can be used as a strategy of choice because actinomycetes are known to produce secondary metabolites that are useful as antibiotics, antifungals, antivirals, and anticancer.

Actinomycetes are one of the gram-positive bacteria that are widespread throughout the world and contain secondary metabolites that are known as antibiotics, antifungal, antiviral, antibiofilm, and anticancer (Wahyuningsih, Eljannah and Mulyati, 2012; Kumari *et al.*, 2013; Ambavane *et al.*, 2014; Wahyuni, Sudarwanto and Lisdiyanti, 2014; Balachandran *et al.*, 2015; Barka *et al.*, 2016). The secondary metabolites produced by actinomycetes vary widely. Active compounds isolated from actinomycetes contain polyene group (Ambavane *et al.*, 2014; Vartak *et al.*, 2014), terpenoids (El-Sayed and Awad, 2013), phenolics (Belghit *et al.*, 2016), polyketides (Oja *et al.*, 2015; Asnani, Ryandini and Suwandri, 2016; Abdelmohsen *et al.*, 2017), phenazine (Abdelmohsen, Bayer and Hentschel, 2014), piperazine (Abdelmohsen *et al.*, 2017), and non-polyene (Augustine, Bhavsar and Kapadnis, 2005) which are proven has antibacterial, antifungal, and antibiofilm activity.

This paper will discuss active compounds isolated from actinomycetes that have antibacterial, antifungal, and antibiofilm activities and their mechanism of action.

DISCUSSION

Actinomycetes

Actinomycetes are Gram-positive bacteria, anaerobic or facultative with a fungal-like morphological appearance, widely distributed in both water and land. Actinomycetes are bacteria with the largest taxonomic unit with diverse phyla diversity related to morphology, physiology, and metabolic ability. The morphology of actinomycetes varies greatly from round (*Micrococcus*), rod-round (*Arthrobacter*), fragmented hyphae (*Nocardia*, *Rothia*) to branched mycelium types (*Micromonospora* and *Streptomyces*) (Kanti *et al.*, 2016). Actinomycetes, especially *Streptomyces*, have colony growth on a specific solid medium that is vegetatively arranged and with antennae or murmur mycelia. In colonies that have not grown mycelia, the surface of the colonies looks shiny. Colonies from *Nocardia* tend to be easily separated by each hypha and break easily like flour (Barka *et al.*, 2016).

Actinomycetes produce the most secondary metabolites compared to other microbes such as fungi and other bacteria. Mohamed *et al.* (2017) isolated 32 actinomycetes from Sahara soil in Algeria and found three isolates with broad-spectrum antimicrobial activity. Arumugam *et al.* (2017) reported that 38 of 144 actinomycetes isolated from soil samples of mangrove forests in India had activity against fungi, Gram-positive bacteria, and Gram-negative bacteria. Arasu *et al.* (2014) screened 130 actinomycetes isolates, and 100 of them were active against one of the Gram-positive bacteria, Gram-negative bacteria, or fungi, and most of them were more active against Gram-negative bacteria. Lee *et al.* (2016) reported that actinomycetes isolated from the Tanjung Lumpur mangrove forest soil samples, Malaysia was only active against Gram-negative bacteria. The difference in antimicrobial activity of actinomycetes could be due to differences in chemical structure, disintegration during the extraction process, and environmental factors such as temperature and pH (Mohamed *et al.*, 2017).

The *Streptomyces* genus is a genus that produces a lot of antibacterial products (50-55%) (Alharbi, 2016). Each *Streptomyces* strain originating from different sources will produce different secondary metabolites even though they have the same molecular identification based on the 16S rRNA gene sequence

(Sottorff *et al.*, 2019). These differences are influenced by the environment and the ability to compete in that environment for a long time so that each strain can produce different secondary metabolites. Sottorff *et al.* (2019) examined 2 *Streptomyces* strains with the same identification based on the 16S rRNA gene sequence but isolated from different locations, and the results show that the two *Streptomyces* strains have the similarities identification in cell morphology. However, microscopically, the two strains are different in terms of pigmentation, hyphal distribution, and colony morphology. Each of these strains has unique secondary metabolites.

Actinomycetes that have antimicrobial activity are commonly found in marine habitats (Fenical and Jensen, 2006), such as in watersheds, river deposits, beach sand, marine microorganisms associated with sponges, mud, marine sediments, and mangrove forests (Eccleston, 2008; Sunaryanto Balai Pengkajian Bioteknologi, Pengkajian dan Penerapan Teknologi Korespondensi Penulis and Sunaryanto, 2012; Fadhilah, Santoso and Yasman, 2018). The soil rhizosphere is the most abundant habitat for various bacteria, especially actinobacteria. Actinomycetes have an essential role in the rhizosphere ecosystem because they can produce various types of antibiotics and extracellular enzymes that support growth and protect plants from pathogens (Sarker, Latif and Gray, 2012). Asnani, *et.al.* (2016) reported that actinomycetes isolated from soil sediments in Segara Anakan could produce various hydrolytic enzymes such as amylase, cellulase, protease, lip, urease, and nitrate reductase. Actinomycetes isolated from the soil rhizosphere (mud) in Segara Anakan were also reported could inhibit MDR bacteria such as *E. coli*, *S. aureus*, *K. pneumonia*, *P. aeruginosa*, and *Enterococcus sp.* (Ryandin, Hendro and Sukanto, 2018).

Isolation and screening of secondary metabolites from actinomycetes methods

Natural ingredients, be it plants, animals or organisms, contain many active compounds that can be used as medicinal ingredients, one of which is antimicrobial. Several strategies can be used to isolate active compounds from natural ingredients, *i.e.*,: (Sarker, Latif and Gray, 2012)

- a. Focus on chemical compounds from these natural materials, not their activities.
- b. Isolation and identification of active compounds were followed by in vivo biological activity tests.
- c. Chemotaxonomy approach.
- d. An ethnopharmacological approach based on information about the efficacy and use of traditional medicine.
- e. Bioassay-guided isolation and identification of active lead compounds (in vitro).
- f. Producing active compounds by cell or tissue culture, genetic, and manipulation.
- g. Focus on its bioactivity.
- h. Through metabolomics approach, dereplication, and fingerprinting.

One strategy that is quite popular and often used for isolating active compounds from natural ingredients is bioassay-guided isolation. Bioassay is a test or test that uses living organisms to determine the effectiveness of living material or organic and inorganic materials against a living organism. There are four main functions of bioassays, according to Suffness and Pezzuto (1990), i.e., pre-screen, screen, monitor, and secondary testing. The pre-screen function is the application of a bioassay on a large number of initial samples to determine whether the sample has bioactivity or not. Screen function is to select compounds/materials for secondary testing. The monitor's function is using bioassays as a guide for the fractionation of crude extracts to isolate active compounds. Finally, the function of secondary testing is the evaluation of lead compounds with various models and test conditions to select compounds to be developed and used in clinical trials.

Bioassay Guided Isolation is the isolation of chemical components based on the activity shown by the bioassay (Weller, 2012). By knowing the activity of a group of chemical components (fraction), compounds can be isolated to obtain a single active compound. For example, extracts of natural ingredients/a mixture of synthetic products are chromatographically fractionated and refracted again to obtain purified compounds/isolates with biological activity. Each chromatographic separation was tested by bioassay to identify which fraction was the most active.

Only the most active fraction will be further processed.

Metabolites from natural materials have high complexity, so a high-throughput, simple, fast, and inexpensive technique is needed in conducting the screening. Currently, spectrometric and chromatographic methods for screening natural ingredients that have antioxidant, antibacterial, and antifungal activities (Ciesla *et al.*, 2015) is still the mainstay because it is included in these criteria.

Bioassay-guided isolation is still the standard procedure for finding antimicrobial compounds from natural materials. This method is quite adequate, fast, and specific. Several screening techniques are developed to isolate the active metabolite based on the bioassay-guided isolation method so that the isolated compound has a high level of purity based on its biological activity. In general, three methods can be used to detect antimicrobial activity, i.e., diffusion, dilution, and bioautography. Bioautography is the most effective method for detecting antimicrobial compounds because it can detect their bioactivity even in complex samples and has been used for more than 60 years (Botz, 2013). Bioautography is a screening method to detect the bioactivity of a compound (e.g., antibacterial) after chromatographic separation (Adhami *et al.*, 2013; Abou-Donia *et al.*, 2014). Thin-layer chromatography (TLC) is the best separation technique for bioautography (Botz, 2013).

The principle of bioautography is based on the agar diffusion technique, where antimicrobial compounds migrate by diffusion from the chromatographic plate to the agar plate that has been inoculated with bacteria. The zone of inhibition can be easily observed by using bacteria living on a suitable medium or by detecting reagents with dehydrogenase activity. Furthermore, the active spot of the antimicrobial can be localized because the sample has been separated by thin-layer chromatography (Botz, Nagy and Kocsis, 2001).

There are three kinds of TLC-bioautography methods, i.e., contact bioautography/contact, agar overlay bioautography/immersion, and direct bioautography/direct (Rios, Recio and Villar, 1988). The principle of contact bioautography is that antimicrobial compounds are transferred from a TLC plate to an agar plate that has been inoculated with bacteria. The principle of the agar overlay method is that the developer plate is covered with an agar medium that has

been inoculated with bacteria. Meanwhile, direct bioautography is microorganisms grown directly on TLC plates. Direct bioautography with TLC is a high-throughput method that can be used for semi-quantitative screening and analysis (Choma and Jesioneck, 2015).

Antimicrobial and antibiofilm compounds isolated from actinomycetes

Actinomycetes produce a wide variety of diverse secondary metabolites and functions to defend themselves from their environment. *Streptomyces* produce most of the antibiotics known today. Several compounds from actinomycetes that have been isolated and have antimicrobial and antibiofilm activity are summarized in Table 1.

Table 1. Antimicrobial and Antibiofilm from Bioactive compounds of actinomycetes

Bioactive compounds	Strain	Sample sites	Activity	Refferences
<i>Halicloclamines A-C</i>	<i>Haliconia sp</i>	the waters of Manado, Indonesia	antibacterial activity against <i>M. smegmatis</i>	Maarisit et al., 2017
<i>Actinomycins XoB, X2, and D</i>	<i>Streptomyces heliomyacin</i>	Saudi Arabia	antibacterial activity against <i>S. aureus</i> , <i>MRSA</i> , <i>B. subtilis</i> , and <i>B. cereus</i>	Wang et al., 2017a
<i>Fenol, 2,4-bis (1,1-dimethylethyl</i>	<i>Nocardiopsis sp</i>		antibacterial activity against <i>Salmonella enterica typhii</i> , <i>S. aureus</i> , <i>B. subtilis</i> , <i>Vibrio cholera</i> , and the fungus <i>Pythium myriotylum</i>	Sabu et al., 2017
<i>n-hexadecanoic acid</i>	<i>Nocardiopsis sp</i>			
<i>1-heptacosanol</i>	<i>Nocardiopsis sp</i>			
<i>Forazoline A</i>	<i>Actinomycete s sp</i>		anti-candida	Abdelmohsen et al.,

Bioactive compounds	Strain	Sample sites	Activity	Refferences
				2017
<i>2,4-Di-tert-butyl-phenol</i>	<i>Streptomyces mutabilis</i>	the soil of the Sahara-desert	antifungal activity against <i>Candida</i> and antibacterial activity against <i>S. aureus</i>	Belghit et al., 2016
<i>Nagelamide Z</i>	<i>Agelas sp</i>		antifungal activity against <i>C. albicans</i>	Tanaka et al., 2016
<i>Agelamides A,B</i>			antibacterial activity	
<i>Nagelamides X, Y, U</i>			antibacterial activity	
<i>Elaiophylin</i>	<i>Streptomyces sp</i>	soil from Jatiroto, East Java, Indonesia	antibacterial activity against <i>S. aureus</i> ATCC 12600 and <i>M. smegmatis</i>	Sheng et al., 2015
<i>11,12 dehydroelaiophylin</i>	<i>Streptomyces sp</i> 7-145		antibacterial activity against <i>MRSA</i> bacteria and <i>vancomycin-resistant enterococci (VRE)</i>	Wu et al., 2013
<i>11,11-o-dimethyl-14-diethyl-14-methylelaiophylin,</i>			antibacterial activity against <i>Methicillin-resistant Staphylococcus epidermidis (MRSE)</i>	
<i>elaiophylin,</i>			antibacterial activity against	

Bioactive compounds	Strain	Sample sites	Activity	Refferences
			<i>MRSA</i> bacteria and <i>vancomycin-resistant enterococci (VRE)</i> , and <i>methicillin-resistant Staphylococcus epidermidis (MRSE)</i>	
<i>11-O-methylelaiophylin</i> ,			antibacterial activity against <i>MRSA</i> bacteria and <i>vancomycin-resistant enterococci (VRE)</i> , and <i>methicillin-resistant Staphylococcus epidermidis (MRSE)</i>	
<i>11,11-O-dimethylelaiophylin</i> ,			<i>methicillin-resistant Staphylococcus epidermidis (MRSE)</i>	
<i>efomycin</i>			antibacterial activity against <i>MRSA</i> bacteria and <i>vancomycin-resistant enterococci (VRE)</i> , and <i>methicillin-resistant Staphylococcus</i>	

Bioactive compounds	Strain	Sample sites	Activity	Refferences
			<i>epidermidis (MRSE)</i>	
<i>Alnumycin-D</i>	<i>Streptomyces albus</i>		antibacterial and antibiofilm activity against <i>S. aureus</i>	Oja et al., 2015.
<i>Granaticin B</i>	<i>Streptomyces violaceoruber</i> DSM-40701		antibacterial and antibiofilm activity against <i>S. aureus</i>	Oja et al., 2015
<i>Kalafungin</i>	<i>Streptomyces tanashiensis</i> CH999		antibacterial and antibiofilm activity against <i>S. aureus</i>	Oja et al., 2015
<i>Medermycin</i>	<i>Streptomyces coelicolor</i>		antibacterial activity against <i>S. aureus</i>	Oja et al., 2015
<i>Caerulomycin..</i>	<i>bacterium Actinoallotichus sp</i>		antifungal activity against <i>Candida</i>	Ambavane et al., 2014.
<i>1,6-phenazinedimethanol</i>	<i>Brevibacterium sp,</i>		antibacterial activity against <i>M. luteus</i>	Abdelmohsen et al., 2014.
<i>1,3-OH-dodecanoic acid</i>			antifungal activity.	Vartak et al., 2014.
<i>32,33-didehydroroflomycoin/DDHR (Dhayabandomycin).</i>	<i>Streptomyces sp. wild-type</i>	Rocky mountain of Himachalpradesh India	broad-spectrum antifungal activity	Vartak et al., 2014
<i>Urauchimycin A and B</i>	<i>Streptomyces Sp strain N180,</i>		antifungal activity against <i>C. albicans</i>	Abdelmohsen et al., 2014.
<i>Helalomycin-1 (Chlorohydronaph</i>	<i>Streptomyces Sp HuGu-11,</i>		antibacterial activity	El-Sayed & Awad,

Bioactive compounds	Strain	Sample sites	Activity	Refferences
<i>thol</i>)			against Gram-positive bacteria (<i>B. subtilis</i> and <i>S. aureus</i>).	2013
<i>Xinghaiamine A</i>	<i>Streptomyces xinghaiensis</i> NRRLB 24674.		antibacterial activity against <i>Acinobacter baumannii</i> , <i>P.aeruginosa</i> , <i>E. coli</i> , <i>S. aureus</i> , and <i>B. subtilis</i>	Jiao et al., 2013.
<i>4-dehydro-4a-dechlorona pyradiomycin A1</i> , <i>3-dichloro-3-bromonapyradiomycin A1</i> , and <i>3-chloro-6, 8-dihydroxy-8-α-lapachone</i>	<i>Streptomyces</i> sp. SCSIO 10428.		antibacterial activity against Gram-positive bacteria (<i>Staphylococcus</i> and <i>Bacillus</i>).	Wu et al., 2013.
<i>5-(2,4-dimethylbenzyl) pyrrolidine-2.</i>	<i>Streptomyces</i> VITSVK5	Bengal, India,	antibacterial activity against <i>E. coli</i> , <i>K. pneumonia</i> , <i>S. aureus</i> , <i>B. subtilis</i> , and antifungal activity against <i>A. niger</i>	Saurav & Kannabiran, 2012

Halicloclamines A-C compounds are new compounds of the 3-alkyl pyridinium dimer alkaloid group, were produced by *Halicona* sp isolated from the waters of Manado, Indonesia. This compound has antibacterial activity against *M. smegmatis* (Maarisit et al., 2017). *Actinomycins* XoB, X2, and D compounds produced by *Streptomyces heliomyacin* isolated from Saudi Arabia. *Actinomycins* X2 was more potent against *S. aureus*, MRSA, *B. subtilis*, and *B. cereus* than ciprofloxacin. *Actinomycins* XoB is more cytotoxic than others (Wang et al., 2017).

The phenolic compounds, 2,4-bis (1,1-dimethyl ethyl), trans-cinnamic acid, benzoic acid, n-hexadecanoic acid, and 1-heptacosanol produced from the endophytic actinomycetes *Zingiber officinale*, i.e., *Nocardiopsis* sp. This compound has excellent antibacterial activity against *Salmonella enterica typhii*, *S. aureus*, *B. subtilis*, *Vibrio cholera*, and the fungus *Pythium myriotylum*. In addition, this compound also has activity against *coagulase-negative staphylococci* (CoNS) with better results than vancomycin, ciprofloxacin, and gentamicin (Sabu, Soumya and Radhakrishnan, 2017).

The polyketide compound, Forazoline A, isolated from Actinomycetes sp, acts as an anti-candida (Abdelmohsen *et al.*, 2017). The compound 2,4-Di-tert-butyl-phenol is an alkylphenol group compound with antifungal activity on *Candida* and antibacterial activity on *S. aureus*. In addition, this compound also has anticancer, antimalarial, and antioxidant activity. This compound was isolated from *Streptomyces mutabilis* taken from the soil of the Sahara desert (Belghit *et al.*, 2016). A new compound derived from Bromopyrrole alkaloids has been isolated from the marine sponge *Agelas* sp. These compounds include Nagelamide Z, which can inhibit *C. albicans* with MIC value of 0.25 µg/mL, and Agelamides A, Agelamides B, Nagelamides X, Y, U, and U W which have moderate activity against several microorganisms (Tanaka *et al.*, 2016).

Elaiohylin compound is a polyketide compound produced by soil bacteria *Streptomyces* sp isolated from soil from Jatiroto, East Java, Indonesia. This compound can inhibit *S. aureus* ATCC 12600 with MIC 0.78-3.13 µg/mL, and *M. smegmatis* with MIC 6.25 µg/mL (Sheng *et al.*, 2015). Wu *et al.* (2013) also succeeded in isolating six compounds derived from elaiophylin from *Streptomyces* sp 7-145, i.e., (1) 11.12 dehydroelaiophylin, (2) 11.11-o-dimethyl-14-diethyl-14-methylelaiophylin, (3) elaiophylin, (4) 11-O-methylelaiophylin, (5) 11,11-O-dimethylelaiophylin, and (6) efomycin. These compounds have activity against Gram-positive bacteria but are not active against Gram-negative bacteria. Compounds 1,3,4, and 6 showed activity against MRSA bacteria and vancomycin-resistant enterococci (VRE) with MICs ranging from 1-4 µg/mL. In addition, compound 3-6 inhibited *methicillin-resistant Staphylococcus epidermidis* (MRSE) with MIC ranging from 2-6 µg/mL.

Alnumycin-D compound from *Streptomyces albus*, a polyketide group, has antibacterial and antibiofilm activity on *S. aureus* (Oja *et al.*, 2015). Pyranonaphthoquinone (PNQ) polyketide compound consisting of Granaticin B produced from *Streptomyces violaceoruber* DSM-40701, Kalafungin produced from *Streptomyces tanashiensis* CH999, and Medermycin produced from *Streptomyces coelicolor* has antibacterial activity against *S. aureus* with MIC values ranging from 0.9-3, 6 μ M, 7-53 M and 0.4-1.7 μ M (Oja *et al.*, 2015). In addition, Granaticin B also has high activity against biofilms with an IC₅₀ value of 3.72 μ M. Kalafungin, and Medermycin had lower activity against biofilms with IC₅₀ values of 27.8 μ M- 24.6 μ M (Oja *et al.*, 2015). Caerulomycin A belongs to the polyene group, which has antifungal against *Candida*. This compound was isolated from marine invertebrates associated with the bacterium *Actinoallotechus sp.* (Ambavane *et al.*, 2014).

The compound 1,6-phenazinedimethanol isolated from *Brevibacterium sp.*, has antibacterial activity against *M. luteus* (Abdelmohsen, Bayer and Hentschel, 2014). 1,3-OH-dodecanoic acid is a compound of the diketopiperazine group with antifungal activity. The compound 32,33-dide-hyd roroflamycoin/DDHR (Dhayabado mycin) from *Streptomyces sp.* wild-type isolated from the Rocky-mountains of Himachalpradesh India. This compound is a new derivative of the antibiotic Roflamycoin and belongs to the polyene macrolide group. This compound has broad-spectrum antifungal activity but does not have antibacterial activity (Vartak *et al.*, 2014). Urauchimycin compounds A and B, isolated from the extract of *Streptomyces Sp* strain N180, are new compounds that have activity against *C. albicans* (Abdelmohsen, Bayer and Hentschel, 2014). Helalomycin-1 (Chlorohydronaphthol), a new derivative of bicyclic sesquiterpenoids, has antibacterial activity against Gram-positive bacteria *B. subtilis* and *S. aureus*. This compound was isolated from *Streptomyces Sp* HuGu-11, a new strain (El-Sayed and Awad, 2013). Xinghaiamine A compound produced by *Streptomyces xinghaiensis* NRRLB 24674. This compound is a new alkaloid group with a sulfoxide group and has broad-spectrum antibacterial activity against *Acinobacter baumannii* (MIC value is 11.04 μ g/mL), *P. aeruginosa* (MIC value is 2.76 μ g/mL), *E. coli* (MIC value is 0.17 μ g/mL), *S. aureus* (MIC value is 0.69 μ g/mL), and *B.*

subtilis (MIC value is 0.35 µg/mL) (Jiao *et al.*, 2013).

Compounds 4-dehydro-4a-dechlorona pyradiomycin A1 (1), 3-dichloro-3-bromonapyradiomycin A1 (2) and 3-chloro-6, 8-dihydroxy-8- α -lapachone (3) are new compounds of napyradiomycins. This compound belongs to the terpenoid/polyketide group, produced by *Streptomyces* sp. SCSIO 10428. This compound is active against Gram-positive bacteria *Staphylococcus* and *Bacillus* with MIC ranging from 0.25-32 µg/mL (Wu *et al.*, 2013). Compound 5-(2,4-dimethylbenzyl) pyrrolidine-2. This compound produced by *Streptomyces* VITSVK5 isolated from Bengal, India, has antibacterial and antifungal activity. Compound 5-(2,4-dimethylbenzyl) pyrrolidine-2 had the highest antibacterial activity against *E. coli* with MIC 187 µg/mL followed by *K. pneumonia* (MIC value is 220 µg/mL), *S. aureus* (MIC >1000 µg/mL), and *B. subtilis* (MIC value is 850 µg/mL). The compound 5-(2,4-dimethylbenzyl) pyrrolidine-2 was more potent than others against the opportunistic fungus *A. niger* with a MIC ranging 1 µg/mL (Saurav and Kannabiran, 2012).

The mechanism of actinomycetes as antimicrobial and antibiofilm

Alkylphenol compounds found in 2,4-Di-tert-butylphenol which have antifungal activity work by inhibiting the spore germination which causes swelling of the germ tube and hyphal branching. This compounds also inhibit the integration of the microtubule spindles and interfering with the chromosome alignment in the metaphase phase, causing the loss of chromatidification of the germ tube and hyphal branching, which results in reduced mycelial growth and spore germination (Dharni *et al.*, 2014). 2,4-Di-tert-butylphenol compounds also inhibit the production of prodigiosin (84.5%), hemolysin (69.9%), phospholipase (84.3%) and proteinase secretion (41.9%) which are virulence factors of *C. albicans* (Padmavathi *et al.*, 2015). The active compound from the endophytic *Zingiber officinale* can inhibit CoNS biofilms because of its inhibitory effect on the synthesis of exopolysaccharides and proteins, thereby affecting the structural integrity of the biofilm and the growth of the biofilm matrix (Sabu, Soumya and Radhakrishnan, 2017). Glycolipid compounds produced from actinobacterium *Brevibacterium casei* MS419 isolated from the sponge *Dendrilla nigra* inhibited the initial

attachment phase of *E. coli* and *Vibrio alginolyticus* biofilm cells (Kiran, Sabarathnam and Selvin, 2010).

Antimycin compounds such as Urauchimycins have a mechanism of action by inhibiting the flow of electrons in the mitochondrial respiratory chain (Mendes *et al.*, 2013). Polyketide compounds including Forazoline A, as an anticandida have a mechanism by damaging the integrity of cell membranes through deregulation of homeostasis from phospholipids. Forazoline A also has a synergistic effect with amphotericin B. Phenolic compounds, 2,4-bis (1,1-dimethylethyl), trans cinnamic acid, benzoic acid, n-hexadecanoic acid, and 1-heptacosanol cause changes in cell morphology (cells become collapse and cells wall deformity occurs) in *Fusarium oxysporum*. Some Actinomycetes species have different polyketide synthase (PKS) and nonribosomal peptide synthetase (NRPS) enzymes, producing hydrolytic enzymes such as amylase, cellulase, protease, lipase, urease and nitrate reductase which have the potential to be antibacterial and antifungal (Asnani, Ryandini and Suwandri, 2016). The compound of actinomycetes is also active against extracellular enzymes (Keikha *et al.*, 2015), and influences nucleic acid so that it can cause morphological changes in fungal cells (Nurkanto and Julistiono, 2014). The polyene glycosylate compound from actinomycetes acts as an antifungal due to its ability to interact with sterols and damage cell membranes so that fungal cells lack ion and other molecules out of cells, causing cell death. Polyene macrolides are still the drug of choice for the treatment of systemic fungal infections because of their broad spectrum and resistance is rarely reported, even though they have toxic side effects (Vartak *et al.*, 2014).

The mechanism of the active compound Actinomycetes in inhibiting biofilms is by inhibiting the proliferation, adhesion and activity of metabolic enzymes (protease and phospholipase), hyphal formation and biofilm development (Guo *et al.*, 2015). Actinomycetes isolated from *Acropora digitifera* coral mucus have activity as an antibiofilm because it can reduce cell surface hydrophobicity which is an important factor in the formation of *S. pyogenes* biofilms (Nithyanand *et al.*, 2010). Younis, et.al. (2016) reported that the active compound Actinomycetes is able to act as an anti-quorum sensing, where quorum sensing is an important factor in biofilm formation and plays a role in the pathogenicity of fungi. The

phenol compound, 2,4-bis (1,1-dimethylethyl) isolated from *Vibrio alginolyticus* bacteria can also inhibit quorum sensing activity (Padmavathi, Abinaya and Pandian, 2014).

The 2,4-Di-tert-butylphenol compounds are effective in inhibiting and destroying *C. albicans* biofilms because of their ability to inhibit hyphal development which is the key to the initial adhesion stage of fungal cells to form biofilms and increase hydration of biofilm cell walls so as to reduce cell attachment biofilm (Padmavathi *et al.*, 2015). Active compounds from endophytic *Zingiber officinale* are able to inhibit CoNS biofilms because of their inhibitory effects on the synthesis of exopolysaccharides and proteins that affect the integrity of the biofilm structure and the growth of the biofilm matrix (Sabu, Soumya and Radhakrishnan, 2017).

Anti-quorum sensing activity of *Streptomyces parvulus* against *C. violaceum* bacteria can inhibit the formation of *P. aeruginosa*, *S. aureus*, and *M. luteus* biofilms. Mulya and Waturangi (2021) also investigated the anti-quorum sensing activity of actinomycetes isolated from Ancol Beach, Jakarta, and several rice fields in the Sleman area. They found that 8 of the 30 actinomycetes studied had anti quorum sensing activity against *C. violaceum* bacteria. The same was reported by Younis, et.al.,(2016), who examined 40 actinomycetes isolated from Iraq, and 15 of them had anti quorum sensing activity. Raissa, et.al., (2020) reported that 16 actinomycetes studied had anti quorum sensing activity against the bacterium *C. violaceum* with the main compound consisting of polysaccharides.

CONCLUSION

Actinomycetes, especially *Streptomyces* species are a source of a variety of new antibacterial, antifungal and antibiofilm compounds. The active compounds that have been isolated from actinomycetes are very diverse and have potency. So, further research must develop the potency of actinomycetes as antibiofilm agents because FDA has approved no antibiofilm drug until now. Meanwhile, it is too hard for treating the related-biofilm infections

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