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Methanol pigment extracts derived from two marine actinomycetes exhibit antibacterial and antioxidant activities

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Abstract. *Mesrian DK, Purwaningtyas WE, Astuti RI, Hasan AEZ, Wahyudi AT. 2021. Methanol pigment extracts derived from two marine actinomycetes exhibit antibacterial and antioxidant activities. Biodiversitas 22: 4440-4447.* In recent years, natural pigments produced by marine microorganisms, especially actinomycetes, have become an important source of medically valuable drugs and other active compounds. Based on that crucial fact, this study aimed to determine the toxicity, antimicrobial, and antioxidant activities of methanolic pigment extract derived from marine actinomycetes HV11.P3 and SCA54.P2. Both extracts exhibited high toxicity activity based on Brine Shrimp Lethality Test (BSLT). The results showed the lethal concentration 50% (LC_{50}) values of actinomycetes HV11.P3 and SCA54.P2 pigment crude extracts were 94.60 µg/mL and 131.22 mg/mL, respectively. The antimicrobial activity of those extracts was also tested against several microbes and showed the best results for inhibiting against *Bacillus subtilis* and *Escherichia coli*. Furthermore, we assessed the antioxidant activity using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals and the results showed the inhibitory concentration 50% (IC_{50}) values of actinomycetes HV11.P3 and SCA54.P2 pigment crude extracts were 231.08 µg/mL and 369.3 µg/mL, respectively. By using GC-MS analysis, we identified chemical compounds of actinomycete pigment crude extracts, revealing that the most dominant compounds were 1H-Purine-2,6-dione, 3,7-dihydro-1,3,7-trimethyl- (CAS) (44.65%) for HV11.P3 and 5-Methoxypyrrolidin-2-one (30.23%) for SCA54.P2. The 16S rRNA gene sequence analysis showed that actinomycete HV11.P3 and actinomycete SCA54.P2 had the closest similarity to *Micromonospora chalcea* strain 1464-217L and *Micromonospora tulbaghiae* strain TVU1, respectively. This study revealed that two pigment crude extracts derived from actinimycetes HV11.P3 and SVA54.P2 had antibacterial, antioxidant, and toxicity activities. The actinomycete SCA54.P2 pigment crude extract had the best activity as an antibacterial against *E. coli* ATCC 8739, while actnomycete HV11.P3 pigment crude extract had the best activity as an antioxidant.

Keywords: Actinomycetes, antimicrobial, antioxidant, pigment, toxicity

INTRODUCTION

In recent years, various problems in the health sector have emerged and attracted the attention of the world. These include resistance to several types of pathogens microbial and increased deaths due to infection with degenerative diseases (Prestinaci et al. 2015; Kharirie and Andriani 2020). Resistance occurs when bacteria, viruses, fungi, and parasites change continuously and reduce the effectiveness of the commonly used antimicrobial agent (Founou et al. 2017). Furthermore, infections that are difficult to treat will increase the risk of disease spread, disease severity, and mortality which have a long-term impact (Roope et al. 2019). Chronic bacterial, viral, and fungal infections might be causative factors for inflammatory and worsen degenerative diseases. Therefore, the discovery of new antimicrobial compounds should be considered in the present and the future.

Degenerative diseases as a result of cell damage due to the aging process or various other factors that affect the number of free radicals in the body (Sahardi and Makpol 2019). The accumulation of free radicals will initiate multiple diseases such as Alzheimer's, Parkinson's, atherosclerosis, cardiovascular disorders, hypertension, and diabetes mellitus type 2 (Stambler 2017). Naturally, the human body can reduce free radicals in a certain amount by antioxidant compounds that have been formed in the body. However, if oxidative stress occurs, which is an imbalance between the number of free radicals and the number of antioxidants in the body, the body needs an external source of antioxidants to prevent the oxidation of the essential constituents of cells (Al-Snafi 2017). The other bioactive compound that can act as a tool against degenerative disease is the toxic compound. It has been proven that the toxic compound tested with the brine shrimp lethality test (BSLT) has a good correlation with cytotoxic activity in some solid human tumors (Mclaughlin et al. 1998).

Actinomycetes are Gram-positive, filamentous aerobic bacteria with high G+C composition (Gong et al. 2018; Bhakyashree and Kannabiran 2018). Actinomycetes isolated from terrestrial were extensively researched, leading to the rediscovery of natural products that have been reported. On the other hand, marine actinomycetes are primarily a new resource of secondary metabolites including terpenes, peptides, sterols, fatty acids, alkaloids, amino acids and their derivatives (Liao et al. 2016; Pudi et al. 2016). The marine environment is capable of producing all of these compounds due to the enormous biodiversity

and diverse environmental conditions (Bibi et al. 2020). Actinomycetes associated with marine sponges such as *Micromonospora* sp, have recently been used to produce many bioactive compounds with novel molecular scaffolds and significant pharmacological activities (Hifnawy et al. 2020). Thus, the genus *Micromonospora* is one of the prolific producer of secondary metabolites, especially as bioactive compounds.

The genus *Micromonospora* is a member of the *Micromonospora*ceae which includes 32 genera. This genus is found in various geographic environments such as soils, mangrove sediments, plants, marine habitats, and extreme environments (Trujillo et al. 2015; Hifnawy et al. 2020). More than 740 strains of *Micromonospora* have been identified to produce various bioactive compounds, one of the bioactive compound comes from the pigment (Berdy 2005). *Micromonospora* colonies have carotenoid pigments including yellow, orange, red, purple, brown, or black (Sineva et al. 2021). Some *Micromonospora* species with their soluble pigments are *M. chalcea* (yellow), *M. halophytica* (red-brown), *M. olivasterospora* (olive-green), *M. purpureochromogenes* (dark-brown), and *M. rosaria* (wine-red) (Genilloud 2015). In the previous study, we have isolated two pigmented actinomycetes from marine sponges, namely actinomycetes HV11.P3 and SCA54.P2 (unpublished data). To determine the potency of pigment crude extract, this study aimed to test the antimicrobial, antioxidant, and toxicity abilities of the pigment methanol extracts produced by the marine actinomycetes HV11.P3 and SCA54.P2.

MATERIALS AND METHODS

Material

Two pigmented marine actinomycetes, namely actinomycete HV11.P3 (greenish-black) and actinomycete SCA54.P2 (orange) used in this study had been isolated from sponges collected from Pramuka Island, Kepulauan Seribu, Indonesia (5°44'46.3 S 106° 36'35.7 E). Five microbes were used for the antimicrobial test, namely *Bacillus subtilis* ATCC 19659, *Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 15442, *Staphylococcus aureus* ATCC 6538, and *Candida albicans* ATCC 10231, which were obtained from IPB Culture Collection, IPB University (Bogor, Indonesia).

Procedures

Pigment extraction

The extraction of pigments refers to method described by Dawoud et al. (2020) with slight modifications. Each of Actinomycete was cultured in 1 L of ISP-4 broth medium (10 g Soluble Starch, 1 g $MgSO_4.7H_2O$, 1 g NaCl, 1 g K_2HPO_4, 2 g $(NH_4)_2SO_4$, 2 g $CaCO_3$, 0.001 g $FeSO_4.7H_2O$, 0.001 g $MnCl_2.4H_2O$, and 0.001 g $ZnSO_4.7H_2O$) and agitated continuously in orbital shaker at 100 rpm for 14 days at room temperatur. The actinomycetes cultures were then centrifuged at 6000 rpm for 15 minutes. The cell biomass were then added with 1 L of methanol as a solvent and then heated at 50°C for 15 minutes in water bath until the cell became colorless. After that, the methanol phase was separated with the cell biomass by centrifugation at 6000 rpm for 15 minutes. The methanol solvent was evaporated using a rotary evaporator at 50°C. The crude extracts were collected and dissolved in 10% dimethyl sulfoxide (DMSO), and then stored at 4ºC for further assay.

Toxicity test

The toxicity test was performed using Brine Shrimp Lethality Test (BSLT) according to Meyer et al. (1982). Twenty *Artemia salina* larvae were placed in each vial containing 4 mL of seawater and pigment crude extracts in 0, 10, 100, 250, 500, 750, and 1000 µg/mL. The vials containing larvae and pigment extracts were incubated under a light condition for 24 hours at room temperature. The dead larvae were counted, and the percentage of mortality was calculated using the following formula:

$$\% \text{ Mortality} = \left(\frac{\sum \text{Sample larvae mortality} - \sum \text{Control larvae mortality}}{\sum \text{Total larvae}}\right) \text{ x } 100$$

A linear regression model and probit analysis were used to determine the LC_{50} value of each extract.

Antimicrobial test

The antimicrobial activity was performed using the disc diffusion method. The pigment extracts were tested against several clinical isolates. *B. subtilis* ATCC 19659, *E. coli* ATCC 8739, *P. aeruginosa* ATCC 15442, and *S. aureus* ATCC 6538 were inoculated into a Mueller-Hinton Agar medium, meanwhile *C. albicans* ATCC 10231 was inoculated into a Potato Dextrose Agar medium. A paper disc (6 mm) containing various concentrations of the extracts (250, 500, 750, and 1000 µg/mL) was placed on the surface of agar plate medium. Tetracycline (100 µg/mL) was used as a positive control and 10% dimethyl sulfoxide (DMSO) was used as a negative control. The plates were incubated for 24 hours at room temperature, and the diameter of inhibition zones formed was measured.

Antioxidant test

The antioxidant activity of the pigment extracts was determined using DPPH radical according to Batubara et al. (2009) with modification. Five hundred microliters of each extract at various concentrations (0, 7.81, 15.63, 31.25, 62.5, 125, 250, and 500 µg/mL) were mixed with 500 µL of 2,2-diphenyl-1-picrylhydrazyl (DPPH) reagent (0.125µM in methanol). The mixture was incubated at room temperature for 30 minutes in the dark condition, then the absorbance of the sample was measured using a spectrophotometer at 514 nm (Thermo spectronic-Genesis 20, Thermo Fisher Scientific, USA). Ascorbic acid was used as a positive control. The percentage of inhibition was calculated using the following formula:

$$\% \text{ Inhibition} = \left[1-\left(\frac{\textit{Sample absorbance} - \textit{Control absorbance}}{\textit{Blanko absorbance} - \textit{Control absorbance}}\right)\right] \text{ x } 100$$

A linear regression model was used to determine the IC_{50} value of each extract.

Chemical compound analysis

The pigment extract of the isolate HV11.P3 and SCA54.P2 was analyzed using Gas Chromatography in Agilent 5977B GC/MSD (Agilent Technologies, USA). 0.6 µL of extract solution diluted in methanol was injected into a column with HP-5MS column type (30 m x 250 µm x 0.25 µm). The initial oven temperature was 80°C and gradually increased at 15°C/min until 300°C and held for 20 minutes. Helium gas was used as a carrier at a 1 mL/min flow rate. The injection temperature was 300°C and the aux temperature was maintained at 300°C. The result was analyzed by the GC-MS Pyrolysis program (WILLEY9THN 08. L).

Molecular identification

The genomic DNA of Actinomycete HV11.P3 and SCA54.P2 were extracted using Presto™ Mini gDNA Bacteria Kit (Geneaid, Taiwan) following the protocol's guidelines. The 16S rRNA gene (1480 bp) were amplified using primers 20F (5'- CAGGCCTAACACATGCA AGTC-3') dan 1500R (5'-GGGCGGWGTGTACAAGGC-3') (Weisberg et al. 1991). The PCR was performed using MyTaq™ HS Red Mix (Bioline, United Kingdom). The PCR conditions were pre-denaturation at 95°C for 2 min, denaturation at 95°C for 30 sec, annealing at 55°C for 30 sec, extension at 72°C for 1 min, and post extension at 95°C for 7 min. PCR products were visualized using 1% (w/v) gel agarose electrophoresis and the DNAs were sequenced in First BASE (Selangor, Malaysia). The sequences were aligned to National Centre or Biotechnology Information (NCBI) database using Nucleotide Blast (https://blast.ncbi.nlm.nih.gov/), and the phylogenetic tree was constructed using Molecular Evolutionary Genetic Analysis X (MEGA X) neighbor joining p-distance model with 1000 bootstrap replication.

RESULTS AND DISCUSSION

The pigment extraction

The Actinomycete HV11.P3 and SCA54.P2 showed greenish-black and orange colonies on ISP-4 agar medium at seven days of incubation at room temperature. The HV11.P3 and SCA54.P2 were cultured in ISP-4 broth medium and incubated for 14 days on a rotary shaker prior to the pigment extraction. The pigment crude extracts showed greenish-black for HV11.P3 and brownish-orange for SCA54.P2 (Figure 1). The pigment extract yield of both actinomycete HV11.P3 and SCA54.P2 were 0.23% and 0.18%, respectively (Table 1).

Toxicity of pigment extracts

Based on the BSLT test, both of the pigment extracts of actinomycete HV11.P3 and SCA54.P2 showed toxicity activity against *Artemia salina*. The toxicity was expressed as LC_{50} value. LC_{50} value is a standard for the lowest concentration that can kill 50% of test organisms (*A. salina*). The lower of LC_{50} value indicated the better of toxicity. The LC_{50} of actinomycete HV11.P3 and SCA54.P2 pigment extracts were 94.53 µg/mL and 131.22 µg/mL, respectively (Table 2).

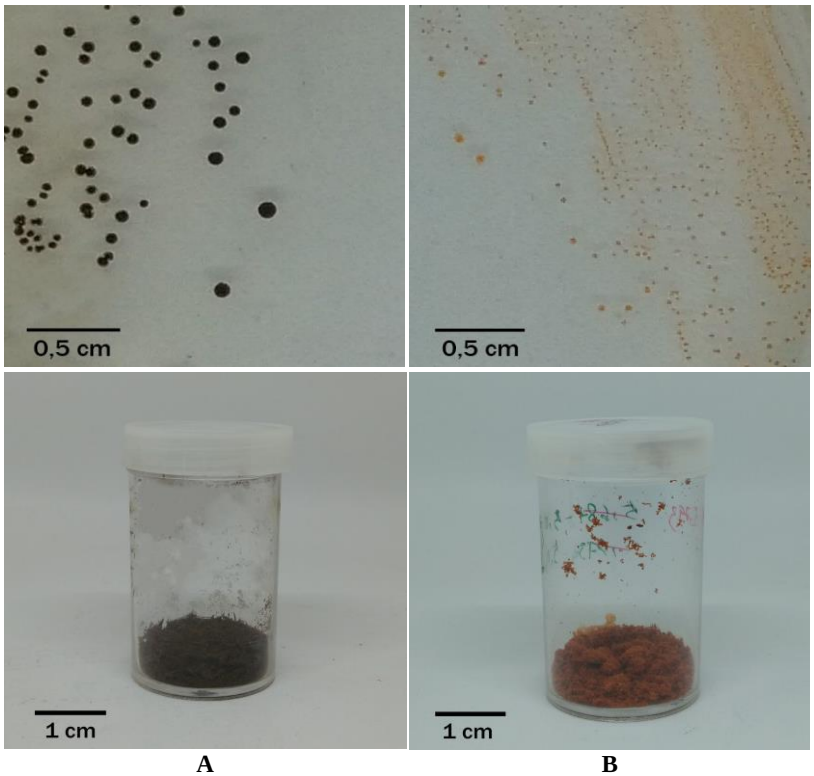

Figure 1. The colonies appearance of actinomycetes on ISP-4 agar medium at 7 days of incubation at room temperature and the pigment crude extract yielded after 14 days of culturing in ISP-4 broth medium: A. Actinomycete HV11.P3; B. Actinomycete SCA54.P2

Table 1. The pigment extract yield derived from Actinomycete HV11.P3 and SCA54.P2.

Pigment Extract	Culture Volume (L)	Weight (g)	Yield (%)
Actinomycete HV11.P3	1	2.33	0.23
Actinomycete SCA54.P2	1	1.82	0.18

Table 2. The toxicity of actinomycete HV11.P3 and SCA54.P2 pigment extracts based on Brine Shrimp Lethality Test

Pigment extract	LC_{50} value (µg/ mL)			
	Replication 1	Replication 2	Replication 3	Average
Actinomycete HV11.P3	88.13	80.04	115.42	94.53 ± 15.14
Actinomycete SCA54.P2	156.96	102.72	133.98	131.22 ± 22.23

Table 3. The antimicrobial activities of actinomycete HV11.P3 and SCA54.P2 pigment extracts (750 µg/ mL) against several tested microbes

Pigment extract	Tested microbes				
	Bacillus subtilis **ATCC 19659**	***Escherichia coli*** **ATCC 8739**	***Pseudomonas aeruginosa*** **ATCC 15442**	***Staphylococcus aureus*** **ATCC 6538**	***Candida albicans*** **ATCC 10231**
Actinomycete HV11.P3	+	+	-	+	-
Actinomycete SCA54.P2	+	++	+	-	-

Note: 0.1-5 mm (+), 6-10 mm (++), and 11-15 (+++)

Table 4. The antioxidant activities of actinomycete HV11.P3 and SCA54.P2 pigment extracts against DPPH as free radical

Pigment extract	IC_{50} value (µg/ mL)			
	Replication 1	Replication 2	Replication 3	Average
Actinomycete HV11.P3	243.40	224.07	225.78	231.08 ± 8.74
Actinomycete SCA54.P2	436.77	373.95	297.18	369.3 ± 57.08

Antimicrobial activities of pigment extracts

The pigment extracts of actinomycetes were tested against both Gram-positive and Gram-negative bacteria, as well as yeast. Based on the antimicrobial test, the pigment extract of actinomycete HV11.P3 and SCA54.P2, exhibited antimicrobial activity against several tested microbes. The HV11.P3 pigment extract possessed positive activities against *B. subtilis*, *E. coli*, and *S. aureus*. On the other hand, SCA54.P2 pigment extract could inhibit *B. subtilis*, *E. coli*, and *P. aeruginosa* activity (Table 3). No inhibition zone was observed for both of those extracts against *C. albicans*.

Antioxidant activities of pigment extracts

Both of the pigment extracts derived from actinomycete HV11.P3 and SCA54.P2 showed scavenging activity against DPPH as free radical. Actinomycete HV11.P3 and SCA54.P2 pigment extracts exhibited an IC_{50} value of 231.08 µg/mL and 369.30 µg/mL, respectively (Table 4). IC_{50} is a standard value for the lowest concentration that can inhibit 50% activity of free radicals.

Chemical compound identification

Gas Chromatography-Mass Spectrometry (GC-MS) analysis was used for investigating the dominant extract compound. The dominant compound in actinomycete HV11.P3 pigment extract was 1H-Purine-2,6-dione,3,7-dihydro-1,3,7-trimethyl- (Figure 2; Table 5), while for actinomycete SCA54.P2 pigment extract the dominant compound was 5-Methoxypyrrolidin-2-one (Figure 3; Table 6).

Molecular identification

Amplification of 16S rRNA genes from actinomycete HV11.P3 and SCA54.P2 genome showed 1480 bp band after visualization by gel electrophoresis (Figure 4). Based on analysis of 16S rRNA gene sequences of the two actinomycetes exhibited the same closest similarity with *Micromonospora* genera (Table 7). The constructed phylogenetic tree of the two 16S rRNA sequences of the two actinomycetes showed their position among other actinomycetes. Actinomycete HV11.P3 showed the closest similarity with *Micromonospora chalcea* strain 1464-217L, while actinomycete SCA54.P2 showed the closest similarity with *Micromonospora tulbaghiae* strain TVU1 (Figure 5).

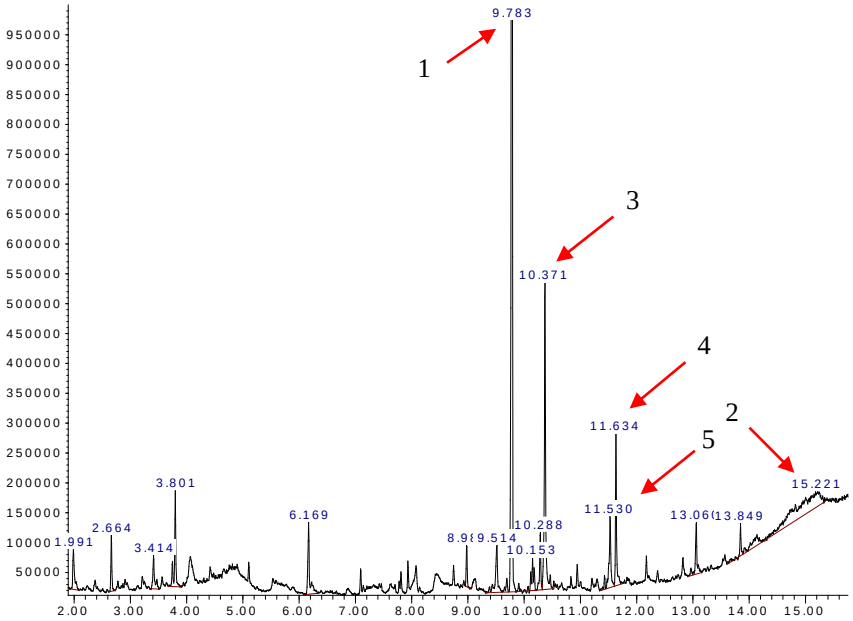

Figure 2. Chromatogram of chemical compounds of pigment extract derived from actinomycete HV11.P3 based on GC-MS analysis showing five dominant compounds as indicated by red arrows

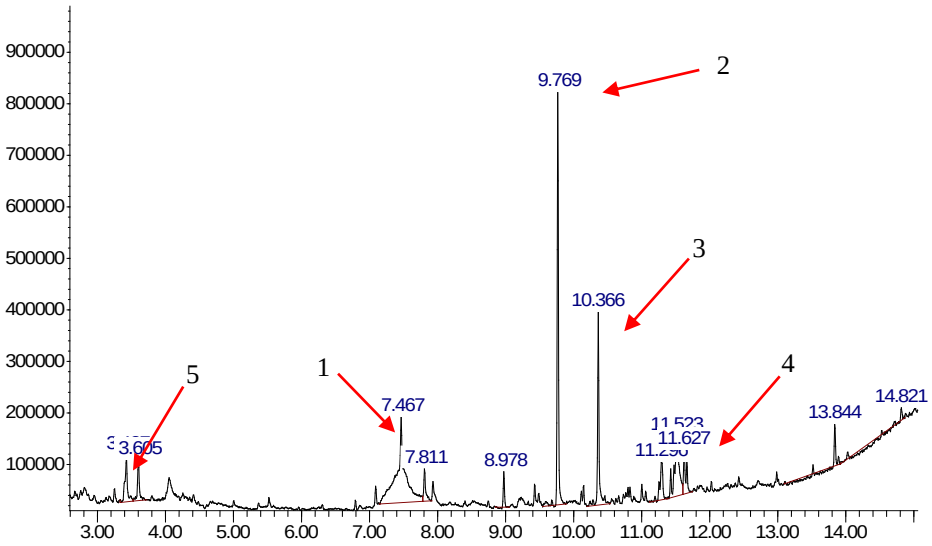

Figure 3. Chromatogram of chemical compounds of pigment extract derived from actinomycete SCA54.P2 based on GC-MS analysis showing five dominant compounds as indicated by red arrows

Table 5. Five major chemical compounds of actinomycete HV11.P3 pigment extract and their biological activities.

Chemical compound	Peak area (%)	Activity	Reference
1H-Purine-2,6-dione, 3,7-dihydro-1,3,7-trimethyl- (CAS)	44.65	Anti-inflammation	Rezaei et al. (2021)
Cyclotrisiloxane, hexamethyl- (CAS)	13.20	Antimicrobial	Keskin et al. (2012)
n-Hexadecanoic acid (CAS)	11.10	Antibacterial, antioxidant, toxic compound	Krishnaveni et al. (2014)
Octadecanoic acid (CAS)	4.46	Antibacterial,	Pradheesh et al. (2017)
9-Octadecenoic acid	3.61	Anti-hyperglycaemic	Kapoor et al. (2019)

Table 6. Five major chemical compounds of actinomycete SCA54.P2 pigment extract and their biological activities.

Chemical compound	Peak area (%)	Activity	Reference
5-Methoxypyrrolidin-2-one	30.23	Antioxidant	Dascalu et al. (2020)
1H-Purine-2,6-dione, 3,7-dihydro-1,3,7-trimethyl- (CAS)	24.27	Anti-inflammation	Rezaei et al. (2021)
n-Hexadecanoic acid	12.93	Antibacterial	Krishnaveni et al. (2014)
9,12,15-Octadecadienoic acid (Z,Z,Z)-	12.26	Antibacterial	Ojinnaka et al. (2015)
4H-Pyran-4-one, 2-hydroxy-3-methyl-(CAS)	5.05	Anti-proliferative, antioxidant	Amatori et al. 2010

Table 7. Molecular identification of the actinomycetes isolates based on the 16S rRNA gene

Isolate	Closest relative	Query cover (%)	E-Value	Percent identification (%)	Accession Number
Actinomycete HV11.P3	*Micromonospora chalcea* strain 1464-217L	99	0.0	97.9	NR_036795.1
Actinomycete SCA54.P2	*Micromonospora tulbaghiae* strain TVU1	100	0.0	100	NR_116241.1

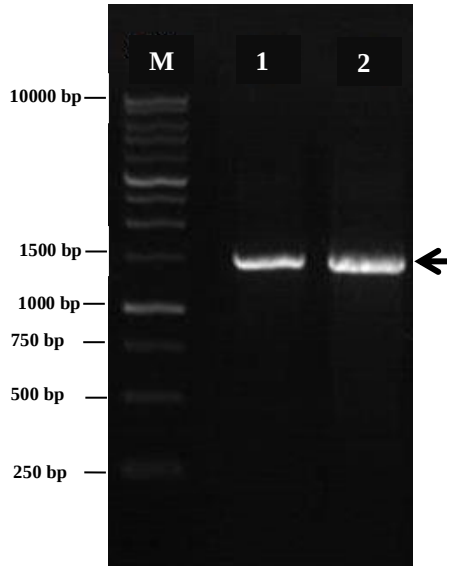

Figure 4. Gel electrophoresis of 16S rRNA genes amplified from actinomycete genomes. 1: actinomycete HV11.P3; 2: actinomycete SCA54.P2; M: Marker 1 Kb ladder

Discussion

In our study, HV11.P3 and SCA54.P2 were found to be capable of producing intracellular pigments that were greenish-black and orange, respectively. Based on the BSLT test, pigment extract of HV11.P3 and SCA54.P2 showed a high *in vivo* toxicity effect with an LC_{50} value of less than 150 µg/mL. BSLT test was used as preliminary test to search for an anticancer agent (Elsyana et al. 2016). Therefore, further study to evaluate the cytotoxic activity against human cells need to be done. Promising antimicrobial abilities will lead to great activity of toxicity (Pandit et al. 2018). Some compound such as antimicrobial peptide (AMP) has been found to have dual action as antimicrobial as well as cytotoxic activity (Felicio et al. 2017). Actinomycete SCA54.P2 and HV11.P3 pigment crude extracts showed a low to moderate activity against the tested bacteria. Both of the extracts could inhibit the Gram-positive and Gram-negative of the tested bacteria. However, none of these extracts had activity against *C. albicans* as a representative of the eukaryotic cell. Pigments derived from actinomycetes have been gaining much attention due to their therapeutic potencies, one of them is antimicrobial activities (Mumtaz et al. 2018).

In order to investigate their antioxidant activity, both of our pigment extracts were tested for their *in vitro* antioxidant activities. Our results revealed that actinomycete HV11.P3 and SCA54.P2 pigment extracts had IC_{50} value of 23.08 µg/mL and 369.30 µg/mL against DPPH radicals, respectively. The antioxidant may decrease the oxidative stress level on the cell. The low level of free radical is undoubtedly reduced the possibility of cell damage and antibiotics effectivity. However, some antioxidant agents were reported to have potent antibacterial activity. The mechanisms involved in this process have been described previously (Naqvi et al. 2019; Dwyer et al. 2014). Interestingly, both our crude extracts also showed antioxidant and antibacterial activity. Our findings were consistent with research done using *Streptomyces* sp. VITSTK7 that possessed both antibacterial and antioxidant activities (Thenmozhi and Kannabiran 2012).

Furthermore, to understand the possible chemical compounds involved in their biological activities, we identified the chemical composition of the pigment extracts by GC-MS analysis. Some of those compounds include benzene, alcohols, esters, fatty acids, and amino acids groups. The presences of n-Hexadecanoic acid (CAS), Octadecanoic acid (CAS), and 9,12,15-Octadecadienoic acid (Z,Z,Z) in our present study may be the main compounds that play a role in inhibiting microbial growth. Our results are in line with the previous study that revealed these compounds can inhibit the growth of bacteria such as *E. coli* (Gram-negative bacteria), *S. aureus* (Gram-positive bacteria), and the fungus *Aspergillus flavus* (Krishnaveni et al. 2014). Meanwhile, n-Hexadecanoic acid (CAS) may also play a significant role along with 5-Methoxypyrrolidin-2-one which may act as an antioxidant agent (Dascalu et al. 2020).

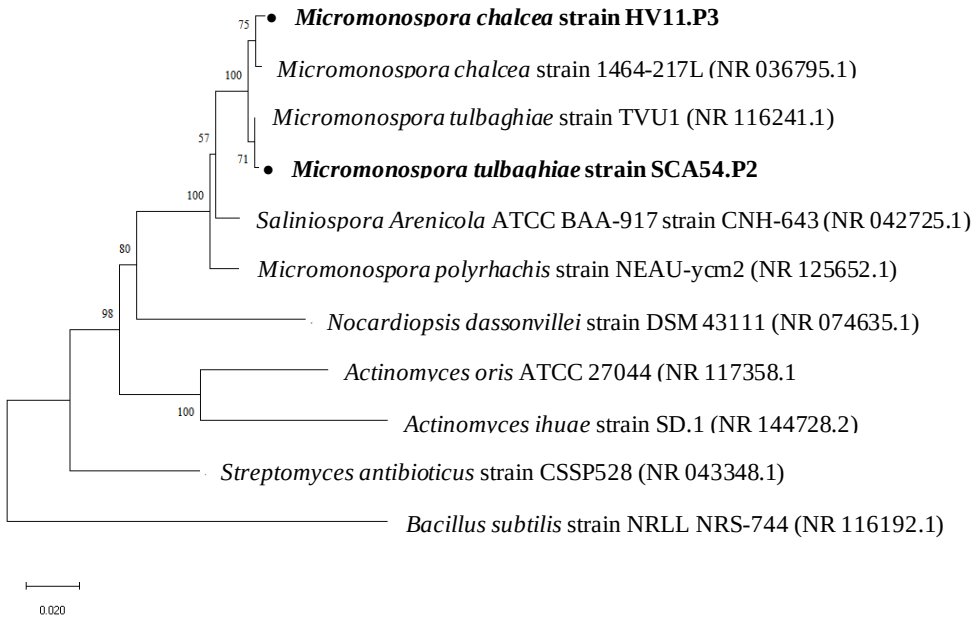

Figure 5. Neighbor-joining phylogenetic tree of the 16S rRNA gene sequence of actinomycete HV11.P3 and SCA54.P2 on bootstrap values 1000x. Both actinomycetes showed the closest similarity with the genus of *Micromonospora.*

Identification of the two actinomycete isolates was performed based on the 16S rRNA gene sequence. As a result, both actinomycetes belong to the genus *Micromonospora*. The actinomycete HV11.P3 showed a similarity percentage of 97.9% with *Micromonospora chalcea* strain 1464-217L, and actinomycete SCA54.P2 showed a 100% similarity percentage with *Micromonospora tulbaghiae* strain TVU1. The genus *Micromonospora* is known to be able to produce various types of bioactive compounds such as oligosaccharides, terpenes, and lanthipeptides that have therapeutic activity as antibacterial (Back et al. 2021), antitumor and antioxidant (Nafie et al. 2021), antifungal (Zhao et al. 2017), and antivirus (Debbab et al. 2010).

Thus, the present results revealed that methanol extract of pigments derived from two actinomycetes identified as *Micromonospora chalcea* HV11.P3 and *Micromonospora tulbaghiae* SCA54.P2 showed antibacterial, antioxidant, and toxicity activities. The best antibacterial activity of the pigment extract was shown by *M. tulbaghiae* SCA54.P2 against *E. coli* ATCC 8739, while the *M. chalcea* HV11.P3 pigment extract showed the best activity as an antioxidant.

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REFERENCES

Al-Snafi AE. 2017. Medicinal plants possessed antioxidant and free radical scavenging effects (part 3)-a review. IOSR J Pharm 7: 48-62. DOI: 10.9790/3013-0704014862

Amatori S, Bagaloni I, Macedi E, Formica M, Giorgi L, Fusi V, Fanelli M. 2010. Malten, a new synthetic molecule showing in vitro antiproliferative activity against tumour cells and induction of complex DNA structural alterations. Br J Cancer 103: 239-248. DOI: 10.1038/sj.bjc.6605745

Back CR, Stennett HL, Williams SE, Wang L, Ojeda Gomez J, Abdulle OM, Duffy T, Neal C, Mantell J, Jepson MA, Hendry KR. 2021. A new *Micromonospora* strain with antibiotic activity isolated from the microbiome of a mid-Atlantic deep-sea sponge. Mar Drugs 19: 1-19. DOI: 10.3390/md19020105

Batubara I, Mitsunaga T, Ohashi H. 2009. Screening antiacne potency of Indonesian medicinal plants: antibacterial, lipase inhibition, and antioxidant activities. J Wood Sci 55: 230-235. DOI: 10.1007/s10086-008-1021-1

Bérdy J. 2005. Bioactive microbial metabolites. J Antibiot 58: 1-26. DOI: 10.1038/ja.2005.1

Bhakyashree K, Kannabiran K. 2018. Anti-MRSA activity of actinomycetes isolated from marine soil sample of ariyaman beach, Tamil Nadu, India. Res J Pharm Technol 11: 2036-2039. DOI: 10.5958/0974-360X.2018.00377.3

Bibi F, Yasir M, Al-Sofyani A, Naseer MI, Azhar EI. 2020. Antimicrobial activity of bacteria from marine sponge *Suberea mollis* and bioactive metabolites of *Vibrio* sp. EA348. Saudi J Biol Sci 27: 1139-1147. DOI: 10.1016/j.sjbs.2020.02.002

Dascalu AE, Ghinet A, Lipka E, Furman C, Rigo B, Fayeulle A, Billamboz M. 2020. Design, synthesis and evaluation of hydrazine and acyl hydrazone derivatives of 5-pyrrolidin-2-one as antifungal agents. Bioorg Med Chem Lett 30: 1-6. DOI: 10.1016/j.bmcl.2020.127220

Dawoud TM, Alharbi NS, Theruvinthalakal AM, Thekkangil A, Kadaikunnan S, Khaled JM, Almanaa TN, Sankar K, Innasimuthu GM, Alanzi KF, Rajaram SK. 2020. Characterization and antifungal activity of the yellow pigment produced by a *Bacillus* sp. DBS4 isolated from the lichen *Dirinaria agealita*. Saudi J Biol Sci 27: 1403-1411. DOI: 10.1016/j.sjbs.2019.11.031

Debbab A, Aly AH, Lin WH, Proksch P. 2010. Bioactive compounds from marine bacteria and fungi. Microb Biotechnol 3: 544-563. DOI: 10.1111/j.1751-7915.2010.00179.x

Dwyer DJ, Belenky PA, Yang JH, MacDonald IC, Martell JD, Takahashi N, Chan CT, Lobritz MA, Braff D, Schwarz EG, Jonathan DY. 2014. Antibiotics induce redox-related physiological alterations as part of their lethality. Proc Natl Acad Sci USA 111: 100-109.

Elsyana V, Bintang M, Priosoeryanto BP. 2016. Cytotoxicity and antiproliferative activity assay of clove mistletoe (*Dendrophthoe pentandra* (L.) Miq.) leaves extracts. Adv Pharmacol Pharm Sci 2016: 1-6. DOI: 10.1155/2016/3242698

Felício MR, Silva ON, Gonçalves S, Santos NC, Franco OL. 2017. Peptides with dual antimicrobial and anticancer activities. Front Chem 5: 1-9. DOI: 10.3389/fchem.2017.00005

Founou RC, Founou LL, Essack SY. 2017. Clinical and economic impact of antibiotic resistance in developing countries: a systematic review and meta-analysis. PLoS One 12: 1-18. DOI: 10.1371/journal.pone.0189621

Genilloud O. 2015. *Micromonospora*. BMSAB 1-28. DOI: 10.1002/9781118960608.gbm00148

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.

Hifnawy SM, Hassan HM, Mohammed R, M Fouda M, Sayed AM, A Hamed A, F AbouZid S, Rateb ME, Alhadrami HA, Abdelmohsen UR. 2020. Induction of antibacterial metabolites by co-cultivation of two red-sea-sponge-associated actinomycetes *Micromonospora* sp. UR56 and *Actinokinespora* sp. EG49. Mar drugs 18:243. DOI: 10.3390/md18050243

Kapoor G, Pathak DP, Bhutani R, Husain A, Jain S, Iqbal MA. 2019. Synthesis, ADME, docking studies and in vivo anti-hyperglycaemic potential estimation of novel Schiff base derivatives from octadec-9-enoic acid. Bioorg Chem 84: 478-492. DOI: 10.1016/j.bioorg.2018.12.004

Keskın D, Ceyhan N, Uğur A, Dbeys AD. 2012. Antimicrobial activity and chemical constitutions of West Anatolian olive (*Olea europaea* L.) leaves. J Food Agric Environ 10: 99-102.

Kharirie K, Andriani L. 2020. The predominance of non-communicable diseases and unhealthy eating patterns. In: Proceeding of National Seminar of Indonesia Biodiversity Community. Sebelas Maret University, Bogor, 12 October 2019. [Indonesian]

Krishnaveni M, Nandhini N, Dhanalakshmi R. 2014. GC-MS analysis of phytochemicals, fatty acids and antimicrobial potency of dry christmas lima beans. Int J Pharm Sci Rev Res 27: 63-66.

Liao L, Chen R, Jiang M, Tian X, Liu H, Yu Y, Fan C, Chen B. 2016. Bioprospecting potential of halogenases from Arctic marine actinomycetes. BMC microbiol 16: 1-9. DOI: 10.1186/s12866-016-0662-2

McLaughlin JL, Rogers LL, Anderson JE. 1998. The use of biological assays to evaluate botanicals. Drug Inf J 32: 513-524. DOI: 10.1177/009286159803200223

Meyer BN, Ferrigni NR, Putnam JE, Jacobsen LB, Nichols DJ, McLaughlin JL. 1982. Brine shrimp: a convenient general bioassay for active plant constituents. Planta Med 45: 31-34. DOI: 10.1055/s-2007-971236

Mumtaz R, Bashir S, Numan M, Shinwari ZK, Ali M. 2018. Pigments from soil bacteria and their therapeutic properties: A mini review. Curr Micribiol 76: 783-790. DOI: 10.1007/s00284-018-1557-2

Nafie MS, Awad NM, Tag HM. 2021. *Micromonospora* species from rarely-exploited Egyptian habitats: chemical profile, antimicrobial, and antitumor activities through antioxidant property. Appl Microbiol Biotechnol 105: 2427-2439. DOI: 10.1007/s00253-021-11177-2

Naqvi SA, Nadeem S, Komal S, Naqvi SA, Mubarik MS, Qureshi SY, Ahmad S, Abbas A, Zahid M, Raza SS, Aslam N. 2019. Antioxidants: Natural Antibiotics. IntechOpen, London.

Ojinnaka, Chukwunonye M, Kelechi IN, Marycolette NE. 2005. The chemical constituents and bioactivity of the seed (Fruit) extracts of *Buchholzia Coriacea* Engler (Capparaceae). J Appl Sci Environ Manag 19: 795-801. DOI: 10.4314/jasem.v19i4.29

Pandit S, Dhawan A, Parthasarathi R. 2018. Emerging computational methods for predicting chemically induced mutagenicity. Academic Press, London. DOI: 10.1016/B978-0-12-809252-1.00008-0

Pradheesh G, Suresh J, Suresh S, Alexramani V. 2017. Antimicrobial activity and identification of potential ethanolic antimicrobial compounds from the medicinal plant *Pisonia grandis* r.br. World J Pharm Pharm Sci 6: 1686-1700. DOI: 10.20959/wjpps20174-8961

Prestinaci F, Pezzotti P, Pantosti A. 2015. Antimicrobial resistance: a global multifaceted phenomenon. Pathog Glob Health 109: 309-318. DOI: 10.1179/2047773215Y.0000000030

Pudi N, Varikuti GD, Badana AK, Gavara MM, Singh SK, Malla R. 2016. Studies on optimization of growth parameters for enhanced production of antibiotic alkaloids by isolated marine actinomycetes. J Appl Pharm Sci 6: 181-188. DOI: 10.7324/JAPS.2016.601025

Rezaei H, Rahimpour E, Zhao H, Martinez F, Jouyban A. 2021. Solubility measurement and thermodynamic modeling of caffeine in n-methyl-2-pyrrolidone+ isopropanol mixtures at different temperatures. J Mol Liq 363: 116519. DOI: 10.1016/j.molliq.2021.116519

Roope LS, Smith RD, Pouwels KB, Buchanan J, Abel L, Eibich P, Butler CC, San Tan P, Walker AS, Robotham JV, Wordsworth S. 2019. The challenge of antimicrobial resistance: What economics can contribute. Science 364: 1-8. DOI: 10.1126/science.aau4679

Sahardi NFNM, Makpol S. 2019. Ginger (*Zingiber officinale* Roscoe) in the prevention of ageing and degenerative diseases: review of current evidence. Evid Based Complement Alter Med 2019: 1-13. DOI: 10.1155/2019/5054395

Stambler I. 2017. Recognizing degenerative aging as a treatable medical condition: methodology and policy. Aging Dis 8: 583-589. DOI: 10.14336/AD.2017.0130

Sineva ON, Bychkova OP, Terekhova LP. 2021. Acidotolerant actinomycetes of the genus *Micromonospora* are producers of antibiotic compounds. In Presented At: The First International Electronic Conference On Antibiotics 8:17.

Thenmozhi M, Kannabiran K. 2012. Antimicrobial and antioxidant properties of marine actinomycetes *Streptomyces* sp. VITSTK7. Oxid Antioxid Med Sci 1: 51-57. DOI: 10.5455/oams.270412.or.005

Trujillo ME, Riesco R, Benito P, Carro L. 2015. Endophytic actinobacteria and the interaction of *Micromonospora* and nitrogen fixing plants. Front Microbiol 6: 1341. DOI: 10.3389/fmicb.2015.01341

Weisberg WG, Barns SM, Pelletier DA, Lane DJ. 1991. 16S ribosomal DNA amplification for phylogenetic study. J Bacteriol 173: 697-703. DOI: 10.1128/jb.173.2.697-703.1991

Zhao S, Liu C, Zheng W, Ma Z, Cao T, Zhao J, Yan K, Xiang W, Wang X. 2017. *Micromonospora parathelypteridis* sp. nov., an endophytic actinomycete with antifungal activity isolated from the root of *Parathelypteris beddomei* (Bak.) Ching. Int J Syst Evol Microbiol 67: 268-274. DOI: 10.1099/ijsem.0.001614