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Bioactive Compound from Extract Filtrat *Streptomyces* sp.Sp1. as Biocontrol of Vibriosis on Larvae of *Macrobrachium rosenbergii* shrimps

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ABSTRACT

Production of *Macrobrachium rosenbergii* shrimp was limited by vibriosis disease. *Streptomyces* can be used as an alternative control of vibriosis which is caused by *Vibrio anguillarum*. Aplication of *Streptomyces* sp.1 culture could give significant different impacts (p<0.05) on the percentage of survival (SR) of shrimp larvae that had been infected by *V. anguillarum* compared to the control. This study was conducted to identify and determine bioactive compound of *Streptomyces* sp.1. The presence of bioactive compound was confirmed by gas chromatography-mass spectrometry (GC-MS) analysis. Identification of *Streptomyces* sp.1 by 16S rRNA partial gene sequencing method. *Streptomyces* sp.1 was identified to be *Streptomyces* sp.Sp1. There were 35 chemical compounds identified, the chemical compounds which have antimicrobial properties identified in the n butanol extract of *Streptomyces* sp.Sp1 are 3-Hexanone, 2-methyl (43%), n Butyl ether (19%) Nonane (CAS) n-Nonane (5.6%), Decane (CAS) n-Decane (3.2%), Hexacosane (CAS) n-Hexacosane (1.9%), Tetracontane (1.3%), Heneicosane (1.9%), Hexadeconoic acid, methyl ester (0.3%), Butane,1,1-dibutoxy (0.3%), and Limonene (0.3%). Benzeneacetic acid, 3-methoxy-.alpha.,4-bis|(tri (0.3%). These results suggested that *Streptomyces* sp.Sp1 had good general antimicroba activity and might have potential biocontrol antagonist *Vibrio anguillarum*.

1. Introduction

Giant prawns are one of the original Indonesian freshwater shrimp. The introduction of this commodity for aquaculture began by the Department of Marine and Fisheries in 2001 as a superior alternative commodity. Giant shrimp is very potential to be used as an alternative commodity because it has a large body size compared to other types of shrimp farming and has high economic value in the market (Priyono *et al.* 2011).

Based on the results of the survey conducted (2017), one of the efforts to seed giant prawn larvae in Bali is carried out by the Bali Province Marine and Fisheries Hatchery Unit in the Klungkung Bali. The amount of ready-stock shrimp production produced by the UPT is generally very volatile. This is influenced by environmental and microbiological conditions of the pond, especially the presence of pathogenic bacteria that cause disease.

Vibriosis in addition to infecting shrimp in aquaculture ponds can also infect shrimp larvae in hatcheries. Infection at the seed level is very dangerous because it can cause high seed mortality and can be a source of entry of pathogens into the pond (Patang 2012). Oanh *et al.* (2008) also reported Vibrio infection in *Macrobrachium rosenbergii* larvae and postlarvae causing significant mortality for 48 hours of testing. Therefore production of *M. rosenbergii* shrimp was limited by vibriosis disease. *Streptomyces* can be used as an alternative control of vibriosis which is caused by *Vibrio anguillarum*. As our previous research showed that application of *Streptomyces* sp.1 culture give significant different impacts (p<0.05) on the percentage of survival (SR) of shrimp larvae that had been infected by *V. anguillarum* compared to the control. Treatment of *Streptomyces* sp.1 culture could also significantly (p<0.05) reduce the total population of Vibrio on the

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maintenance media compared to the control (Bintari *et al*. 2017).

Most *Streptomyces* spp. can produce antibiotic such as cephamycin, chloramphenicol, kanamycin, tetracycline, spectinomycin, streptomycin, clavulanic acid, and monensin (Barrios *et al*. 2003) Lowicki and Huczynski (2013) showed monensin that isolated from *S. cinnamonensis* can inhibit the growth of bacteria genus *Micrococcus, Bacillus, and Staphylococcus.*

Gas chromatography–mass spectrometry (GC–MS) analyses have been developed for the analysis and characterization of active compounds from micro-organisms. This study aimed to identify *Streptomyces* sp.1 with 16S rRNA and determine the GC-MS analysis of the n butanol extracts of bioactive compound of *Streptomyces* sp.1.

2. Materials and Methods

Streptomyces sp.1 (collection of Microbiology Lab. FMIPA Unud, Figure 1), molecular identification using 16S rRNA with primers 63f (5'-CAG GCC TAA CAC ATG CAA GTC-3') and primer reverse 1387r (5'-GGG CGG WGT GTA CAA GGC-3') was conducted in the Laboratory of Microbiology, Bogor Agricultural Institute (Institut Pertanian Bogor/IPB).

2.1. Isolation of Genomic DNA

Rejuvenation of bacterial isolates was done by using Yeast Salt Agar (ISP6) media and the cultures were incubated at room temperature for four days. Five colony circles (diameter 5 mm) were added to Yeast Salt Broth (ISP7) media and incubated in an incubator shaker at a temperature of 25°C for four days. Isolation of DNA was done by CTAB method according to Marchesi *et al.* (1988).

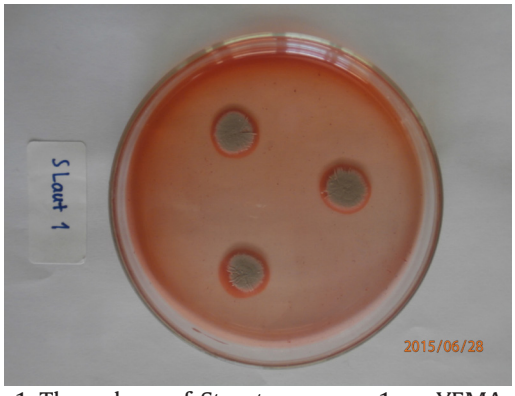

Figure 1. The colony of *Streptomyces* sp.1 on YEMA media (Bintari *et al*. 2017)

2.2. DNA Amplification by PCR

16S rRNA gene was amplified by Polymerase Chain Reaction machines (Perkin Elmer GeneAmp PCR system 2400, Germany) using specific primers of prokaryotic actinomycetes, namely 63f (5'-CAG GCC TAA CAC ATG CAA GTC-3'), and primer reverse 1387r (5'-GGG CGG WGT GTA CAA GGC-3'). The composition of the PCR reaction consisting of Taq DNA polymerase enzyme La 0.5 ml, 25 ml 2X GC buffer, dNTP mixture 8 ml, 20f primer (10 pmol) 1.5 µl, 1387r primer (10 pmol) 1.5 µl, ddH2O 9.5, and 4 µl DNA template. PCR conditions used are predenaturation (94°C, 5 min), denaturation (94°C, 1 min), annealing (57°C, 1 min), elongation (72°C, 1 minute and 10 seconds), and the post-PCR (72°C, 7 min) with the number of cycles of 30 cycles. The separation of DNA PCR products is done on a mini-gel electrophoresis machine using 1% agarose on 75 volt power supply voltage for 45 minutes. Visualization of DNA was done over the UV transilluminator using Ethidium Bromide (EtBr) dye.

2.3. DNA Sequence and Alignment

The amplified Deoxyribose Nucleic Acid (DNA) was partially sequenced to determine the nucleotide sequences using the services of PT Macrogen, Korea. The nucleotide sequences, as the result of the sequencing process, would then be aligned with the GeneBank data using BLAST-N program (Basic Local Alignment Search Tool-Nucleotides) from the NCBI website (National Center for Biotechnology Information).

2.4. Preparation of *Streptomyces* sp.1 Extract

Streptomyces sp.1 was grown for five days at Yeast Malt Agar (YEMA) media and incubated at 28°C ± 2°C, then taken using cork borer (diameter of 5 mm) by 5 pieces and put in an erlenmeyer (250 ml) filled with 50 ml Yeast Malt Extract Broth (1% malt extract, 0.4% dextrose, 0.4% yeast extract, 2% agar; pH 7.0) media. Then it was incubated on a incubator shaker at temperature of 28°C ± 2°C at 125 rpm speed for 14 days. Filtrate from culture was then collected, followed by centrifugation at 11,000 rpm speed for 15 minutes and was filtered using filter paper 0.45 µm. Filtrate partition was done by pouring the filtrate into a 1 litre separation bottle and then n butanol solvent was added at ratio of 1:1 (v/v), followed by evaporation to separate n butanol with filtrate using evaporator machine (Buchi Rotavapor R-210, Japan). Then it was homogenized and left undisturbed for 24

hours to separate between water phases and ethyl acetate phase. Each of these phases (water and ethyl acetate phase) was separated by separating funnel and then n butanol phase was evaporated with an evaporator machine at a temperature of 40°C (Buchi Rotavapor R-210, Japan) to obtain an extract used for further testing.

2.5. Purification of Active Compound Extract *Streptomyces* sp.1

The purification of active compounds were separated by thin layer chromatography (TLC) method (Sharon *et al.* 2013). The extract was spotted on a TLC plate (silica gelplat Merck 60 F254) developed with a mixture of chloroform-ethyl acetate-acetic acid (7:3:1, v/v) and node visualized under UV light λ254 nm and λ365 nm. Each band were scrapped out separately and collected in different vials. Then the each band compounds were checked again for bioactive metabolites by agar well diffusion method. Testing is done by testing the activity of the extract of *Streptomyces* sp.1 against *V. anguillarum*. Petri dish that already contains 10 ml of media NA and 200 ml of the suspension of against *V. anguillarum* allowed to solidify and after solid diffusion wells were made using a cork borer.

2.6. GC-MS Analysis

Identification of active compounds of *Streptomyces* sp.1 that had bactericidal activity against *Vibrio anguillarum* causing vibiosis in *Macrobrachium rosenbergii* shrimp was identified using gas chromatography-mass spectroscopy (GC-MS). A snapshot of most active and a relatively pure fraction was analyzed by gas chromatography-mass spectrometry. Through suitability of molecular weight and fragmentation pattern of the isolated compounds with the compounds in the library (WILEY or NIST) in the GC-MS system then isolated compounds could be known of the name, formula and molecular structure (Gopalakrishnan and Vadivel 2011). Gas chromatography-mass spectroscopy test conducted at the Joint Laboratory of the Faculty of Mathematics and Natural Sciences University of Udayana, Badung, Bali. GC-MS tool used was GC-MS-QP2010 Ultra SHIMADZU. Column temperature was programmed between 80°C and 250°C at a rate of 1:18 ml/min. The temperatures in the injector and detector were respectively 250°C and 220°C.

3. Results

3.1. Molecular Identification of *Streptomyces* sp.1 using 16S rRNA

Genome amplification of bacterial samples using PCR with primers 63F and 1387r produced DNA fragments measuring approximately 1300 bp (Figure 2). The results of the gene sequence alignments of 16S rRNA isolates sp.1 to the data available at NCBI was available in Table 1, and Phylogenetic tree construction of *Streptomyces* sp.1 was showed in Figure 3. Based on molecular identification, the *Streptomyces* sp.1 can be identified as *Streptomyces* sp.Sp1.

3.2. Purification of Active Compound Extract *Streptomyces* sp.1

The result of the extract fractionation using a hexane solvent, Ethyl acetate and n butanol, 8 fractions were found. Based on Retention Fraction (RF) value, it was found 4 fraction (Table 2). To the four active compounds, antagonist test was conducted with pathogen *V. anguillarum*. The result showed that RF mixture (RFG1, RFG2, RFG3, and RFG4) has the clear zone even it has inhitory zone 22 mm (Table 3 and Figure 4) and then analyzed for the compounds by using GCMS as indicated on Table 4 and Figure 5.

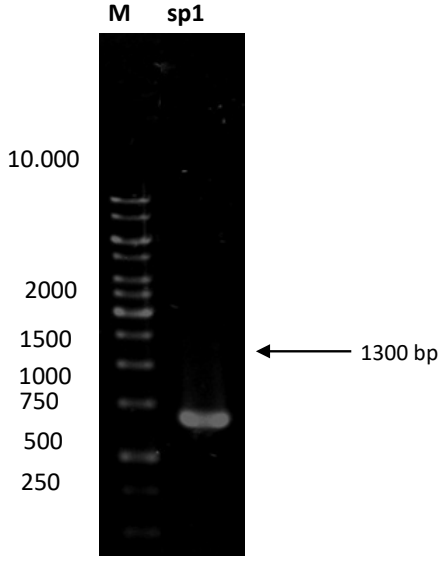

Figure 2. PCR amplification of the gene 16S rRNA with primers 63F and 1,387r primer; M= marker 1 Kb ladder; sp1= PCR product samples of the bacterium

Table 1. The results of the gene sequence alignments of 16S rRNA isolates sp.1 to the data available at NCBI (BLASTN)

Description	Max score	Total score	Quary cover	E value	Identity	Acces number
Streptomyces sp. LCJ8A 16S ribosomal RNA gene, partial sequence	2,289	2,289	100	0.0	99	KU870433.1
Streptomyces djakartensis strain TTIO5.3 16S ribosomal RNA gene, partial sequence	2,289	2,289	100	0.0	99	KT726172.1
Streptomyces rochei strain H3-1 16S ribosomal RNA gene, partial sequence	2,289	2,289	100	0.0	99	KR023965.1
Streptomyces enissocaesilis strain SAS05 16S ribosomal RNA gene, partial sequence	2,289	2,289	100	0.0	99	KP986572.1
Streptomyces minutiscleroticus strain KMF-2 16S ribosomal RNA gene, partial sequence	2,289	2,289	100	0.0	99	KJ020685.1

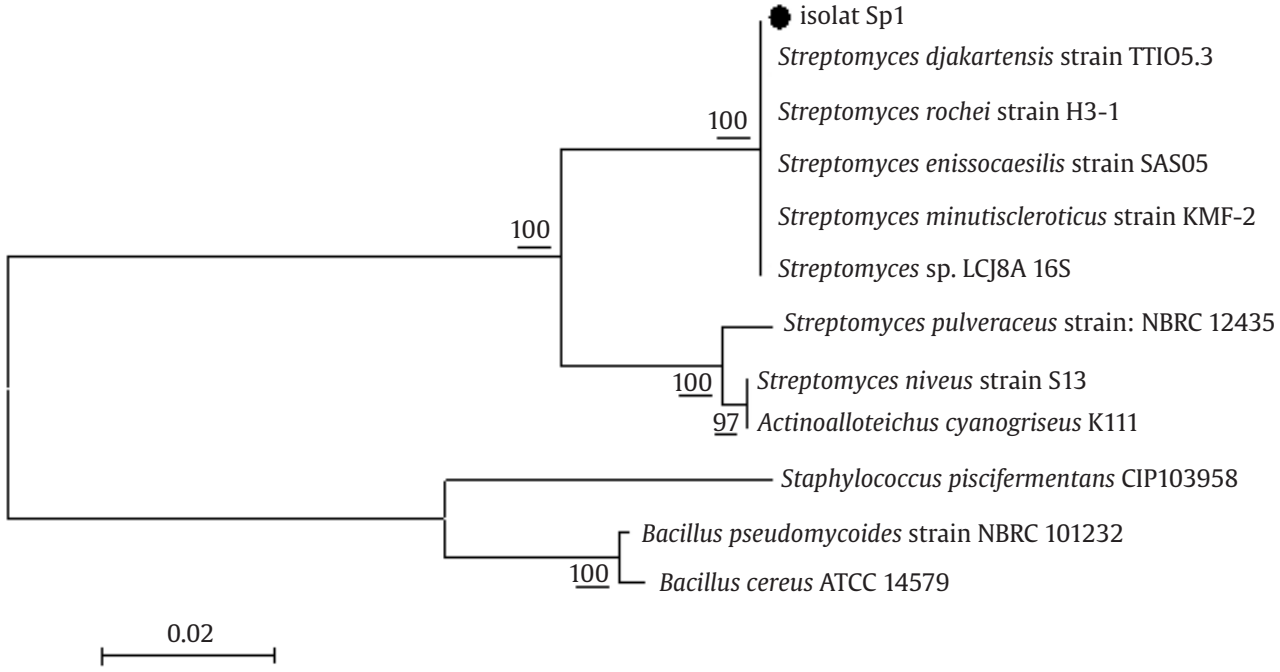

Figure 3. A phylogenetic tree that illustrates how close isolate Sp.1 against other bacteria in one clade or another clade (outer group). The construction is based on neighbor-joining tree method with bootstrap value of 1,000x repetition

Table 2. Retention fraction (RF) Value of extract filtrat *Streptomyces* sp.Sp1

Fraction	RF value
1	0.97
2	0.97
3	0.93
4	0.87
5	0.87
6	0.87
7	0.98
8	0.98

Table 3. The antagonist test of the active compound of *Streptomyces* sp.Sp1 againts *V. anguillarum*

Active compound	Inhibitory (mm)
RFG1	7
RFG2	24
RFG3	20
RFG4	19
RF mixture (RF1, RF2, RF3, RF4)	22

3.3. GC-MS Analysis

Gas chromatogram analysis results showed 35 peaks and each peak was identified more with mass spectroscopy in which each compound had a specific mass fragmentation pattern (Figure 5 and Table 4). Compounds identification at each peak was made by comparing the mass spectrum of each peak in the mass spectrum of compounds that had been known and programmed in the data base of GC-MS. Eleven active compound have antimicrobial activities (Table 5), Spectrum and structure of the 11 antimicrobial compounds were presented on Figure 6 and Figure 7.

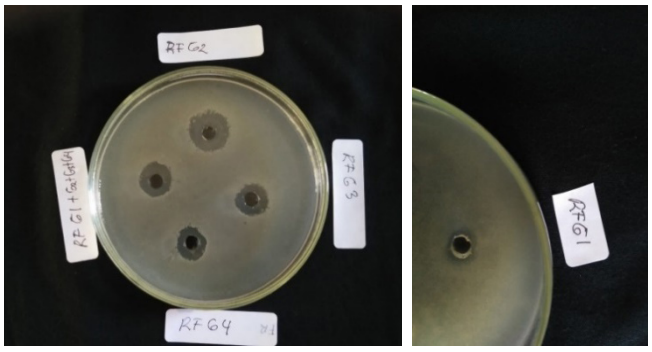

Figure 4. Antagonist test active compound with pathogen *V. anguillarum*

4. Discussion

After the phylogenetic- and the 16S rRNA gene sequence analyses, it is known that the strain with a significant antimicrobial capability was recognized as *Streptomyces* sp.Sp1. As one of the most reliable methods, a number of researchers commonly applied the Gas chromatography-mass spectrometry (GC-MS) to characterize the components of volatile compounds[6]. Through this technique it is identified that the extract of *Streptomyces* sp.Sp1 have 35 major peaks with different retention time.

Table 4. Components detected in the n butanol extract of *Streptomyces* sp.Sp1

Compound name	Molecular formula	Molecule wight	Peak area (%)
3-Hexanone, 2-methyl	C7H14O	43	43
2-t-Butyl-6-chloromethyl-[1,3]dioxan-4-one	C9H15CLO3	103	3.0
Butane, 1,1'-oxybis- (CAS) n-Butyl ether	C8H18O	57	3.3
n Butyl ether	C8H18O	57	19
Nonane (CAS) n-Nonane	C9H20	43	5.6
Propanoic acid, 2-methyl-, 2-methylpropyl ester	C8H16O2	71	0.8
Propanoic acid, 2-methyl-, butyl ester	C8H16O2	43	0.3
3-Hexen-2-one (CAS) 1-Butenyl methyl ketone	C6H10	43	1.0
Pentane, 1-butoxy-	C9H20O	57	1.6
Decane (CAS) n-Decane	C10H22	43	3.2
l-Limonene	C10H16	68	0.2
Butane, 1,1-dibutoxy- (CAS) Lageracetal	C12H26O2	57	0.3
Cyclopentasiloxane, decamethyl-	C10H30O5Si5	73	0.2
Benzeneacetic acid, 3-methoxy-.alpha.,4-bis[(tri	C14H24O3Si2	163	0.2
Tetradecane	C14H30	57	0.3
Cyclohexasiloxane, dodecamethyl-	C12H36O6Si6	73	0.3
Trimethylsilyl 3-methoxy-2-(2-oxo-2-((trimethy	C16H26O6Si2	73	0.4
Hexadecane (CAS) n-Hexadecane	C16H34	57	0.5
1, 1, 3, 3, 5, 5, 7, 7, 9, 9, 11, 11-dodecamethylhexasilo	C12H36O5Si6	73	0.1
Oxalic acid, cyclohexylmethyl tridecyl ester	C22H40O4	97	0.4
Nonadecane	C19H40	57	0.3
Cyclooctasiloxane, hexadecamethyl-	C16H48O8Si8	73	0.1
Hexadecanoic acid, methyl ester	C7H34O2	74	0.3
4-TERT-BUTYL-2-(1-METHYL-2-NITRO-ET	C13H23NO3	97	0.3
Heptasiloxane, hexadecamethyl- (CAS) Hexadecamet	C16H48O6Si7	73	0.3
Heptasiloxane, hexadecamethyl-	C16H48O6Si7	73	0.3
Heneicosane	C20H42	57	1.9
9-Octadecenoic acid (Z)-, methyl ester	C19H36O2	55	1.1
Heptasiloxane, hexadecamethyl-	C16H48O6Si7	73	1.9
TETRACOSAMETHYLCYCLODODECASIL	C24H72O12Si12	73	1.7
Hexacosane (CAS) n-Hexacosane	C20H45	57	1.9
Tetracontane	C40H82	57	1.3
Hexatriacontane	C36H74	57	1.3
Benzeneacetic acid, 3-methoxy-.alpha.,4-bis[(tri	C17H30O5S12	163	0.3

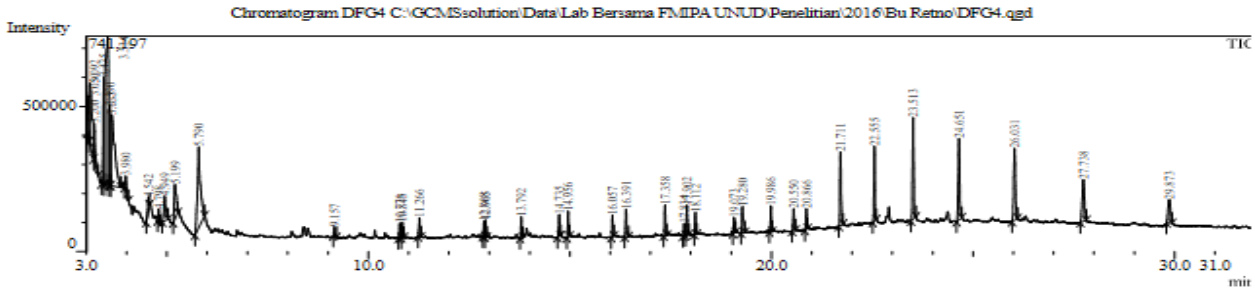

Figure 5. GC-MS chromatogram of n butanol extract of *Streptomyces* sp.Sp1

Table 5. Antimicrobial activities identified in the n butanol extract of *Streptomyces* sp.Sp1

Name of the compound	Nature of compound	Activitya*
3-Hexanone, 2-methyl	Organic compound	Antimicrobial
n Butyl ether	Organic compound	Antimicrobial
Hexacosane(CAS) n Hexacosane	Organic compound	Antimicrobial
Tetracontane	Organic compound	Antimicrobial
l-Limonene	Organic compound	Antimicrobial
Heneicosane	Organic compound	Antimicrobial
Decane (CAS) n-Decane	Organic compound	Antimicrobial
Hexadeconoic acid, methyl ester	Organic compound	Antimicrobial
Butane, 1, 1-dibutoxy	Organic compound	Antimicrobial
Nonane(CAS)n-Nonane	Organic compound	Antimicrobial
Benzeneacetic acid, 3-methoxy-.alpha.,4-bis[(tri	Organic compound	Antimicrobia

*Source: Pubchem/open chemistry database

The chemical compounds which have antimicrobial properties identified in the n butanol extract of *Streptomyces* sp.Sp1. are 3-Hexanone, 2-methyl (43%), n Butyl ether (19%) Nonane (CAS) n-Nonane (5.6%), Decane (CAS) n-Decane (3.2%), Hexacosane(CAS) n-Hexacosane (1.9%), Tetracontane (1.3%), Heneicosane (1.9%), Hexadeconoic acid, methyl ester (0.3%), Butane,1,1-dibutoxy (0.3%), and Limonene (0.3%). Benzeneacetic acid, 3-methoxy-.alpha.,4-bis [(tri (0.3%). The main chemical compounds (known as ketones group) is 3-Hexanone, 2-methyl (43%).

Chemical compound 2-Methyl-3-hexanone is an organic compound belonging to the ketone group. The presence of a group of ketone compounds in *Streptomyces*, previously reported by Zothanpuia *et al.* (2017). In this study, *Streptomyces cyaneofuscatus* was isolated from fresh water sediments. Based on GCMS analysis, it was reported that the contents of the amines, ketones, aldehydes, and alkanes in the crude extract methanol from *Streptomyces cyaneofuscatus* were reported. Also carried out extract crude antibacterial tests, where the results have strong antibacterial activity against Gram positive bacteria, Gram negative bacteria, and pathogenic yeast. The results showed the highest activity against *E. coli* (5.42 µg/ml to 15.89 µg/ml) followed by *C. albicans* (4.12 µg/ml to 12.6 µg/ml), *B. subtilis* (3.96 µg/ml to 11.2 µg/ml), and *P. aeruginosa* (3.17 µg/ml to 8.0 µg/ml).

The antibacterial activity of hexacosane compounds was tested by Rukaiyat *et al.* (2015). This compound is one of the active ingredients contained in the extract of the ethyl acetat plant stem *Sanseveria liberica*. The results of the study mentioned the presence of antibacterial activity against *Klebsiela pnemoniae*, *Salmonela typhi*, *Mithecithinne staphaureus,* and *Proteus vulgaris*. Dehjurian *et al.* (2017) identified the presence of hexacosane compounds in *Cirsium arvense* flower extract in Iran. Antibacterial activity is proven against *C. arvense*. Another study by Martins *et al.* (2015), identified this compound as one of the compounds contained in *Kielmeyera coriacea* which has antibacterial activity against aerobic and non-aerobic bacteria. Rajalakshmi and Mahesh (2014) also reported the content of Hexacosane (CAS) hexacosane in *Aspergillus terreus* extract which was shown to have antibacterial activity.

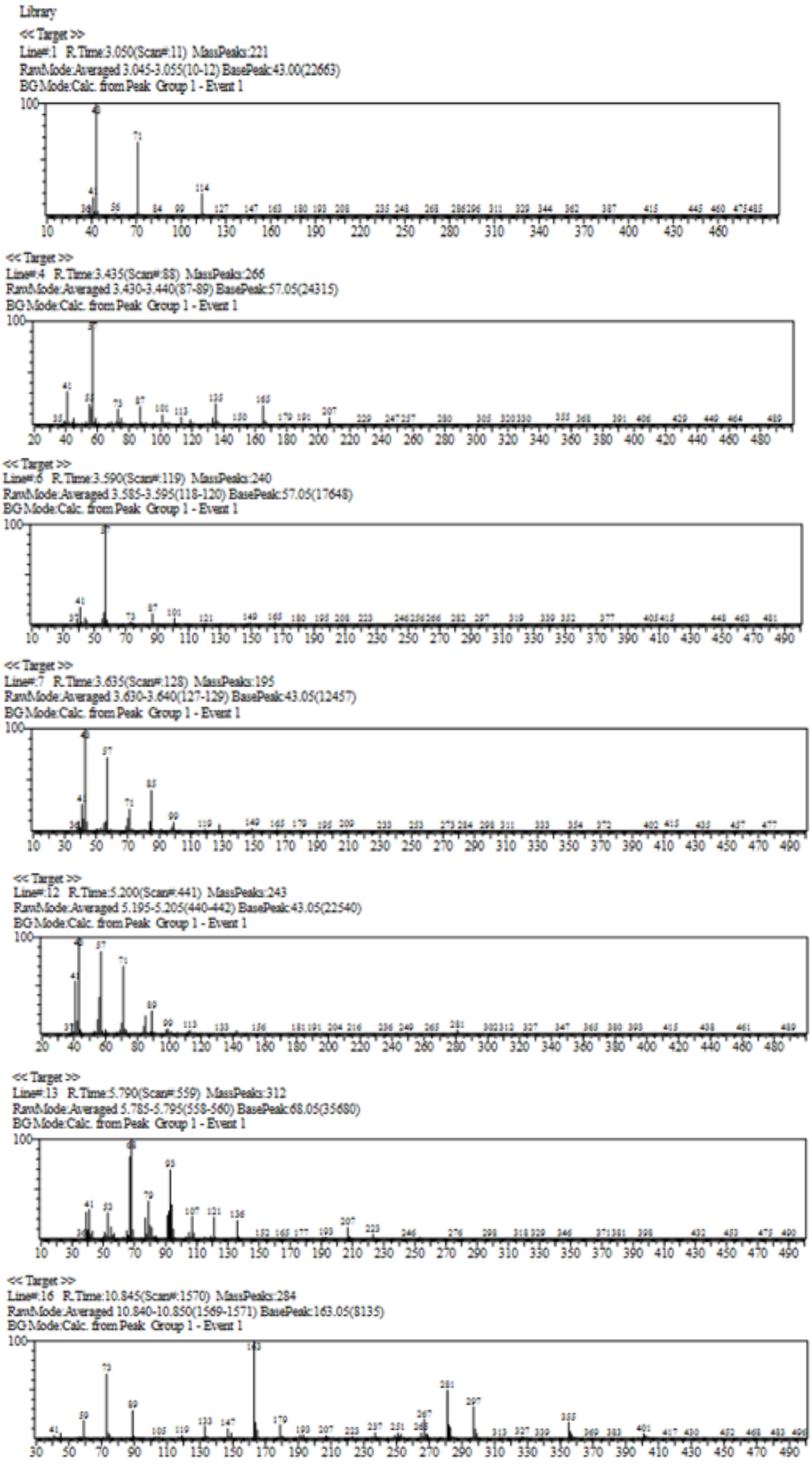

Figure 6. Spectrum of the 11 antimicrobial compounds detected in n butanol extract of *Streptomyces* sp.Sp1

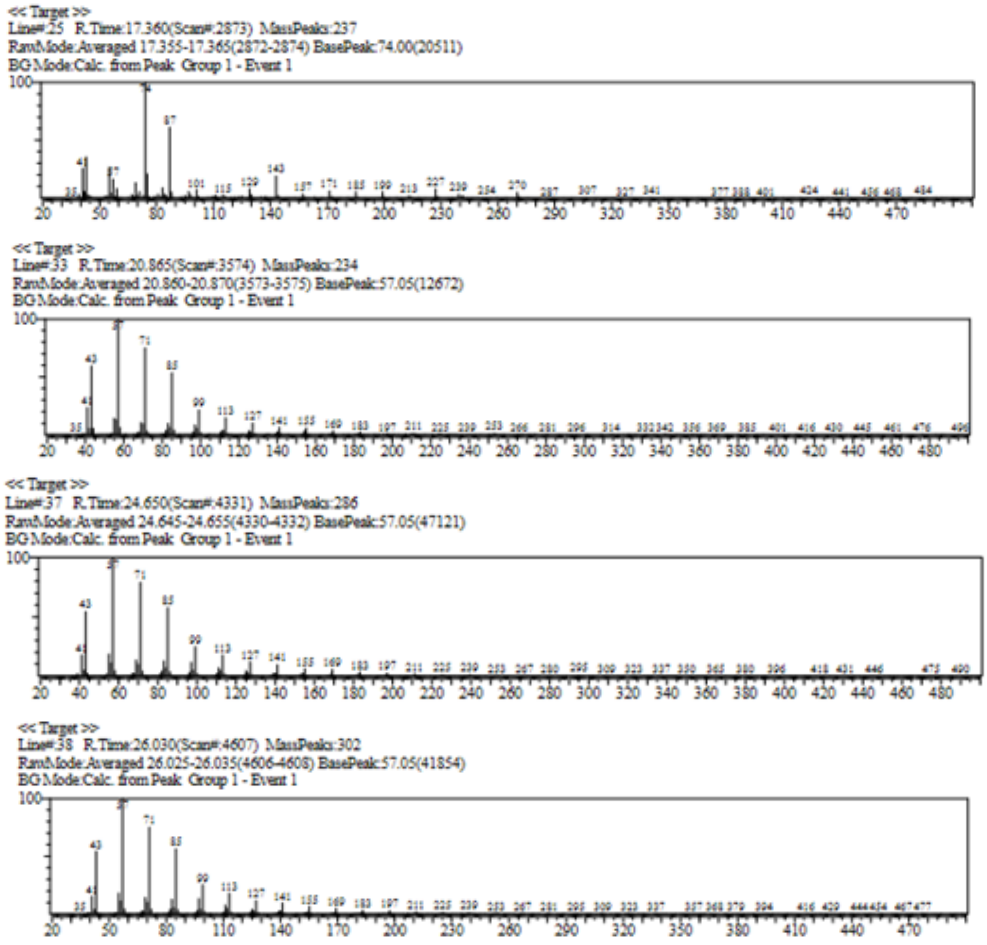

Figure 6. Continued

Tetracontane compounds are found as ingredients in various secondary metabolites which have antibacterial activity. Johnson *et al*. (2012) identified the presence of tetracontane, 3, 3, 2, 4-trimethyl from methanol extract of marine sponge *Zygomycale* sp. which was isolated from Kanyakumari beach (southeast coast of India). It was reported that the extract had antibacterial activity against *Bacillus megaterium*, *Klebsiella pneumoniae,* and *Streptococcus pyogenes* and antifungi against *Aspergillus niger*, *Aspergillus fumigatus*, *Candida albicans*, and *Rhizomucor miehei*. Tetracontane compounds in the form of tetracontane, 3,5,24-trimethyl- are identified as the content of chloroform extract of *Hugonia mystax* L. (Linaceae) by Vimalavady and Kavadul (2013), where the extract has antimicrobial activity, antifungal, and antioxidants.

Several studies have reported the activity of limonene compounds as antibacterial. Research by Haiyan *et al*. (2016) examined the antibacterial activity of limonene emulsions and acetone limonene solutions in three food pathogenic species. The tube dilution test in this study showed that limonene was effective for *Y. enterocolitica* and *S. aureus*, but was not effective for *L. monocytogenes*. Espina *et al*. (2013) reported the inhibitory of limonene mechanism for *E. coli* MC4100 by increasing permeability of the outer membrane and changing the structure of bacterial proteins. Obidi *et al*. (2013) tested the antimicrobial activity of citrus sinensis oil. The inhibitory effects were shown in all test microorganisms (*S. aureus* 001, *S. aureus* ATCC 25923, *Enterococcus feacalis* 002, *E. feacalis* ATCC 295212, *P. aeruginosa* 003, *E. coli* 004, *E. coli* ATCC 29522, *C. albicans* 010, and *C. albicans* ATCC 90028). GCMS results indicate the presence of D-limonane.

Ma *et al.* (2014) reported the presence of hyenicosane compounds as one of 87 compounds contained in tomato seed oil, which had antibacterial activity against *S. aureus*, *E. coli*, *S. flexneri,* and *P. mirabilis*.

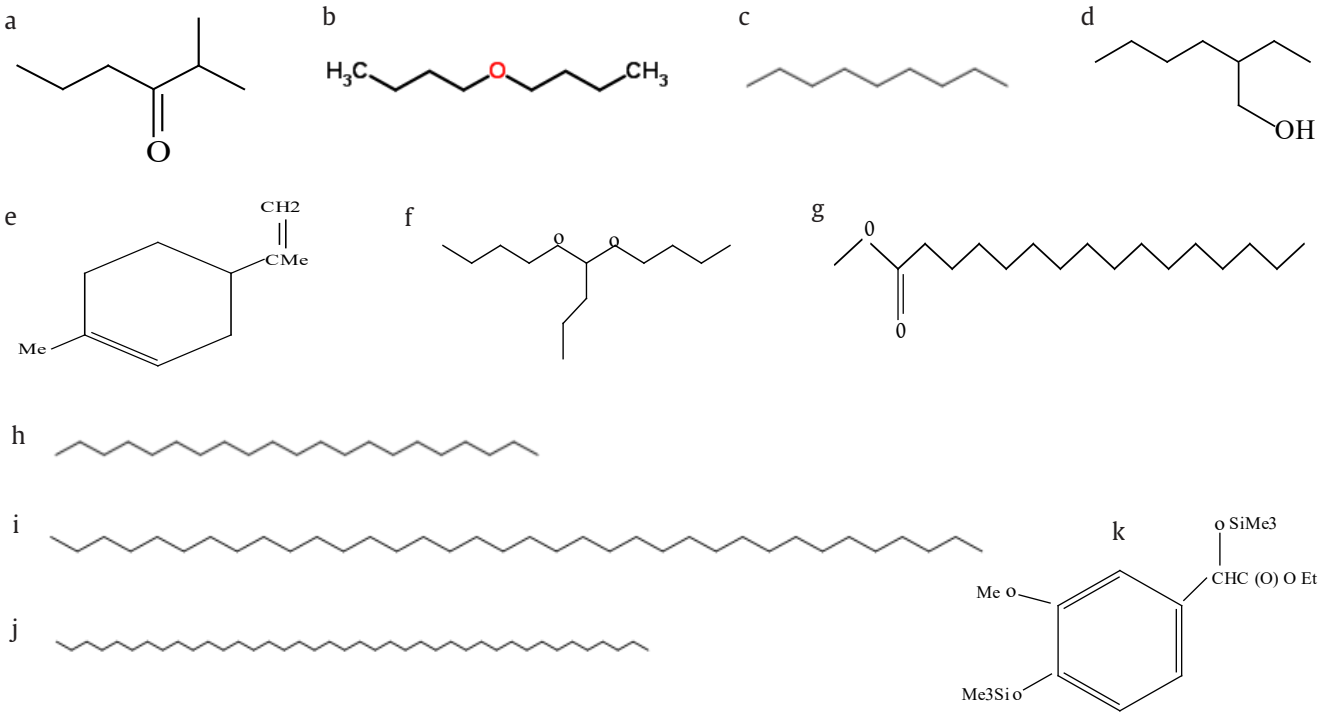

Figure 7. Structure of (a) 3-Hexanone, 2-methyl-, (b) n Butyl ether, (c) Nonane (CAS) n-Nonane, (d) Decane (CAS) n-Decane, (e) l-Limonene, (f) Butane, 1,1-dibutoxy- (CAS) Lageracetal, (g) Hexadecanoic acid, methyl ester, (h) Heneicosane, (i) Hexacosane (CAS) n-Hexacosane, (j) Tetracontane, (k) Benzeneacetic acid, 3-methoxy-.alpha.,4-bis[(tri

Previous research by Dadasoglu *et al.* (2011) identified the presence of n-henecosane in Origanium extract (*O. acutidens*, *O. rotundifolium,* and *O. vulgare*) which is known to have antibacterial activity.

Research by Nahar *et al.* (2016) reported the content of decane as the main component of *Trigonella foenumgraecum* seed essential oil collected from Jessore Road in Bangladesh. The essential oils obtained were tested for antibacterial activity and showed antibacterial activity against *Bacillus subtilis* IFO 3026, *Sarcina lutea* IFO 3232, *Xanthomonas campestris* IAM 1671, *Proteus vulgaris* MTCC 321, and *Pseudomonas denitrificans* KACC 32026. Wang *et al.* (2013) identified decane compounds in the Bicyclo form decane, 2-methylene-5 (1-methylvinyl) -8-methyl-, 0.27 from *Streptomyces alboflavus* TD-1 which was reported to have antifungal activity against *Fusarium moniliforme* Sheldon, *Aspergillus flavus*, *Aspergillus ochraceus*, *Aspergillus niger*, and *Penicillum citrinum* in vitro.

Raningsih *et al.* (2015) identified the content of hexadeconoic acid, methyl ester in the filtrate of *Nocardia* sp. isolates. 1 which is reported to have antibacterial activity against Methicillin Resistance *Staphylococcus aureus* (MRSA). Another study by Jaluldeen *et al.* (2015) also identified the presence of hexadeconoic acid, methyl ester compounds from crude extect ethyl acetat *Streptomyces* species which were isolated as endophytic bacteria in the rhizosphere of chili plants. Furthermore Nandhini *et al.* (2015) also reported that GC-MS analysis of extract *S. cacaoi* strain SU2 presence Heneiconsane, Dodecane, Eicosane, and Cetene that have antimcrobial activity to pathogenic bacteria and fungi.

As reported by El-Gendy *et al.* (2008) several substances namely 3,5 -Dichloro- 2pyridone, Benzeneacetic acid, Heptadecane, Phenol. 2.4-bis (1.1-dimethylethyl), Dodecanoic Acid Methyl, Ester, Hexadecanoic Acid Methyl Ester, 1-(+)-Ascorbic Acid 2.6 Dihexadecanoate were identified by GC-MS characterization technique. These chemical compounds are the isolate substance derived from *Streptomyces galbus* TP2 and *Streptomyces humidus*, which are recognized as antifungal, and have a significant role in medical. Pramisandi *et al.* (2018) reported that Benzeneatisc acid is expected as precursor during *Pinicillium chrysogenum* fermentation to produce antibiotic penicillin G.

Severeral *Actinomycetes* isolated from different soil are proven to inhibit pathogenic resistance bacteria

such as *Escherichia coli* and *Vancomycin-Resistant Enterococci*. Furthermore, several substances such as 3,5 -Dichloro- 2 pyridone, Benzeneacetic acid, Heptadecane, Phenol. 2.4-bis(1.1-dimethylethyl), Dodecanoic Acid Methyl Ester, Hexadecanoic Acid Methyl Ester, 1-(+)-Ascorbic Acid 2.6 Dihexadecanoate were identified using the GC-MS characterization (Singh *et al*. 2012).

Moreover, Actinomycetes commonly synthetizes the Dodecanoic acid methyl ester and hexadecanoic acid methyl ester. The existence of those substances can be used as an indicator in *Actinomycetes* group classification (Susuki 1988). The mechanism of secondary metabolite of *Actinomycetes occurs* by damaging cell wall and obstructing cell division (Ambavane *et al*. 2014).

5. Conclusion

Streptomyces sp.Sp1 showed various bioactive properties, which highlighted its importance as potential biocontrol agents on Vibriosis desease. Hence, there were new bioactive compound in the extract, which might provide a basis for further development of novel compound from *Streptomyces* sp.Sp1. This also provided a new insight towards the development of good candidates for bioactive natural products.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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