Presence of Biosynthetic Gene Clusters (NRPS/PKS) in Actinomycetes of Mangrove Sediment in Semarang and Karimunjawa, Indonesia

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ARTICLE INFO

Received: 31 Mar 2021
Received in revised: 9 Jun 2021
Accepted: 9 Jun 2021
Published online: 26 Jul 2021
DOI: 10.32526/ennrj/19/202100050

Keywords:
Antibiotic/ Actinomycetes/ Pathogenic bacteria/ Sediments

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ABSTRACT

Actinomycetes are a group of bacteria that are widely distributed in soil, litter, water, and other natural sources. These Gram positive bacteria can produce hundreds of bioactive compounds, especially antibiotics. This research isolated culturable actinomycetes from mangrove sediments in the Semarang and Karimunjawa Island areas. The isolates that produce potential antibacterial compounds were identified by qualitative screening using the Biosynthetic Gene Cluster (NRPS/PKS) prediction approach. This research was conducted from June to November 2020. A total of 19 actinomycetes from Semarang and 17 actinomycetes from Karimunjawa were found to have at least one type of Biosynthetic Gene Cluster (NRPS, Type I or Type II PKS), but only three isolates had antibacterial activity against *S. aureus*, *E. coli*, and *L. monocytogenes*. Molecular identification found that the bacteria were similar to *Brachybacterium paraconglomeratum* (99.92%), *Streptomyces pluripotens* (100%), and *Micromonospora chersina* (99.08%). Results of the study concluded that the three bacterial isolates that had bacterial activity have similar genes with known antibiotic-producing genes and can potentially provide new antibiotic candidates.

1. INTRODUCTION

Mangroves have many marine organisms (Ariyanto et al., 2020) and have good nutrition sources (Ariyanto, 2019) and amino acid contents (Ningsih et al., 2020). This is supported by the physicochemical factors and litter dynamics of mangroves (Ariyanto et al., 2019) and the decomposition of leaves over time (Ariyanto et al., 2018). Pathogenic microbes in the world have evolved for their survival, such as the development of resistance mechanisms to drug compounds. Pathogenic microbial resistance is predicted to kill 10 million people by 2050 if not handled immediately (Romano et al., 2018). However, there is a solution if humans can adeptly utilize natural conditions because nature is the main provider of bioactive materials needed by humans to overcome various kinds of disease (Sharma and Thakur, 2020). More than 100,000 types of natural bioactive compounds during the last 150 years have been identified, such as polyketides, alkaloids, non-ribosomal peptides, isoprenoids, and phenylpropanoids (Carbonell et al., 2016). Some types of natural bioactive compounds that have been identified have come from the actinomycetes group of microorganisms. Natural materials produced by microorganisms are very structurally diverse and are considered important sources in the search for new drugs for various diseases in humans including infections and cancer (Sekurova et al., 2019).

Actinomycetes are Gram-positive bacteria that are known to be capable of producing potential compounds, especially in pharmacology. Some of the compounds produced by actinomycetes are used as antibiotics and can kill cancer cells. The bioactive compounds produced by actinomycetes are proven to be useful as drugs for infections caused by fungi, viruses, and bacteria. They are also used as drugs to treat various types of cancer. Bioactive compounds are usually formed as products of secondary metabolites of organisms, thus they are often referred to as secondary metabolites. According to Katz and Baltz (2016), one type of actinomycetes can produce 30-50 types of secondary metabolite compounds based on the information from the genomic sequences results.

Citation: Anggelina AC, Pringgenies D, Setyati WA. Presence of Biosynthetic Gene Clusters (NRPS/PKS) in actinomycetes of mangrove sediment in Semarang and Karimunjawa, Indonesia. Environ. Nat. Resour. J. 2021;19(5):391-401. (https://doi.org/10.32526/ennrj/19/202100050)

In general, secondary metabolite compounds are produced by enzymes encoded in Biosynthetic Gene Groups (Biosynthetic Gene Clusters) (Romano et al., 2018). Examples of biosynthetic gene groups frequently found are PKS and NRPS.

Actinomycetes are commonly found growing naturally on land and water, including in mangrove sediments. Sediment from mangrove ecosystems is known to be a good place to live for various types of microorganisms because it provides various kinds of nutrients that can be used by actinomycetes to live (Thatoi et al., 2012). Hence, the target location of this research was in the mangrove ecosystem area of Semarang waters and Karimunjawa Islands (Nyamuk island), Central Java. Accordingly, the research objectives were to identify a number culturable-actinomycetes isolated from mangrove sediments in the Semarang and Karimunjawa Island areas. Isolates with the potential to produce anti-bacterial compounds were found by using Biosynthetic Gene Cluster (NRPS / PKS) prediction approach.

2. METHODOLOGY

2.1 Study material

This research was conducted from June to November 2020. Sediment samples were collected from Tapak, Tugurejo Village, Semarang mangrove forest and Nyamuk Island, Karimunjawa mangrove forest. Samples were inserted into sterile ziplock bags, then dried at 26-32°C for two weeks in the laboratory. The treatment method used to isolate actinomycetes from the sediment samples was a modification of the method of Davies-Bolorunduro et al. (2019). Isolation of actinomycetes was done using spread plate method by serial dilution. Each 1 g dry sediment sample was diluted into 9 mL of sterile seawater, then 1 mL of the solution diluted into 9 mL of sterile seawater repeatedly to make a 10^{-3} dilution series. A total of 50 µL from the sample dilutions was flattened on the surface of different medium types, namely Zobell (Zobell 2216 (HiMedia, India)) 40.25 g; agar (Oxoid, England) 15 g), Zobell + Humic Acid (humic acid 1 g (diluted in 10 mL 0.2 N NaOH)), International Streptomyces Project 1 (ISP 1) (yeast extract 3 g; tryptone 5 g; agar (Oxoid, England) 15 g), ISP 1 + Humic Acid (humic acid 1 g (diluted in 10 mL 0.2 N NaOH)), and Humic Acid Vitamin Agar (HVA) (humic acid 1 g (diluted in 10 mL 0.2 N NaOH); Na_2HPO_4 0.5 g; KCl 1.71 g; $MgSO_47H_2O$ 0.05 g; $FeSO_47H_2O$ 0.01 g; vitamin B complex 3.75 mg; $CaCO_3$ 0.02 g; agar (Oxoid, England) 18 g) (Hayakawa and Nonomura, 1987). Each medium was added with 60 mg/L of antibiotic compounds (Nalidixic Acid and Nystatin). Samples were incubated at 29-37°C for 1-5 weeks. Representative isolates were grown on new medium using the streak plate method until pure cultures were obtained.

2.2 Antibacterial activity screening

Actinomycetes antibacterial screening was carried out by modifying the agar plug method (Messaoudi et al., 2020). Actinomycetes cultures grown for two weeks were cut into a cylindrical shape (about 8 mm in diameter), then affixed to the surface of Mueller Hinton Agar (MHA) medium that had been inoculated, which include the types of: (*Staphylococcus aureus*, *Escherichia coli*, and *Listeria monocytogenes*). The test sample was incubated at 29-34°C.

2.3 DNA extraction

Bacterial DNA extraction was carried out using Chelex method. Bacterial colonies were included in a mixture of 500 µL of 0.5% saponin solution (in Phosphate Buffer Saline) and 100 µL of ddH_2O. Samples were soaked for 12-24 h at 4°C in order to lyse the bacterial cell walls. Samples that had been soaked in saponins were centrifuged at 9,000 rpm for 15 min. The supernatant from centrifugation was discarded, then the pellets were added with 1 mL of PBS solution, then vortexed until homogeneous. The homogeneous mixture of natant and PBS was then centrifuged again for 10 min. The supernatant was discarded again. 100 µL of ddH_2O and 50 µL of 20% Chelex solution (vortex Chelex solution before use) were added. The samples were then heated at 95°C for 5 min then vortexed, then heated again at 95°C for 5 min. The samples were re-centrifuged for 15 min, then the supernatants were transferred to new 1.5 mL microtubes ready for use as a DNA Template.

2.4 NRPS and PKS gene type II cluster amplification

NRPS gene cluster detection was done using Thermo Scientific 2X Phire Plant Direct PCR Master Mix with A2gam F (5´-AAGGCNGGCGSBGCSTAY STGCC-3´) and A3gamR (5´-TTGGGBIKBCCGGTS GINCCSGAGGTG-3´) primer pair (Radjasa et al., 2005). The PCR condition was 98°C for 5 min for initial denaturation; then 40 cycles consisting of a denaturation stage at 98°C for 5 sec, annealing stage at 70°C for 5 sec, extension stage at 72°C for 1 min,

and a final extension stage at 72°C for 1 min and cooling stage at 4°C.

Type I PKS gene cluster detection was used Thermo Scientific 2X Phire Plant Direct PCR Master Mix with MDPQQR f (5'-RTRGAYCCNCAGCAIC G-3') and HGTGT r (5'-VGTNCCNGTGCCRTG-3') primer pair (El Samak et al., 2018). The PCR condition was 98°C for 5 min for initial denaturation; then 10 cycles consisting of a denaturation stage at 98°C for 5 sec, annealing stage at 60°C (temperature reduced 2°C per cycle) for 5 sec, extension stage at 72°C for 1 min, followed with 30 cycles with a denaturation stage at 98°C for 1 min, annealing stage at 40°C for 5 sec, extension stages at 72°C for 1 min, and a final extension stage at 72°C for 1 min and a cooling stage at 4°C.

PKS-II gene amplification was carried out by mixing the primary pair of PF6 (5'-TSGCSTGCTTGG AYGCSATC-3 ') and PR6 (5'TGGAANCCGCCGAA BCCGCT-3') (El Samak et al., 2018), 1 µL each at a concentration of 10 mM, with 1 µL of extracted template DNA, 10 µL of Thermo Scientific2X Phire Plant Direct PCR Master Mix, and 8 µL of ddH_2O. PCR amplification process was carried out in 40 cycles with the following stages: initial denaturation stage (98°C, 5 min), followed by denaturation (98°C, 5 sec), annealing (70°C, 5 sec), extension stage (72°C, 1 min), and the final extension (72°C, 1 min).

2.5 Amplification of 16S rRNA from active isolates and DNA visualization

Amplification of the 16S rRNA gene was carried out by mixing 1 µL of template DNA, primary pair of 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1429R (5'-GGTTACCTTGTTACGACTT-3') (El Samak et al., 2018), 1 µL each at a concentration of 10 mM, 12.5 µL of Thermo Scientific2X Phire Plant Direct PCR Master Mix, and 9.5 µL of ddH_2O. PCR process was carried out in 40 cycles with the following stages: initial denaturation (98°C, 5 min), denaturation (98°C, 5 sec), annealing (55°C, 5 sec), extension (72°C, 1 min), and final extension (72°C, 1 min).

Electrophoresis of the DNA samples was done in an agarose gel medium. The agarose gel concentration used in this research was 1% agarose gel in a buffer solution of TAE (Tris Acetate EDTA) mixed with GelRed dye to make it easier to visualize with UV light. The electrophoresis process was carried out at a voltage of 100 volts and a strong current of 400 A for 30 min. Then, the agarose gel is transferred to a UV Transilluminator to process the visualization of the formed DNA bands.

The amplified sample was carried out in sequencing process that aims to determine the nucleotide bases sequence using Sanger Deoxy Method. The sequencing data were edited using MEGA 7.0 software, then the data from 16s rDNA primers were matched with the data from GenBank NCBI. The 16S rRNA sequence data from the samples were deposited on GenBank with access numbers MW750399, MW750400, and MW750401.

2.6 Biosynthetic Gene Cluster (BGC) mapping simulation

Biosynthetic Gene Cluster (BCG) mapping simulation was carried out by submitting the whole genome sequence of actinomycetes species which was similar to the result of molecular identification of active isolates in the AntiSMASH 6.0 program (https://antismash.secondarymetabolites.org/).

3. RESULTS AND DISCUSSION

3.1 Actinomycetes isolation and Biosynthetic Gene Cluster (BGC) screening

Thirty six actinomycetes isolates were successfully obtained from the mangrove sediments of Semarang and Karimunjawa with five different growth medium (Zobell, Zobell + Humic Acid, ISP 1, ISP 1 + Humic Acid, and Humic Acid Vitamin Agar (HVA)), namely 19 isolates from Semarang consisting of 3 isolates growing from ISP 1 medium; 1 isolate grown from Zobell medium; 9 isolates grown from HVA medium; 6 isolates grew from ISP 1 + Humic Acid medium and 17 Karimunjawa Isolates consisting of 3 isolates grown from ISP 1 medium; 4 isolates grown from Zobell medium; 5 isolates grew from HVA medium; 2 isolates grew from ISP 1 medium + Humic Acid; and 3 isolates grown from Zobell + Humic Acid medium (Figure 1(a) and (b)).

Selective media is an important step to enhance the isolation process. Furthermore, growth medium composition affects the biological activities for the isolates, for example, antimicrobial properties (Dhanasekaran et al., 2009). HVA has been indicated as the best medium for isolating actinomycetes from mangrove sediments based on the number of actinomycetes isolates obtained from the two sampling locations compared to the other four types of medium. This is because the HVA media is specifically designed to isolate actinomycetes from the

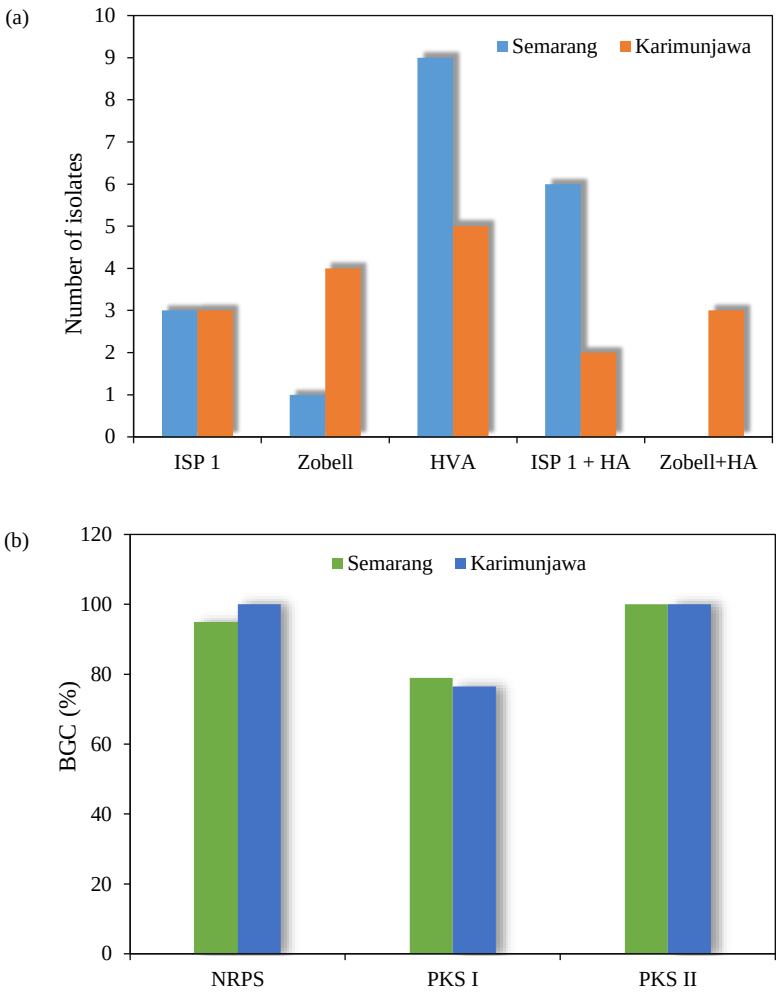

Figure 1. (a) Number of actinomycetes isolates successfully grown based on the type of growth medium and (b) percentage of the number of actinomycetes isolates detected to have Biosynthesis Gene Clusters (BGC) from Semarang and Karimunjawa

soil by providing sufficient nutrients to support growth and sporulation for actinomycetes and to inhibit the development of other bacteria (Hayakawa and Nonomura, 1987). ISP 1 contains tryptone and yeast extract provides nutrition that is necessary for bacterial metabolism (https://himedialabs.com/TD/M356.pdf). Zobell medium contains peptone and yeast as nutrients as well as minerals like seawater. Some actinomycetes have adapted to the salinity of marine areas (seawater and sediments). High salinity adaptation is needed to survive and grow as a fundamental biological process (Rashad et al., 2015). Furthermore, the average number of actinomycetes isolated from the modified ISP 1 + Humic Acid medium was greater than the average number of actinomycetes isolated from the ISP 1 medium. Meanwhile, the average number of actinomycetes isolated from Zobell + Humic Acid modified medium was less than the average number of actinomycetes from Zobell medium. This means that the addition of humic acid as one of the soil constituent components has a more positive effect when it is added to actinomycetes isolation medium (ISP 1) compared to when it is added to the isolation medium of universal marine bacteria (Zobell).

The number of actinomycetes isolated from the mangrove sediments of Tapak, Tugurejo, Semarang was higher than the number of actinomycetes isolated from the mangrove sediments of Nyamuk Island, Karimunjawa. This can be caused by the supply of nutrients derived from factory waste, household waste, shipping activity waste, and waste from various other human activities in Semarang (Siregar and Koropitan, 2016) which provide more nutrients for actinomycetes when compared to nutrients in the waters of Nyamuk Island which are not yet abundant. It is influenced by human activities due to its relatively smaller population, less diverse community activities in terms of industry, and the remote location of the islands (Karimunjawa National Park, 2019).

Screening results for the presence of Biosynthetic Gene Clusters (BGC) consisting of NRPS genes, PKS type I genes, and PKS type II genes in actinomycetes isolates that were isolated from mangrove sediments in Tapak, Tugurejo, Semarang and Nyamuk Island, Karimunjawa obtained detailed results; 18 out of 19 (94.74%) actinomycetes isolates from Semarang were detected to have the NRPS gene while all Karimunjawa actinomycetes isolates (100%) were detected to have the NRPS gene. Results of PKS type I genes detection from Semarang isolates showed that 15 out of 19 (78.95%) isolates had genes detected, while 13 out of 17 (76.47%) of Karimunjawa isolates had these genes. Results of PKS type II genes detection showed that all isolates (100%) from both locations were detected to have the gene.

3.2 Antibacterial activity

Inhibition zone values from the antibacterial activity screening of mangrove sediment actinomycetes isolates in Semarang and Karimunjawa against pathogenic bacteria *Staphylococcus aureus* (PN.SB.11:1.67±0 cm), *Escherichia coli* (PN.SB. 11.1:0.57±0.15 m), and *Listeria monocytogenes* (S.SK.8.1:0.65±0.40 cm; PN.SB.11.3:1.72±0.29 cm) are shown in Figure 2.

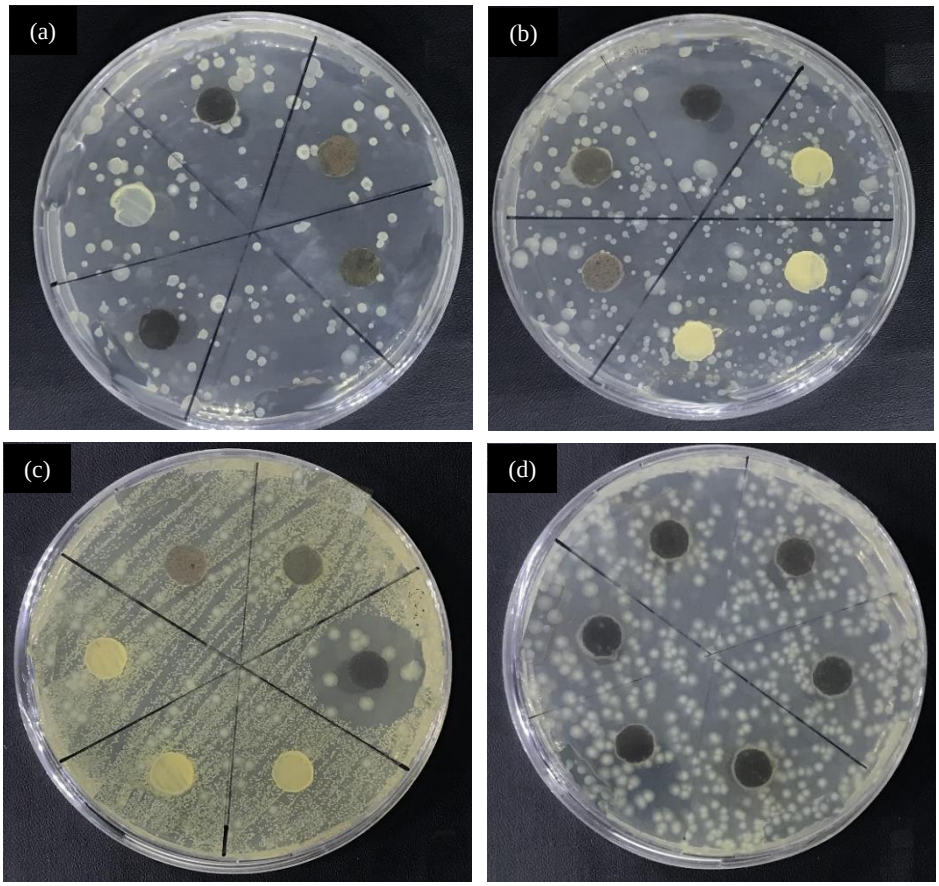

Figure 2. Antibacterial activity screening clear zone data of actinomycetes isolates (a) S.SK.8.1 against *L. monocytogenes*, (b) PN.SB.11.3 against *L. monocytogenes*, (c) PN.SB.11.3 against *S. Aureus*, and (d) PN.SB.11.1 against *E. coli*

Although all isolates were detected to have Biosynthetic Gene Clusters (BGC), only three actinomycetes isolates had antibacterial activity with the tested bacteria of *E. coli*, *S. aureus*, and *L. monocytogenes*. The actinomycetes isolate of mangrove sediment from Semarang which has antibacterial activity against *L. monocytogenes* was the isolate designated by code S.SK.8.1. Actinomycetes isolates of mangrove sediment from Karimunjawa that were able to inhibit the growth of pathogenic bacteria were the isolate code PN.SB.11.1 against *E. coli* and isolate code PN.SB.11.3 against *S. aureus* and *L. monocytogenes*. Although almost all isolates were detected to have a Biosynthetic Gene Cluster, the causes of the lack of active isolates against pathogenic bacteria have been reported in several previous studies. It has been stated that the presence of Biosynthetic Gene Clusters (PKS and NRPS) in the

genomic DNA of an organism is an indication of the organism's potential to produce bioactive compounds, however, the products produced by these organisms might have other activities besides antibacterial, such as antioxidants or antitumors (El Samak et al., 2018). It can also be caused by Biosynthetic Gene Clusters that are not expressed (silent) under laboratory culture conditions (*in vitro*) (Kalkreuter et al., 2019).

3.3 Molecular identification of 16S rRNA active isolates

Molecular identification results of actinomycetes active isolates that were isolated from the mangrove sediments of Tapak, Tugurejo, Semarang and Nyamuk Island, Karimunjawa based on 16S rRNA gene sequencing are shown in Table 1.

Table 1. Bacteria identification results at different locations

Isolate code	Species identification (BLAST)	Access number	Sequence length (bp)	Ident (%)	Query cover (%)
S.SK.8.1	*Brachybacterium paraconglomeratum*	MW750399	870	99.08%	100%
PN.SB.11.1	*Streptomyces pluripotens*	MW750400	1354	100%	100%
PN.SB.11.3	*Micromonospora chersina*	MW750401	1316	99.92%	100%

Notes: S.SK=Semarang; PN.SB=Nyamuk Island, Karimunjawa

The active actinomycetes isolates of mangrove sediment from Semarang with code S.SK.8.1 was identified molecularly as *Brachybacterium paraconglomeratum* with the data equation in MT214268.1 sequence at 99.08%. Meanwhile, the actinomycetes isolate code PN.SB.11.1 was identified molecularly as *Streptomyces pluripotens* with a 100% similarity percentage to the data with access number CP022433.1. Actinomycetes code PN.SB.11.3 was identified as *Micromonospora chersina* with a 99.92% similarity percentage to GenBank data with access number EU274367.1.

Brachybacterium paraconglomeratum is a gram-positive bacterium belonging to the actinobacteria phylum which was first introduced by (Takeuchi et al., 1995). This actinobacteria species is anaerobic facultative, pale brown, with coccoid-shaped cells during the stationary phase, and irregular rod-shaped during the exponential phase. *Streptomyces pluripotent* is a species of actinomycetes belonging to the genus Streptomyces which was first isolated from mangrove sediments (Lee et al., 2014). This species is reported to be able to produce Bacteriocin compounds (compounds that can inhibit the growth of MRSA bacteria). *Micromonospora chersina* is a species of actinomycetes belonging to the genus Micromonospora. The genus has characteristics such as gram-positive, spore-forming capability, generally aerobic, and ability to form branched mycelium. Several species of the genus are known as important sources of antibiotics (Hirsch and Valdés, 2010).

3.4 Biosynthetic Gene Cluster (BGC) mapping simulation using AntiSMASH 6.0 program

Mapping simulation results of Biosynthetic Gene Cluster (BGC) in the whole genome from GenBank of the same species with the molecular identification results of the *Brachybacterium paraconglomeratum* sample (NZ_QOCI00000000.1) are shown in Table 2.

Table 2. Mapping simulation of Biosynthetic Gene Cluster (BGC)

Region	Region location (Nucleotides)	Type	Most similar known cluster	Similarity	References
6.1	177714-211682	NAA, Ectoine	Ectoine	75%	Zaccai et al. (2016)
10.1	141523-152335	Siderophore	-	-	-
16.1	141523-152335	Terpene	Carotenoid	50%	Maoka (2019)

Mapping simulation of Biosynthetic Gene Cluster (BGC) using AntiSMASH 6.0 in the whole genome of the same species with the *Brachybacterium paraconglomeratum* sample (S.SK.8.1) has detected three secondary metabolite producing regions with estimates that the resulting product was included in the NAPAA (Non-Alpha Poly-Amino group Acids), ectoine, siderophore group, and terpene group. The

three regions have similarities with the gene clusters that produce active compounds including the cosmetic active ingredient compound Ectoine at 75% (Region 6.1) (Zaccai et al., 2016) and carotenoid pigment compounds at 50% (Region 10.1) (Maoka, 2019). Another region, namely Region 10.1, which was thought to produce sideropore compounds, has not yet been known to have similarities with the gene clusters that produce other secondary metabolite compounds.

Mapping simulation results of Biosynthetic Gene Cluster (BGC) using AntiSMASH 6.0 in the whole genome of the same species with *Streptomyces pluripotens* sample (PN.SB.11.1) has detected 31 BGC regions with estimates of the composing products in the types of butyrolactone, lanthipeptide-class iii, terpene, PKS Type 3, PKS Type 2, PKS Type 1, NRPS, betalactone, hglE-KS (heterocyst glycolipid synthase-like PKS), ectoine, LAP (Linear Azol (in) e-containing Petide), melanin, siderophore, ladderane, RiPP-like, NRPS-like, lanthipeptide-class v, thiopeptide, and nucleoside (Table 3). Some of these regions had similarities with the gene clusters that produce antibiotic compounds such as Cyphomycin at 5% (Region 1) (Chevrette et al., 2019), Cinnamycin at 14% (Region 6) (Widdick et al., 2003), Enduracidin at 10% (Region 7) (Inoue et al., 2010), Glycinocin A at 16% (Region 15) (Corcilius et al., 2018), Alba-flavenone at 100% (Region 16) (PubChem NCBI), Toxoflavin/Fervenulin at 14% (Region 21) (Lee et al., 2016), Formicamycins AM at 11% (Region 24) (Qin et al., 2017), Daptomycin at 6% (Region 27) (WHO, 2018), Platencin at 9% (Region 30) (PubChem NCBI), and Toyocamycin at 30% (Region 31) (PubChem NCBI) as shown in Table 3.

Table 3. Mapping simulation results of the Biosynthetic Gene Cluster (BGC) of the *Streptomyces pluripotens* sample

Regions	Region location (Nucleotides)	Type	Most simillar known cluster	Similarity	Reference
Region 1	72991-81978	Butyrolactone	Cyphomycin	5%	Chevrette et al. (2019)
Region 2	490073-512793	Lanthipeptide-class-iii	Informatipeptin	85%	-
Region 3	702613-724773	Terpene	Isorenieratene	54%	Maresca et al. (2008)
Region 4	771946-811237	Type 3 PKS	Herboxidiene	6%	Hasegawa et al. (2011)
Region 5	823634-935178	Type 1 PKS, NRPS, Betalactone	Sporolide A/B	46%	Nicolaou et al. (2009)
Region 6	953402-1003799	Type 1 PKS, hgIE-KS	Cinnamycin	14%	Widdick et al. (2003)
Region 7	1006575-1060635	NRPS	Enduracidin	10%	Inoue et al. (2010)
Region 8	1694597-1705001	Ectoine	Ectoine	100%	Zaccai et al. (2016)
Region 9	2013395-2085904	Type 2 PKS, LAP	Spore pigment	83%	-
Region 10	2584611-2593666	Melanin	Melanin	60%	El Obeid et al. (2017)
Region 11	2692924-2703013	Siderophore	Desferrioxamin B/E	83%	Hoffman et al. (2013)
Region 12	3134085-3176400	Ladderane	-	-	-
Region 13	3178040-3227594	NRPS	Caniferolide A/B/C/D	4%	Alvarino et al. (2019)
Region 14	4120523-4131467	Butyrolactone	Scieric Acid	29%	-
Region 15	4881960-4962501	NRPS	Glycinocin A	16%	Corcilius et al. (2018)
Region 16	5214968-5235513	Terpene	Albaflavenone	100%	PubChem NCBI
Region 17	5851988-5862506	Siderophore	-	-	-
Region 18	5974148-6040769	Type 1 PKS	4-Z-Annimycin	77%	Kalan et al. (2013)
Region 19	6256914-6266782	RiPP-Like	-	-	-
Region 20	6285291-6303587	Terpene	Geosmin	100%	Neff (2018)
Region 21	6357767-6400888	NRPS-Like	Toxoflavin/Fervenulin	14%	Lee et al. (2016)
Region 22	6437681-6450991	Siderophore	-	-	-
Region 23	6475041-6486984	RiPP-Like	-	-	-
Region 24	6489293-6533506	Lanthipeptide-class-v and ii	Formicamycins A-M	11%	Qin et al. (2017)
Region 25	6733259-6759994	Terpene	Hopene	92%	PubChem NCBI
Region 26	6870824-6959482	Type 1 PKS	E-837	100%	PubChem NCBI
Region 27	6994914-7036098	Type 3 PKS	Daptomycin	6%	WHO (2018)

Table 3. Mapping simulation results of the Biosynthetic Gene Cluster (BGC) of the *Streptomyces pluripotens* sample (cont.)

Regions	Region location (Nucleotides)	Type	Most simillar known cluster	Similarity	Reference
Region 28	7229421-7239861	Melanin	Melanin	71%	El Obeid et al. (2017)
Region 29	7376037-7480384	NRPS, Type 1 PKS	Antimycin	100%	Seipke and Hutchings (2013)
Region 30	7482780-7511674	Thiopeptide, LAP	Platencin	9%	PubChem NCBI
Region 31	7516137-7536499	Nucleoside	Toyocamycin	30%	PubChem NCBI

Table 3 reveals the similarity level from region 1 to region 31, ranging from 0% to 100%. Based on the similarity level in the database, it showed a similarity level of 0%, which means the function of the gene sequence has not yet been known. This can be seen in Region 22 which was 0%, in contrast to regions 7, 16, 20, and 26 which showed 100% similarity, meaning that it can be utilized. Meanwhile, other regions had similarities with the pigment-producing gene clusters consisting of Isorenieratene at 54% (Region 3); *spore pigment* at 83% (Region 9); also Melanin at 60% and 71% (Region 10 and 28), Herboxidiene antitumor at 6% (Region 4), cosmetic active ingredients of Ectoine at 100% (Region 8), Fe and Al Desferrioxamin B/E binders at 83% of (Region 11), Caniferolide A-D antioxidant at 4% (Region 13), Annymycin 4-Z sporulation inhibitor at 77% (Region 18), Antimycin toxin at 100% (Region 29), earthy aroma Geosmin, and several other compounds those function has not yet known.

Based on the mapping simulation of Biosynthetic Gene Cluster (BGC) in the whole genome of the same species on *Micromonospora chersina sample* (PN.SB.11.3) (Table 4), 18 regions of the gene clusters that produce secondary metabolites were estimated to have products included in the types of PKS Type 3, NRPS, PKS Type 2, PKS Type 1, lanthipeptide-class i and iii, terpenes, betalactone, siderophore, RiPP-like, and PKS-like.

Table 4. Mapping simulation results of Biosynthetic Gene Cluster (BGC) on *Micromonospora chersina* samples

Regions	Region location (Nucleotides)	Type	Most simillar known cluster	Similarity	Reference
Region 1	36142-152907	Type 3 PKS, NRPS	Enduracidin	33%	Inoue et al. (2010)
Region 2	242721-315352	Type 2 PKS	Pradimicin-A	25%	PubChem NCBI
Region 3	489117-511699	Lanthipeptide-class-iii	SapB	100%	PubChem NCBI
Region 4	655663-675976	Terpene	-	-	-
Region 5	1101595-1122775	Lanthipeptide-class-i	-	-	-
Region 6	1216411-1244997	Betalactone	Cyphomicin	2%	Chevrette et al. (2019)
Region 7	1620792-1687557	NRPS, Type 1 PKS	Nostopeptolide A2	25%	PubChem NCBI
Region 8	2026564-2038336	Siderophore	Desferrioxamin B/E	100%	Hoffman et al. (2013)
Region 9	2176471-2235139	NRPS	Lysocin	9%	Hamamoto et al. (2015)
Region 10	2237017-2299646	NRPS, Type 1 PKS	Bleomycin	6%	Hindra et al. (2017)
Region 11	3364961-3379746	NAGGN	-	-	-
Region 12	3930661-3951079	Terpene	Phosphonoglycans	3%	Yu et al. (2014)
Region 13	4018620-4039932	Terpene	Isorenieratene	25%	Maresca et al. (2008)
Region 14	4468081-4513783	Type 1 PKS	Dynemicin A	55%	Tuttle et al. (2005)
Region 15	4669198-4710247	Type 3 PKS	Alkyl-O-dihidrogeranyl-methoxyhydroquinones	71%	PubChem NCBI
Region 16	6186452-6207402	Terpene	-	-	-
Region 17	6345774-6356598	RiPP-Like	Lymphostin/Neolymphostinol B/ Lymphostinol/Neolymphostin B	33%	Miyanaga et al. (2011)
Region 18	6508782-6549804	PKS-Like	-	-	-

Table 4 also shows the simulation of the level of similarity in the Biosynthetic Gene Cluster (BGC) on *Micromonospora chersina* samples. The results showed that Region 2 and Region 8 have 100% similarity. However, a 0% similarity level was also found, namely Region 4, Region 5, Region 11, Region 16, and Region 18. The higher the Mapping simulation results of Biosynthetic Gene Cluster (BGC) on *Micromonospora chersina* samples, the greater the usefulness value and vice versa.

There were several regions similar to the antibiotic-producing regions such as Enduracidin at 33% (Region 1), Cyphomycin at 2% (Region 6), and Lysocin at 9% (Region 9). In addition to regions that were similar to those producing antibiotics, it has also detected that several regions were similar to other active compounds producers such as antifungal Pradimicin A at 25% (Region 2) (PubChem NCBI), an important compound in the formation of aerial mycelium SapB at 100% (Region 3), Fe and Al Desferrioxamin E binders at 100% (Region 8), Bleomycin anticancer at 6% (Region 10), Polysaccharides Phosphonoglycans at 3% (Region 12), Isorenieratene pigment at 25% (Region 13), Dynemicin A antitumor at 55% (Region 14), *Lymphostin immunosuppressant* at 33% (Region 17), and several other compounds those function has not yet known.

BGC mapping simulation results are used to estimate the compounds that can be produced by actinomycetes isolates based on the sequence of nucleic bases in their genome. The three actinomycetes isolates were detected to have gene regions that are similar to the antibiotic-producing genes. Thus, based on the BGC mapping simulation, it can be seen that the active isolates of actinomycetes may potentially produce new candidates of antibiotic compounds. It is necessary to do further research on the types of compounds that have antibacterial activity produced by each of these actinomycetes isolates.

4. CONCLUSION

The number of culturable-actinomycetes isolates of mangrove sediment in the Semarang was 19 isolates that consist of 3 isolates grown from ISP 1 medium; 1 isolate grown from Zobell medium; 9 isolates grown from HVA medium; 6 isolates grown from ISP 1 + Humic Acid medium and in Karimunjawa was 17 isolates that consist 3 isolates grown from ISP 1 medium; 4 isolates grown from Zobell medium; 5 isolates grown from HVA medium; 2 isolates grown from ISP 1 + Humic Acid medium, and 3 isolates grown from Zobell + Humic Acid medium. All isolates were detected to have at least one type of Biosynthetic Gene Cluster, but only three isolates had antibacterial activity against *S. aureus*, *E. coli*, and *L. monocytogenes*, namely one isolate from Semarang and two isolates from Nyamuk Island, Karimunjawa. Results of molecular identification found the types of *Brachybacterium paraconglomeratum* (99.08%), *Streptomyces pluripotens* (100%), and *Micromonospora chersina* (99.92%). Biosynthetic Gene Cluster (BGC) mapping simulation results showed that these three species have similar genes with antibiotics producing genes that potentially could be new antibiotic candidates.

ACKNOWLEDGEMENTS

Part of this paper covers the work conducted for the author's Master degree at Diponegoro University, Semarang-Indonesia. The authors would like to express gratitude to Mrs. Desy Wulan Triningsih (Toyama Prefectural University, Japan) for her technical support.

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