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

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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 *Brachy bacterium 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.

Keywords: Antibiotic/ Actinomycetes/ Pathogenic bacteria/ Sediments

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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 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.
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 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 NaOH)), and Humic Acid Vitamin Agar (HVA) (humic acid 1 g (diluted in 10 mL 0.2 NaOH); Na₂HPO₄ 0.5 g; KCl 1.71 g; MgSO₄·7H₂O 0.05 g; FeSO₄·7H₂O 0.01 g; vitamin B complex 3.75 mg; CaCO₃ 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 (Messaudi 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 ddH₂O. 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 ddH₂O 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’-AAGGCGGCGSBGCTAY STGCC-3’) and A3gamR (5’-TTGGGBIKBCCGGS 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’-RTRGAYCCNGCAIC G-3’) and HGTGT r (5’-VGTNCCTGCCRTG-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’TGGGANCCCGCCGAA 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 ddH2O. 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’-AGAGTTTGTATCCCTGCTCAG-3’) and 1492R (5’-GGTTACCTTGTACAGCTT-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 ddH2O. 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 base 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...
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](image)

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 pluripotens* 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).

### 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 (NZ_QOCI00000000.1) are shown in Table 2. 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) of the *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, hgIE-KS (heterocyst glycolipid synthase-like PKS), ectoine, LAP (Linear Azol (in) e-containing Petide), melanin, siderophore, ladderane, RiPPElike, NRPS-like, lanthipeptide-class v, thiopptide, 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), Albaflavenone 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.

| Regions | Region location (Nucleotides) | Type | Most similar 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 | 16945097-1705001              | 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   | Desferrioxamine 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 | 6475041-6486894              | 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 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, lantipeptide-class i and iii, terpenes, betalactone, siderophore, RiPP-like, and PKS-like.

| Regions | Region location (Nucleotides) | Type | Most similar 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 4. Mapping simulation results of Biosynthetic Gene Cluster (BGC) on *Micromonospora chersina* samples

| Regions | Region location (Nucleotides) | Type | Most similar 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% | Chevrete 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 | 336461-3379746 | 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 | Alky-O-dihdrogeranyl-methoxyhydroquinones | 71% | PubChem NCBI |
| Region 16 | 6186452-6207402 | Terpene | - | - | - |
| Region 17 | 6345774-6356598 | RiPP-Like | Lymphostin/Neolymphostinol B/ Lymphostinol/Neolymp hostin 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 anticanter 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 cultivable-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.

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