BIODIVERSITAS
Volume 24, Number 4, April 2023
Pages: 2374-2383

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d240452

Phylogenetic analysis of *Streptomyces* producing antimicrobial agent isolated from Kukup Beach Sand, Yogyakarta, Indonesia

AMBARWATI AMBARWATI[1,♥], BROTO SANTOSO[2], AAN SOFYAN[3]

[1]Department of Biology Education, Faculty of Teacher Training and Education, Universitas Muhammadiyah Surakarta. Jl. Ahmad Yani, Sukoharjo 57169, Central Java, Indonesia. Tel.: +62-271-717417, ♥email: ambarwati@ums.ac.id

[2]Laboratory of Biology Pharmacy, Faculty of Pharmacy, Universitas Muhammadiyah Surakarta. Jl. Ahmad Yani, Sukoharjo 57169, Central Java, Indonesia

[3]Department of Nutrition, Faculty of Health Sciences, Universitas Muhammadiyah Surakarta. Jl. Ahmad Yani, Sukoharjo 57169, Central Java, Indonesia

Manuscript received: 26 February 2023. Revision accepted: 28 April 2023.

Abstract. *Ambarwati A, Santoso B, Sofyan A. 2023. Phylogenetic analysis of* Streptomyces *producing antimicrobial agent isolated from Kukup Beach Sand, Yogyakarta, Indonesia. Biodiversitas 24: 2374-2383.* Microorganisms such as bacteria, fungi, and *Streptomyces* are sources of bioactive compounds. *Streptomyces* is known as the largest antibiotic-producing genus. This study aimed to determine the antimicrobial activity of *Streptomyces* isolates and analyze the relationship between *Streptomyces* isolates based on the 16S rRNA gene sequence. The *Streptomyces* isolates were screened for their antimicrobial activity based on their ability to inhibit test bacteria growth using the agar block method. The selected isolates that showed antimicrobial activity were molecularly characterized using 16S rRNA gene sequence analysis. The result showed 32 *Streptomyces* isolates successfully isolated on SCA and Raffinosa Histidine Agar media. Among 32 isolates, it was observed that five isolates demonstrated the ability to inhibit the test bacteria growth on 9 to 25 mm of inhibition zone diameters. The BRI-18 isolate showed the highest antimicrobial activity inhibiting *Bacillus subtilis* G. FNCC 0060 on 25 mm of inhibition zone diameter (strong inhibition category). Based on 16S rRNA sequences, it was known that five isolates belonged to *Streptomyces*. BLAST analysis on the ARA-5 isolate revealed that it was closely related to *Streptomyces griseoincarnatus* strain JCM 4381 with 99.62% sequence similarities. The AR3-29 isolate was a sister clade identical to *Streptomyces rochei* strain NRRL B-1559 with a 99.35% similarity level. The BRI-18 and BRI-19 isolates were the most similar to *Streptomyces fradiae* strain NBRC 12773 on 96.87% and 95.15% index similarity, respectively, whereas the BRI-35 isolate demonstrated the highest similarity to *Streptomyces zhihengii* strain YIM T102 (97.02%). The research demonstrated that *Streptomyces* isolates from Kukup beach sand showed the potential as an antimicrobial agent.

Keywords: Antimicrobial, Kukup Beach sand, molecular identification, *Streptomyces*

INTRODUCTION

Antibiotics are widely known as drugs to control infectious diseases. However, there have been many reports of resistance of pathogenic bacteria to existing antibiotics. This has prompted research to find new types of antibiotics. In addition, microorganisms, including bacteria and fungi, are sources of bioactive compounds that will never run out to be studied.

Several studies have been conducted to find the antimicrobial substance from fungi, including: endophytes (Bhardwaj et al. 2017) and bacteria (Nayaka et al. 2019; Ambarwati et al. 2019), endophyte bacteria (Leonita et al. 2015; Yuwantiningsih et al. 2015; Mohammed et al. 2017; Syed et al. 2017; Gislin et al. 2018; Moi et al. 2018), Actinomycetes (Wulandari and Rahayu. 2015; Retnowati et al. 2017; Nayaka et al. 2018; Al-Ansari et al. 2019), and *Streptomyces* (Beiranvand et al. 2017; Ambarwati et al. 2020).

In addition, ten isolates of Actinomycetes from the Merapi Mount have antibacterial activity against *E. coli*, with the strongest isolate being D (S4), with a zone of irradiation with a diameter of 17.25 mm (Wulandari and Rahayu. 2015). Furthermore, 77 of 167 Actinomycetes isolates from different mangrove and rhizosphere environments exhibited antibacterial properties (Retnowati et al. 2017).

Gram-positive and Gram-negative infections were effectively combated by the chosen *Streptomyces* sp ES2 (Al-Ansari et al. 2019). *Streptomyces* sp CRB46, found in the rhizosphere of *Cyperus rotundus* L. in the Cemoro Sewu highlands of Indonesia, inhibited 11 out of 12 examined microorganisms with a diameter of the inhibition zone about 17 to 35 mm (Ambarwati et al. 2020).

Streptomyces is a member of Actinomycetes, widely known for producing secondary metabolite bioactive compounds. Bioactive compounds produced by *Streptomyces* can act as enzyme inhibitors (El-Hadedy et al. 2015), antioxidants (Siddharth and Vittal. 2018), growth-promoting factors (Fatmawati et al. 2019; Wahyudi et al. 2019; Saraylou et al. 2021), antitumor (Ahmad et al. 2017), anticancer (Osama et al. 2022), and especially as antibiotics.

Actinomycetes, particularly *Streptomyces*, can be isolated from various places, including: rice rhizosphere soil (Ambarwati et al. 2012a), soil (Nayaka et al. 2018; Muthuraj et al. 2021), sediment river-soil samples (Nayaka et al. 2020a), soybean rhizosphere soil (Mariastuti et al.

2018; Fatmawati et al. 2019), corn rhizosphere (Wahyudi et al. 2019), *Alternanthera sessilis* (L.) DC. rhizosphere soil (Nayaka et al. 2020b). In addition, *Streptomyces* is also found in mangroves (Retnowati et al. 2018), ocean (Kamjam et al. 2017; Paderog et al. 2020; Guerrero-Garzón et al. 2020; Tenebro et al. 2021), freshwater sediment (Nayaka et al. 2020c) and also in the desert (Ouchari et al. 2019).

The sand beach is one of the extreme habitats for microorganism life. It's caused by environmental factors that do not support microorganisms' growth, such as high salt content, temperature, and humidity. The microorganism density living in extreme environments is generally not abundant. Nevertheless, it can usually produce bioactive substances. In extreme habitats, microorganisms can generally be found that can produce secondary metabolite bioactive compounds (Sivalingam et al. 2019).

This study aimed to determine the antibiotic activity of *Streptomyces* isolates and analyze the relationship between *Streptomyces* isolates based on the 16S rRNA gene sequence. Several studies have succeeded in isolating and molecularly identifying Actinomycetes, especially *Streptomyces*. Therefore, 87 actinobacteria can be isolated from mangrove forests in Malaysia (Lee et al. 2014). A total of 8 species of Actinobacteria have antimicrobial activity (SuPei et al. 2014). Research by Retnowati et al. (2018) has successfully isolated Actinomycetes isolates from the Torosiaje Mangrove Forest, Gorontalo. Sivalingam et al. (2019) also successfully identified one isolate as *Streptomyces* sp. BDUSMP 02 isolated from mangrove sediments.

MATERIALS AND METHODS

Sample collection

Sand samples were taken from Kukup Beach of Kemadang Village, Tanjungsari Subdistrict, Gunungkidul District, Yogyakarta, Indonesia (Figure 1). Sampling sites were located at coordinates -6.9958-S; 110.3694-E. Sand samples were collected at a depth of about 10 cm by a soil core. Sand samples were collected from three sites and placed on sterile plastic.

Isolation and purification

Isolation was performed using standard procedures on Starch-Casein Agar (SCA) and Raffinose-Histidine Agar (RHA) media with 50.0 gmL^{-1} cycloheximide and 50 gmL^{-1} nystatin, and then a serial dilution was made up to 10^{-4}. Next, the incubation is conducted at 28°C for 4 to 14 days. Finally, the purification was conducted on each colony, showing different appearances; this was conducted on SCA media.

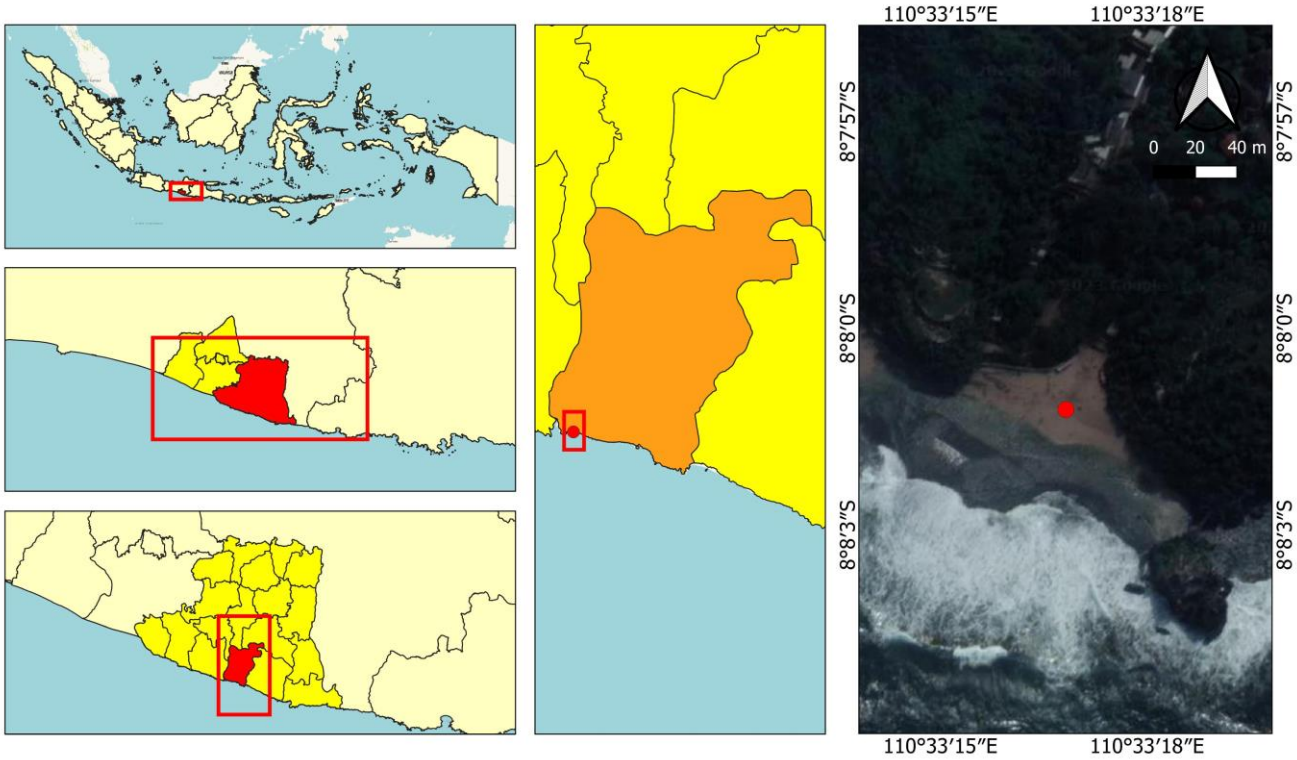

Figure 1. Location of sand samples, Kukup Beach, Tanjungsari Subdistrict, Gunungkidul District, Yogyakarta, Indonesia (Google maps, 2023)

Antimicrobial activity assay

Antimicrobial activity assay was carried out on 32 purified *Streptomyces* isolates against four test bacteria, namely: *Staphylococcus aureus* Rosenbach (*S. aureus*) ATCC 25923, *Bacillus subtilis* G. (*B. subtilis*) FNCC 0060, *Escherichia coli* E. (*E. coli*) ATCC 35218 and *Pseudomonas aeruginosa* A. *(P. aeruginosa)* ATCC 27853. The antimicrobial activity was based on the agar block method on 6 mm of block diameter (Nedialkova and Naidenova 2005). The test bacteria culture was then incubated at 30°C for 24 hours. Antimicrobial activity was determined based on the diameter of the inhibition area that showed by the clear area around the agar block. Furthermore, the size of the inhibition area is categorized into: (i) weak if the diameter of the inhibition area is between 7-15 mm, (ii) moderate if the diameter of the inhibition area is 16-25 mm, and (iii) strong if it is more than 25 mm (Nedialkova and Naidenova 2005).

Identification of *Streptomyces* isolates

The potential *Streptomyces* isolates were identified based on cell morphology by Gram staining and spore chain morphology by Scanning Electron Microscope (SEM) (JEOL JSM 5310 LV).

DNA extraction of bacterial isolates

Before DNA isolation, the *Streptomyces* isolate was first grown in 20 mL of liquid Raffinosa Histidine medium. It was then incubated in an incubator shaker and shaken at 160 rpm at 28°C for seven days. After that, the *Streptomyces* culture isolate was centrifuged at 4,000 rpm for 5 minutes to separate the pellets from the supernatant. Next, *Streptomyces* isolate pellets were put into an Eppendorf tube, and 2.0 mg glass beads and 1.0 mL lysis buffer were added. Furthermore, DNA extraction of *Streptomyces* isolates was carried out using the standard method (Magarvey et al. 2004).

PCR amplification of 16S rRNA gene

The 16S rRNA gene was amplified by using universal primers 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-TACGGCTACCTTGTTACGACTT-3') on BIO-RAD T100 Thermal Cycler (Magarvey et al. 2004). The total volume of the PCR reaction was as much as 25 µL, including: 1-2 µL of genomic DNA on 20 ngμL^{-1} of concentration; 12.5 µL Go Taq®Green (Bioline) Master MIX; 1 µL primer 27F and 1 µL primer 1492R and 8.5-9.5 µL ddH_2O. The PCR reaction was carried out under the following conditions: pre-denaturation at 96ºC for 5 minutes followed by 30 cycles at 95ºC for 1 minute, annealing at 54ºC for 2 minutes, elongation at 72ºC for 2 minutes, and then the final elongation at 72ºC for 10 minutes. First, the PCR product was visualized by agarose gel electrophoresis by adding 5 µL of cyber. Next, the PCR program was run at 90 volts for 40 minutes in 1X TBE buffer, then the DNA band was observed under UV light. In addition, the amplification used was a 1 kb DNA ladder and 100 bp plus DNA ladder as markers.

DNA sequencing

The 16S rRNA gene sequencing was conducted at First BASE Laboratories Bhd in Selangor, Malaysia. The PCR purification KIT BigDye® Terminator v3.1 Cycle Sequencing Kit was used to purify the PCR product. The ABI PRISM 3730xl Genetic Analyzer, developed by Applied Biosystems, USA, was used for sequencing. The assembled, examined, and altered DNA sequences were assembled using the CAP3 software program and compared to bacteria sequences from the NCBI database. The sequences were then aligned with the available database, and the program BLAST (Basic Local Alignment Search tool) was used to analyze and find sequence similarity (Altschul et al. 1990).

Reconstruction of the phylogenetic tree

The DNA sequences of the potential *Streptomyces* isolates were aligned with CLUTALX multiple alignments (Thompson et al. 1997). The phylogenetic tree was constructed using the MEGA version 7 program (Kumar et al. 2016), performed using the neighbor-joining method, and validated by 1000X bootstrapping as described by Saitou and Nei (1987).

RESULTS AND DISCUSSION

The density of *Streptomyces* isolates

The *Streptomyces* population growing on RHA media was more than on SCA media. The results of the *Streptomyces* density measurements are presented in Table 1.

Purification results

The purification results obtained as many as eight pure isolates growing on SCA medium and 24 pure isolates growing on RHA. The results of *Streptomyces* purification from samples of Kukup sand beach on SCA and RHA medium are presented in Table 2. The results of five potential *Streptomyces* isolates from samples of Kukup sand beach are presented in Figure 2.

The potential *Streptomyces* isolates as antimicrobial agent

Among five of 32 *Streptomyces* isolates potential antimicrobial agents. The results of the five *Streptomyces* isolates inhibition test are presented in Table 3 and Figure 3. Table 3 shows that the BRI-18 isolate was the best in inhibiting *Bacillus subtilis* FNCC 0060 with a diameter of an inhibition zone of 25 mm (the clear zone is categorized as moderate).

Table 1. The density of *Streptomyces* isolates on sand samples from Kukup Beach, Gunungkidul District, Yogyakarta, Indonesia

Isolation media	Density average of Streptomyces isolates (Cfu/g-dw)
SCA	610,924.666 ± 553,424.232 (6.1±5.5 X 10^5)
RHA	770,878.222 ± 621,552.658 (7.7±6.2 X 10^5)

Table 2. Number of *Streptomyces* isolates on sand of Kukup Beach, Gunungkidul District, Yogyakarta, Indonesia

Medium	Total isolate	Isolate code
SCA	8	AS3-1, AS3-2, AS3-3, AS3-4, BS3-1, BS3-3, BS3-5, BS3-6
RHA	24	ARA-1, ARA-5, AR3-23, AR3-29, BR3-5, AR2-1, AR2-2, AR2-28, AR1-2, BR1-18. BR1-19, BR1-35, BR3-3, BR3-6, CR3-25, CR2-1, CR2-2, CR2-3, CR2-4, CR2-5, CR2-6, CR2-7, CR3-44, CR3-46
Total	32	

Table 3. Inhibition test of *Streptomyces* isolates from sand on Kukup Beach, Gunungkidul District, Yogyakarta, Indonesia

Isolate code	Inhibition diameter (mm)			
	S. aureus ATCC 25923	*B. subtilis* FNCC 0060	*E. coli* ATCC 5281	*P. aeruginosa* ATCC 27853
ARA5	12	-	-	-
AR3-29	-	17	-	-
BR1-18	16	25	15	15
BR1-19	13	24	13	15
BR1-35	9	-	-	-

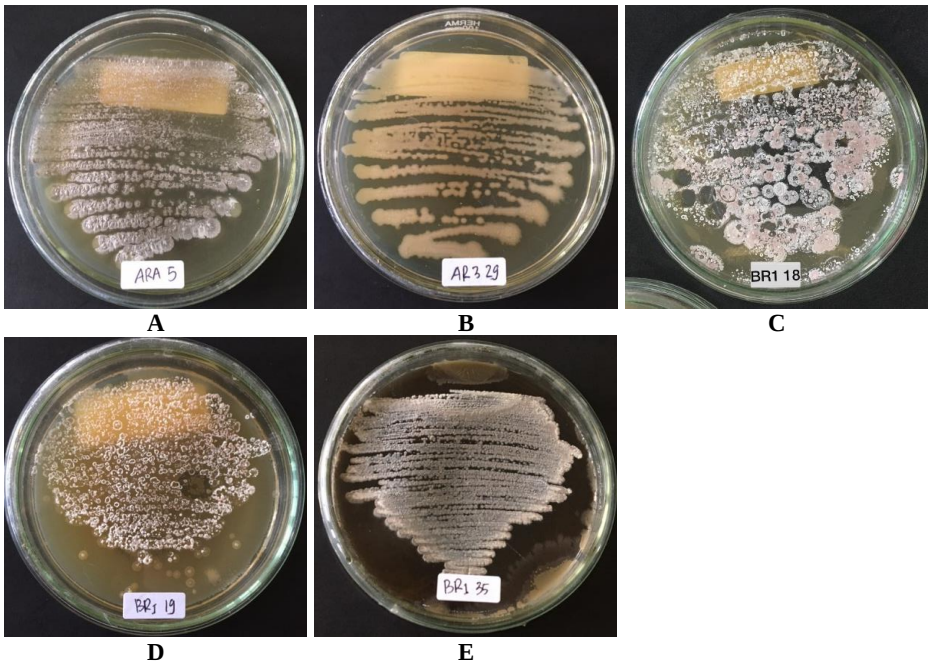

Figure 2. The five potential *Streptomyces* isolates from Kukup Sand Beach, Gunungkidul District, Yogyakarta, Indonesia. A. ARA 5. B. AR3-29. C.BR1-18. D. BR1-19. E. BR1-35

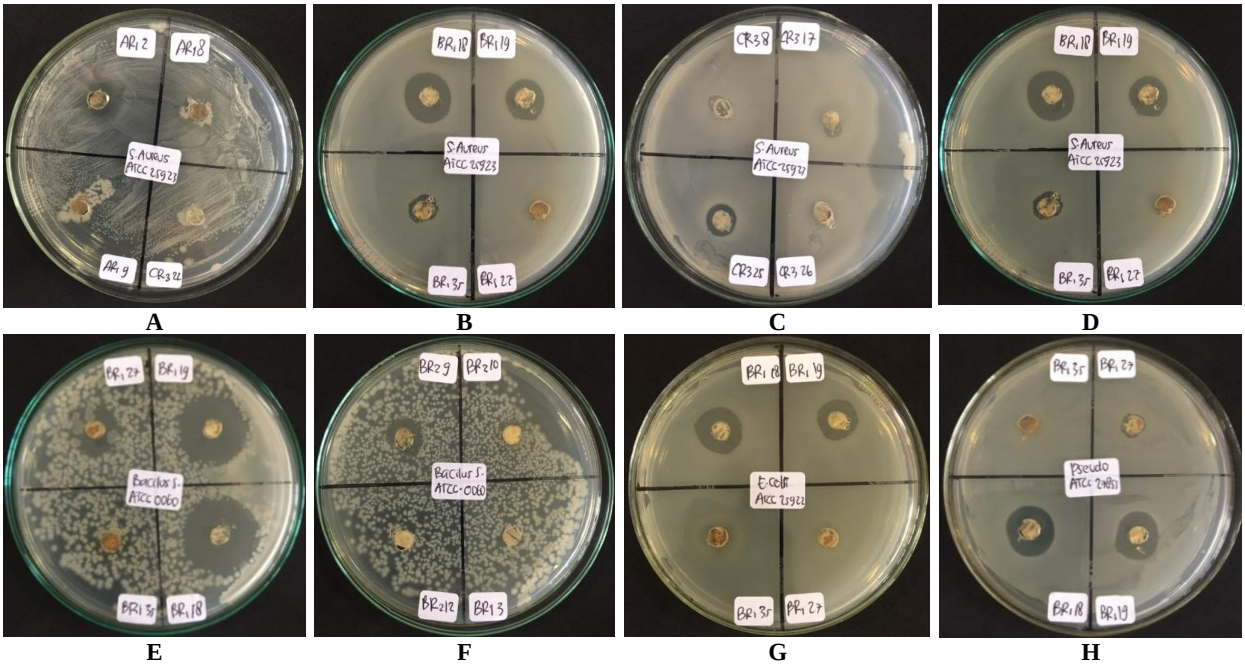

Figure 3. The inhibition of *Streptomyces* isolates from Kukup Beach, Gunungkidul District, Yogyakarta, Indonesia sand against test bacteria

Cell and spores morphology of Streptomyces *isolates*

The results of cell morphology observations showed that the five isolates found were in the form of branched rods, purple, and Gram-positive, a characteristic of *Streptomyces* (Figure 4). The spores morphology of five potential *Streptomyces* isolates are presented in Figure 5.

Molecular identification of Streptomyces *isolates*

Molecular identification is done through DNA isolation, PCR, and sequencing. The sequencing result is presented in Table 4, and the phylogenetic tree of *Streptomyces* isolates is presented in Figure 6.

Discussion

SCA and RHA media are two selective media used to grow *Streptomyces*. Both media were also used to isolate *Streptomyces* from the rhizosphere of *Cyperus rotundus* L. (Ambarwati et al. 2012b). In addition, SCA was also used to isolate Actinomycetes from soil samples and various rhizosphere (Retnowati et al. 2017; Vishwanatha et al. 2017). Microorganisms can use raffinose, starch, and casein, including *Streptomyces*, as a carbon source. In addition to selective media, cycloheximide and nystatin were added (Ser et al. 2016). Both are antifungal functioning to prevent the growth of fungi (Charousoviá et al. 2015; Priyadarshini et al. 2016). This is performed to avoid confusion, as *Streptomyces* has filamentous colonies morphology similar to fungi.

Based on Table 1, *Streptomyces* density on the sand sample of Kukup Beach was 6.1±5.5 X 10^5 in SCA media and 7.7±6.2 X 10^5 in RHA media. The study by Patil et al. (2016) concluded that the average population of Actinomycetes in the sediment of Thoothukkudi east coast India was 1.89 X 10^5 CFU/g while in the seawater was 1.96 X 10^4 CFU/g. Moreover, to produce antibacterial substances, the activity tests were carried out on four test bacteria, two Gram-positive and two Gram-negative, to determine the ability of *Streptomyces* isolates. Those tests bacteria, namely *S. aureus* ATCC 25923**,** *B. subtilis* FNCC 0060, *E. coli* ATCC 5281, and *P. aeruginosa* ATCC 27853**.** Based on the result of the research, it was known that among 32 *Streptomyces* isolates, only five isolates could inhibit the test bacteria. Therefore, the antimicrobial activity-test results produced by the *Streptomyces* isolates from the Kukup sand beach will be analyzed quantitatively and qualitatively. Quantitative analysis was carried out by measuring the size of the inhibition area formed on the test microorganism. In comparison, the qualitative analysis was carried out by classifying the size of the inhibition area.

Table 4. The comparison of *Streptomyces* isolates sequencing with comparative *Streptomyces* sequence

Isolate	Closest comparison	Query	Identity	Accession number
ARA 5	*Streptomyces griseoincarnatus strain JCM 4381*	93%	99.62%	MT760527.1
AR3-29	*Streptomyces rochei strain NRRL B-1559*	100%	99.35%	NR_116078.1
BR1-18	*Streptomyces fradiae strain NBRC 12773*	99%	95.15%	NR_112270.1
BR1-19	*Streptomyces fradiae strain NBRC 12773*	100%	96.87%	NR_112270.1
BR1-35	*Streptomyces zhihengii strain YIM T102*	99%	97.02%	NR_146828.1

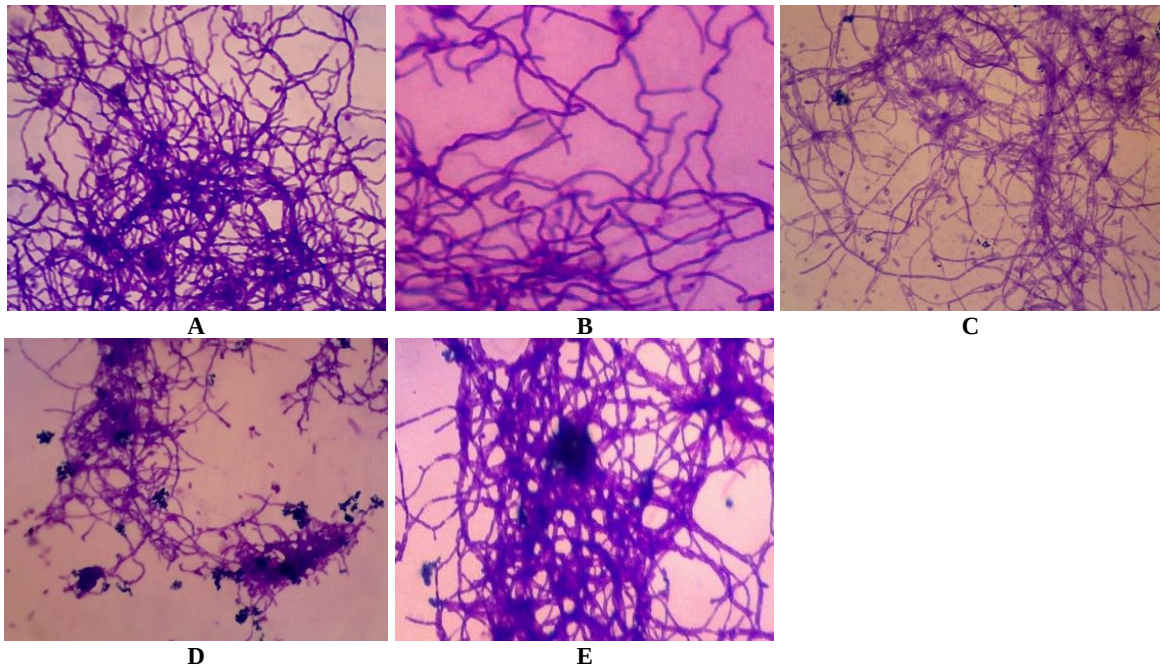

Figure 4. Identification of *Streptomyces* cell morphology by Gram staining. A. ARA-5. B. AR3-29. C. BR1-18. D. BR1-19. E. BR1-35

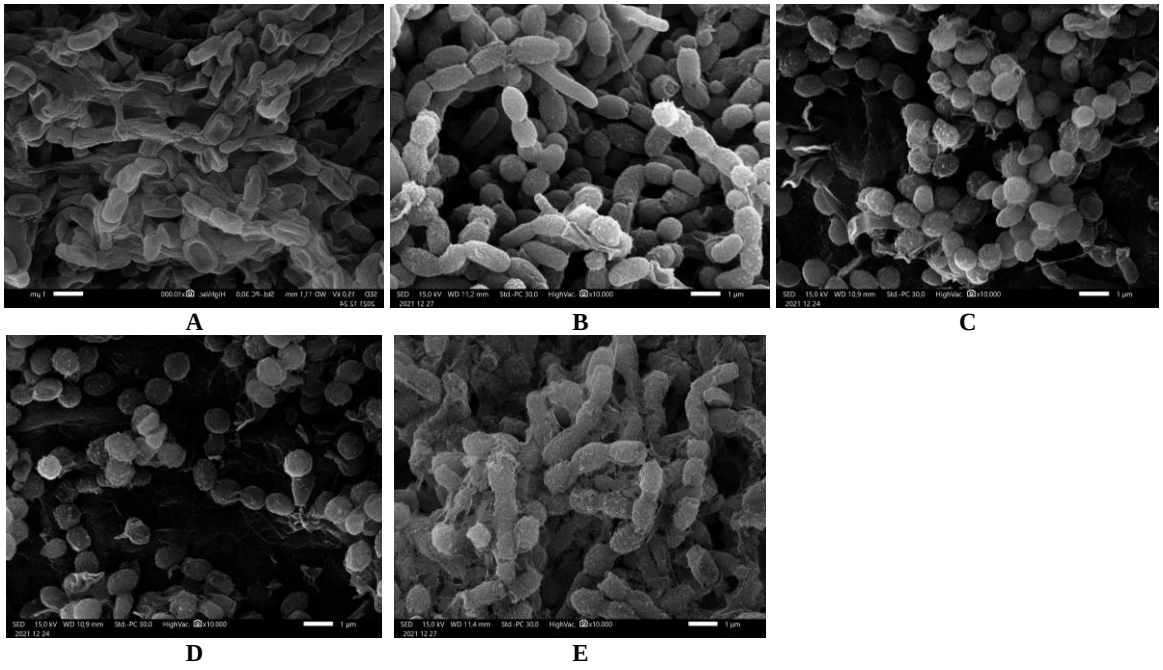

Figure 5. Identification of *Streptomyces* spores morphology by scanning electron microscope. A. ARA-5. B. AR3-29. C. BR1-18. D. BR1-19. E. BR1-35

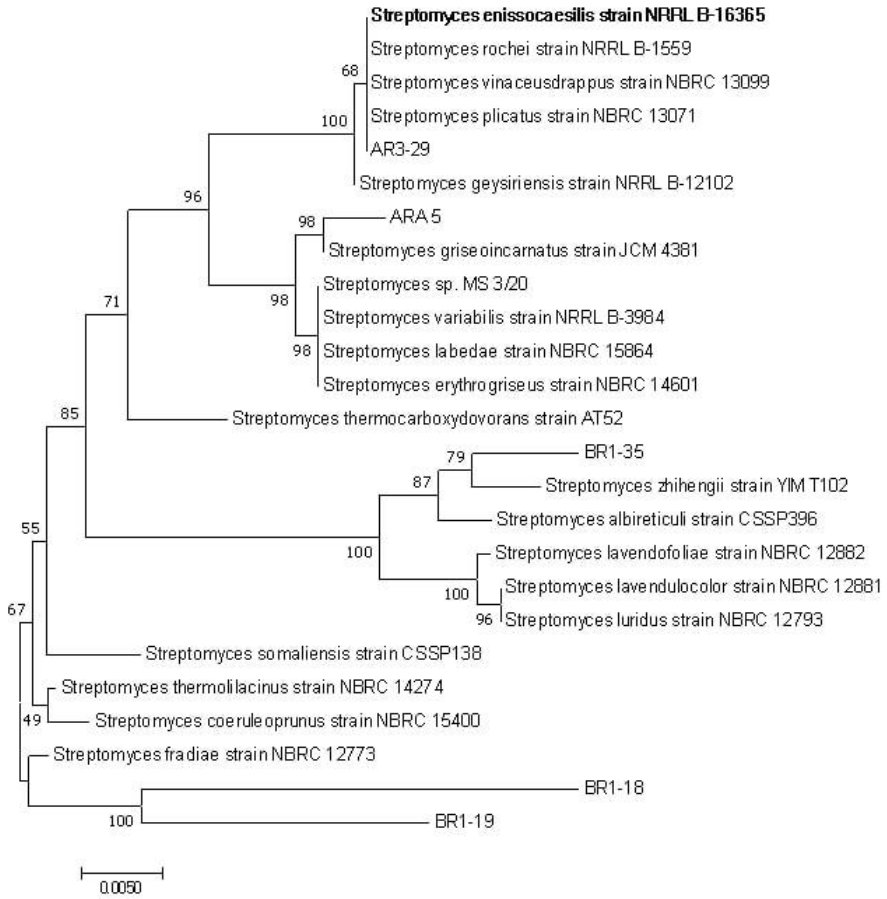

Figure 6. Phylogenetic tree of *Streptomyces* isolates from sand of Kukup Beach, Gunungkidul District, Yogyakarta, Indonesia

Based on Gram staining, it was observed that the five potential isolates from Kukup sand beach showed characteristics as members of *Streptomyces*, branched-rod, purple, and Gram-positive (Figure 4). In addition, the five isolates' spore chain morphology and surface ornamentation showed *Streptomyces* characteristics; the minimum spore chain length is three. Based on the SEM result, it was observed that: isolates ARA-5 had spore morphology of rods with warty spore surfaces; AR3-29 had spore morphology of rods with smooth spore surfaces; BR1-18 and BR1-19 had a similarity, both were with smooth surfaces; BR1-35 had spore morphology of rods with a hairy spore surface (Figure 5).

Table 3 shows that the four isolates could inhibit S. aureus ATCC 25923, three could inhibit *B. subtilis* FNCC 0060, and two could inhibit *E. coli* ATCC 5281 and *P. aeruginosa* ATCC 27853, respectively. The study indicates that the inhibition of *Streptomyces* isolates on Gram-positive is better than on Gram-negative bacteria. It can be observed regarding the number of test bacteria that can be inhibited and the size of the inhibition area. Two isolates, namely BRI-18 and BR1-19 isolates, have high potential as antimicrobials. Judging from the number of microorganisms tested and their inhibitory strength, they could inhibit the growth of four tested bacteria. It was found that BR1-18 isolates were superior to BR1-19 isolates. The BR1-18 was the best isolate in inhibiting *B. subtilis* FNCC 0060 with a diameter inhibition zone of 25 mm (moderate inhibition category).

Several previous studies have succeeded in finding microorganisms from marine as producers of antimicrobial compounds. For example, 47 out of 186 *Streptomyces* isolates from marine sediments collected in British Columbia, Canada's temperate cold waters, showed antibacterial activity (Dalisay et al. 2013). Although 16 Actinomycetes isolates from marine sediments were found along the southern coast of the Bay of Bengal, at least one of the test organisms was resistant to their antifungal effects (Mohan et al. 2014).

A study by Burhamzah et al. (2016) concluded after being fermented in starch nitrate broth for 11 days, isolate GLS-01, a marine actinomycetes isolate obtained from marine sediment of the Galesong Coast, exhibited antibacterial activity against *S. aureus* and *E. coli*. Another study concluded that most deep-sea Actinomycetes substances had antibacterial properties (Kamjam et al. 2017). Among 59% of the 163 Actinobacteria isolates from Merzouga sand demonstrate activity against one or more of the three types of microorganisms: Gram-positive, Gram-negative, and the yeast *Candida albicans* (C.P.Robin) Berkhout (Ouchari et al. 2019). With a 96.4% growth inhibition rate, the crude extract of *Streptomyces griseorubens* strain DSD069, isolated from marine sediments collected in Romblon, Philippines, exhibits the highest antibacterial activity (Paderog et al. 2020). Sabido et al. (2020) found that one isolate of actinomycetes from maritime sediments collected near the shore of Islas de Gigantes, Iloilo, Philippines, showed strong activity against the multidrug-resistant *S. aureus* (MRSA). Six *Streptomyces* strains were found in the sea sand of a beach in northern Portugal. They can inhibit *C. albicans* with MIC values between 3.90 and 125 g mL^{-1} (Ribeiro et al. 2020).

One isolate (M3) from ten Actinomycetes was found in a mangrove habitat on the Red Sea coast. It exhibited broad antagonistic activity against *B. subtilis* ATCC 6633, *E. coli* ATCC 19404, *S. aureus* ATCC 6538, *P. aeruginosa* ATCC 9027, and *C. albicans* ATCC 10231, with inhibition zones ranging from 12.0±0.9 to 20.0±1.9 mm (Hamed et al. 2021). *Streptomyces* were found in maritime sediments in the west-central Philippines, where 92 of the 2,212 isolates showed antibacterial activity against multidrug-resistant *S. aureus*, *P. aeruginosa*, and *E. coli* (Tenebro et al. 2021). According to several researches, marine and sea sand Actinomycetes and *Streptomyces* isolates can potentially serve as antibacterial agents.

Molecular identification in this study was performed with 16S rRNA gen sequencing. The sequencing result for the fifth isolate *Streptomyces* had been sent to NCBI, and it already obtained the accession number as follows: ARA-5 isolate with accession number OL635580.1. AR3-29 isolate (accession number OL635579.1). BR1-18 isolate (accession number OL635581.1). BR1-19 isolate (accession number OL635598.1). And BR1-35 isolate (accession number OL635591.1). Based on the phylogenetic tree analysis, ARA-5 isolate is a sister clade to *Streptomyces griseoincarnatus* (Preobrazhenskaya et al. 1957) Pridham et al. 1958 strain JCM 4381 with accession number MT760527.1 (NCBI 2022b), having similarity of 99.62%. *S. griseoincarnatus* strain JCM 4381 was isolated from the soil and grew at 28°C. AR3-29 isolate has a familial relationship with *Streptomyces rochei* Berger et al. 1953 strain NRRL B-1559(NR_116078.1) (NCBI 2022c), having similarity of 99.35%. *S. rochei* strain NRRL B-1559 was also isolated from the soil and grew at 28°C. BR1-18 isolate has a familial relationship with BR1-19, and both are closely related to *Streptomyces fradiae* (Waksman & Curtis 1916) Waksman & Henrici 1948 strain NBRC 12773 (NR_112270.1) (NCBI 2022a), each of them has the similarity of 96.87% and 95.15%, respectively. *S. fradiae* strain NBRC 12773 was isolated from the soil and grew at 28°C. BR1-35 isolate is a sister clade from *Streptomyces zhihengii* Huang et al. 2017 strain YIM T102 (NR_146828.1) (NCBI 2022d). In addition, being a similarity of 97.02% with *S. zhihengii* strain YIM T102 include mesophilic bacteria isolated from the soil of rhizosphere *Psammosilene tunicoides* W.C.Wu & C.Y.Wu. These findings suggest a close link between the *Streptomyces* from the soil and the beach sand. This shows that *Streptomyces* is widespread across a variety of environments.

A previous study found six alkaliphilic *Streptomyces* strains isolated from four different sites along a transect of a beach and dune sand system of 60 meters. The six strains shared more than 99% of their 16S rRNA gene sequences with representative strains of *Streptomyces griseus* I and one another (Antony-Babu et al. 2008). Another study found one isolate (InaCC A765) of three Actinomycetes isolates from a sand beach in Pramuka Island, Kepulauan Seribu, Jakarta, Indonesia, had antimicrobial activity

against *S. aureus* ATCC 6538, *B. subtilis* BTCC B-612, and *E. coli* BTCC B-614, with zones of inhibition measuring 20, 18 and 20 mm, respectively. InaCC A765 is closely related to *Nocardia otitidiscaviarum* Snijders 1924 strain NBRC 14405 (MN826189); it has a similarity of 96.06% (Setiawati et al. 2021).

Among five isolates, BR1-18 isolate and BR1-19 isolate can be considered a new species since it has similarity under 98.65%. According to Kim et al. (2014), an unknown isolate can be considered one species with the reference species if it has a similarity level of ≥98.65%. Kusuma et al. (2020) found a novel *Streptomyces* species, namely *Streptomyces harenosi* sp. nov., isolated from Indonesian sand dune soil collected at Parangkusumo, Yogyakarta Province, Java, Indonesia. *Streptomyces marincola* sp. nov. is proposed as a unique species name comprising two *Streptomyces* isolates, SCSIO 64649^T (isolated from *Favites* sp. from the South China Sea) and SCSIO 03032 (isolated from deep-sea sediment from the Bay of Bengal in the Indian Ocean). Thirty-seven potential biosynthetic gene clusters are encoded by strains SCSIO 64649^T and SCSIO 03032, and their metabolic crude extract exhibited potent antibacterial action (Shi et al. 2022). The studies demonstrated the potential of *Streptomyces* isolates from sandy beaches and the sea as a novel species.

ACKNOWLEDGEMENTS

The authors would like to thank the Indonesian Ministry of Research, Technology, and Higher Education, for providing a research fund with "*Penelitian Dasar Unggulan Perguruan Tinggi*" grant number 80.1/A.3-III/LPPM/V/2021, 18th Mart 2021. We are also grateful to Mr. Muhammad Miftahussurur for his assistance in analyzing the 16S rRNA gene sequence data.

REFERENCES

Ahmad MS, El-Gendy AO, Ahmed RR, Hassan HM, El-Kabbany HM, Merdash AG. 2017. Exploring the antimicrobial and antitumor potentials of *Streptomyces* sp. AGM12-1 isolated from Egyptian soil. Front Microbiol 13 (8): 438. DOI: 10.3389/fmicb.2017.00438.

Al-Ansari M, Alkubaisi NP, Vijayaragavan KM. 2019. Antimicrobial potential of *Streptomyces* sp. to the Gram positive and Gram negative pathogens. J Infect Public Health 12 (6): 861-866. DOI: 10.1016/j.jiph.2019.05.016.

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. 1990. Basic local alignment search tool. J Mol Biol 215: 403-410. DOI: 10.1016/S0022-2836(05)80360-2.

Ambarwati A, Azizah TS, Sembiring L, Wahyuono S. 2012a. Uji aktivitas antifungi isolat actinomycetes yang berasosiasi dengan rizosfer padi (*Oriza sativa*). Jurnal Kesehatan 5 (2): 139-148. [Indonesian]

Ambarwati A, Sembiring L, Soegihardjo CJ. 2012b. Antibiotic produced by Streptomycetes associated with rhizosphere of purple nut sedge (*Cyperus rotundus* L.) in Surakarta, Indonesia. Afr J Microbiol Res 6 (1): 52-57. DOI: 10.5897/AJMR11.832.

Ambarwati A, Wahyuono S, Moeljopawiro S, Sembiring L, Yuwono T. 2019. Antimicrobial activities and phylogenetic study of bacteria associated with *Cyperus rotundus* rhizosphere from Cemoro Sewu Plateau, Indonesia. Biodiversitas 20 (8): 2206-2212. DOI: 10.13057/biodiv/d200814.

Ambarwati A, Wahyuono S, Moeljopawiro S, Yuwono T. 2020. Antimicrobial activity of ethyl acetate extracts of *Streptomyces* sp. CRB46 and the prediction of their bioactive compounds chemical structure. Biodiversitas 21 (7): 3380-3390. DOI: 10.13057/biodiv/d210763.

Antony-Babu, Stach J, Goodfellow M. 2008. Genetic and phenotypic evidence for *Streptomyces griseus* ecovars isolated from a beach and dune sand system. Antonie van Leeuwenhoek. 94: 63-74. DOI: 10.1007/s10482-008-9277-4.

Beiranvand M, Amin M, Hashemi-Shahraki A, Romani B, Yaghoubi S, Sadeghi P. 2017. Antimicrobial activity of endophytic bacterial populations isolated from medical plants of Iran. Iran J Microbiol 9 (1): 11-18.

Bhardwaj A, Chaman S, Verma S. 2017. Production of antibacterial agent from fungi isolated from pharmaceutical soil sample by fermentation under optimized conditions. Asian J Pharm Clin Res 10 (7): 110-115. DOI: 10.22159/ajpcr.2017.v10i7.18258.

Burhamzah R, Djide M, Rante H, Elmi, Z. 2016. Isolation and screening of antimicrobial-producing Actinomycetes from marine sediment of Galesong Coast, Indonesia. Asian J Microbiol Biotech Env Sci 18 (1): 31-34.

Charousoviá I, Javoreková S, Wink J. 2015. Isolation and characterization of *Streptomyces rishiriensis* (VY31) with antibiotic activity against various pathogenic microorganisms. J Microbiol Biotechnol Food Sci 04 (Special 01): 23-27. DOI: 10.15414/jmbfs.2015.4.special1.23-27.

Dalisay DS, Williams DE, Wang XL, Centko R, Chen J, Andersen RJ. 2013. Marine sediment-derived *Streptomyces* bacteria from british Columbia, Canada are a promising microbiota resource for the discovery of antimicrobial natural products. PLoS ONE 8 (10) e77078. DOI: 10.1371/journal.pone.0077078.

El-Hadedy DE, El-Sayed ME, Saad MM. 2015. *Streptomyces lavendulae* protease inhibitor: Purification, gene overexpression, and 3-dimensional structure. J Chem Article 2015: 963041. DOI: 10.1155/2015/963041.

Fatmawati U, Meryandini A, Nawangsih AA, Wahyudi AT. 2019. Screening and characterization of actinomycetes isolated from soybean rhizosphere for promoting plant growth. Biodiversitas 20 (10): 2970-2977. DOI: 10.13057/biodiv/d201027.

Gislin D, Sudarsanam D, Raj GA, Baskar K. 2018. Antibacterial activity of soil bacteria isolated from Kochi, India and their molecular identification. J Genet Eng Biotechnol 16 (2): 287-294. DOI: 10.1016/j.jgeb.2018.05.010.

Guerrero-Garzón JF, Zehl M, Schneider O, Ruckert C, Busche T, Kalinowski T, Bredholt H, Zotchev SB. 2020. *Streptomyces* spp. from the marine sponge *Antho dichotoma*: Analyses of secondary metabolite biosynthesis gene clusters and some of their products. Front Microbiol 11: 437. DOI: 10.3389/fmicb.2020.00437.

Hamed MM, Abdrabo MAA, Fahmy NM, Abdelfattah LS, Kelany MS, Abd-El latif HH, Abou El ela GM, Abd-Elnaby HM, Hassan SWM. 2021. Distribution and characterization of Actinomycetes in mangrove habitats (Red Sea, Egypt) with special emphasis on *Streptomyces mutabilis* M3MT483919. J Pure Appl Microbiol 15 (1): 246-261. DOI: 10.22207/JPAM.15.1.19.

Kamjam M, Sivalingam P, Deng Z, Hong K. 2017. Deep sea Actinomycetes and their secondary metabolites. Front. Microbiol 8: 760. DOI: 10.3389/fmicb.2017.00760.

Kim M, Oh HS, Park SC, Chun J. 2014. Towards a taxonomic coherence between average nucleotide identity and 16S rRNA gene sequence similarity for species demarcation of prokaryotes. Intl J Syst Evol Microbiol 64 (2): 346-351. DOI: 10.1099/ijs.0.059774-0.

Kumar S, Stecher G, Tamura K. 2016. MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. Mol Biol Evol 33 (7): 1870-1874. DOI: 10.1093/molbev/msw054

Kusuma A, Nouioui I, Klenk HP, Goodfellow M. 2020. *Streptomyces harenosi* sp. nov., a home for a gifted strain isolated from Indonesian sand dune soil. Intl J Syst Evol Microbiol 70 (9): 1-10. 10.1099/ijsem.0.004346.

Lee LH, Zainal N, Azman AS, Eng SK, Goh BH, Yin WF, Mutalib NSA, Chan KG. 2014. Diversity and antimicrobial activities of actinobacteria isolated from tropical mangrove sediments in Malaysia. Sci World J 2014: 698178. DOI: 10.1155/2014/698178.

Leonita S, Bintang M, Pasaribu FH. 2015. Isolation and identification of endophytic bacteria from *Ficus variegata* Blume as antibacterial compounds producer. Biosaintifika 10 (3): 546-552. DOI: 10.29244/cb.2.3.116-128.

Magarvey NA, Keller JM, Bernan V, Dworkin M, Sherman DH. 2004. Isolation and characterization of novel marine-derivated Actinomycete taxa rich in bioactive metabolites. Appl Environ Microbiol 70 (12): 7520-7529. DOI: 10.1128/AEM.70.12.7520-7529.2004.

Mariastuti HD, Listiyowati S, Wahyudi AT. 2018. Antifungal activity of soybean rhizosphere actinomycetes producing bioactive compounds against *Fusarium oxysporum*. Biodiversitas 19 (6): 2127-2133. DOI: 10.13057/biodiv/d190619.

Mohammed A, Seid ME, Gebrecherkos T, Tiruneh M, Moges F. 2017. Bacterial isolates and their antimicrobial susceptibility patterns of wound infections among inpatients and outpatients attending the university of Gondar Referral Hospital, Northwest Ethiopia. Intl J Microbiol 2017: 8953829. DOI: 10.1155/2017/8953829.

Mohan YJ, Sirisha B, Prathyusha KR, Rao PS. 2014. Isolation, screening and characterization of Actinomycetes from marine sediments for their potential to produce antifungal agents. Glob J Biol Agric Health Sci 3 (4): 131-137.

Moi MY, Kusdiyantini E, Pujiyanto S. 2018. Endophytic bacteria from Faloak plant seed (*Sterculia comosa*) as antibacterial agent. J Pure Appl Microbiol 12: 1473-1481. DOI: 10.15294/biosaintifika.v10i3.15361.

Muthuraj R, Bhat MP, Dhanyakumara SB, Halaswamy HM, Shashiraj KN, Chakraborty B, Pallavi SS, Nayaka S. 2021. Isolation, identification and characterization of antimicrobial activity exhibiting Actinomycete *Streptomyces paradoxus* Strain KUASN-7 from Soil. Intl J Curr Microbiol Appl Sci 10 (8): 164-176. DOI: 10.20546/ijcmas.2021.1008.021.

Nayaka S, Dandin CJ, Girish BK, Hospet R, Pallavi SS, Chakaraborty B. 2018. Molecular characterization and antimicrobial efficacy of *Streptomyces gancidicus* Strain SN-3 from soil samples. J Microbiol Res 8 (4): 97-102. DOI: 10.5923/j.microbiology.20180804.02.

Nayaka S, Chakaraborty B, Pallavi SS, Meghashyama PB, Dattatraya A, Dhanyakumara SB, Muthuraj R, Halaswamy H, Shashiraj KN, Chaitra M. 2019. Isolation, characterization, and functional group analysis of *Pseudoxanthomonas indica* RSA-23 from rhizosphere soil. J Appl Pharm Sci 9: 101-106. DOI: 10.7324/JAPS.2019.91113

Nayaka S, Shashiraj KN, Chakraborty B, Bhat MP, Pallavi SS, Airodagi D, Halaswamy HM, Muthuraj R, Dhanyakumara SB, Anusha P. 2020a. Antimicrobial and enzymatic potential of *Streptomyces* sp. KAS-1 isolated from the microbiologically unexplored estuary of Kali river ecosystem. Intl J Res Pharm Sci 11 (2):1655-1666. DOI: 10.26452/ijrps.v11i2.2048.

Nayaka S, Hiremath H, Chakraborty B, Swamy PS, Basavarajappa DS, Nagaraja SK, Rudrappa M, Bhat MP, Airodagi D, Murigennavar MS. 2020b. Isolation, *cha*racterization, and identification of *Streptomyces cinereoruber* strain RSA-14. J Appl Biol Biotechnol 8 (04): 001-006. DOI: 10.7324/JABB.2020.80401.

Nayaka S, Muthuraj R, Chakaraborty B, Bhat MP, Pallavi SS, Shashiraj KN, Halaswamy HM, Dhanyakumara SB, Airodagi D, Haged K. 2020c. A potential bioactive secondary metabolites and antimicrobial efficacy of *Streptomyces thermocarboxydus* strain KSA-2, isolated from Kali River, Karwar. Curr Res Microbiol Infect 1 (1): 5-13. DOI: 10.31559/CRMI2020.1.1.2.

NCBI (National Centre for Biotechnology Information). 2022a. *Streptomyces fradiae* strain NBRC 12773 16S ribosomal RNA, partial sequence. https://www.ncbi.nlm.nih.gov/nuccore/NR_112270.1

NCBI (National Centre for Biotechnology Information). 2022b. *Streptomyces griseoincarnatus* strain JCM 4381 16S ribosomal RNA gene, partial sequence. https://www.ncbi.nlm.nih.gov/nuccore/AY999749.1

NCBI (National Centre for Biotechnology Information). 2022c. *Streptomyces rochei* strain NRRL B-1559 16S ribosomal RNA, partialsequence. https://www.ncbi.nlm.nih.gov/nuccore/NR_116078.1

NCBI (National Centre for Biotechnology Information). 2022d. *Streptomyces zhihengii* strain YIM T102 16S ribosomal RNA, partial sequence. https://www.ncbi.nlm.nih.gov/nuccore/NR_146828.1

Nedialkova D, Naidenova M. 2005. Screening the antimicrobial activity of Actinomycetes strains isolated from antarctica. J Cult Collect 4: 29-35.

Osama N, Bakeer W, Raslan M, Soliman HA, Abdelmohsen UR, Sebak M. 2022. Anticancer and antimicrobial potential of five soil *Streptomycetes*: a metabolomics-based study. Royal Soc Open Sci 9 (2): 211509. DOI: 10.1098/rsos.211509.

Ouchari L, Boukeskasse A, Bouizgarne B, Ouhdouch Y. 2019. Antimicrobial potential of actinomycetes isolated from the unexplored hot merzouga desert and their taxonomic diversity. Biol Open 8 (2): bio035410. DOI: 10.1242/bio.035410.

Paderog MJV, Suarez AFL, Sabido EM, Low ZJ, Saludes JP, Dalisay DS. 2020. Anthracycline shunt metabolites from philippine marine sediment-derived *Streptomyces* destroy cell membrane integrity of multidrug-resistant *Staphylococcus aureus*. Front Microbiol 11: 743. DOI: 10.3389/fmicb.2020.00743.

Patil R, Jeyaskaran G, Shanmugan SA. 2016. Occurrence and antibacterial activity of Actinomycetes isolated from marine samples from Thoothukkudi, Tamil Nadu, on the east coast of India, against fish pathogens. Intl J Adv Biol Res 6 (3): 338-347.

Priyadarshini A, Singdevsachan SK, Tripathy SK, Mohanta YK, Patra JK, Sethi S. 2016. Isolation and identification of Actinomycetes from mangrove soil and extraction of secondary metabolites for antibacterial activity. British Biotechnol J 12 (2): 1-13. DOI: 10.9734/BBJ/2016/24102

Retnowati Y, Sembiring L, Moeljopawiro S, Djohan TS, Soetarto ES. 2017. Diversity of antibiotic-producing Actinomycetes in mangrove forest of Torosiaje, Gorontalo, Indonesia. Biodiversitas 18 (4): 1453-1461. DOI: 10.13057/biodiv/d180421.

Retnowati Y, Moeljopawiro S, Djohan TjS, Soetarto ES. 2018. Antimicrobial activities of actinomycetes isolates from rhizospheric soil on different mangrove forests of Torosiaje, Gorontalo, Indonesia. Biodiversitas. 19 (6): 2196-2203. DOI: 10.13057/biodiv/d190627.

Ribeiro I, Girão M, Alexandrino DAM, Ribeiro T, Santos C, Pereira F, Mucha AP, Urbatzka R, Leão PN, Carvalho MF. 2020. Diversity and bioactive potential of Actinobacteria isolated from a coastal marine sediment in northern Portugal. Microorganisms 8: 1691. DOI: 10.3390/microorganisms8111691.

Sabido EM, Tenebro CP, Suarez AFL, Ong SDC, Trono DJVL, Amago DS, Evangelista JE Jr, Reynoso AMQ, Villalobos IGM, Alit LDD, Surigao CF, Villanueva CA, Saludes JP, Dalisay DS. 2020. Marine sediment-derived *Streptomyces* strain produces angucycline antibiotics against Multidrug-Resistant *Staphylococcus aureus* harboring SCC*mec* type 1 gene. J Mar Sci Eng 8 (10): 734. DOI: 10.3390/jmse8100734.

Saitou N, Nei M. 1987. The neighbor-joining method: A new method for reconstructing phylogenetic trees. Mol Biol Evol 4 (4): 406-425.

Saraylou M, Ghomsheh H, Enayatizamir N, Angzan N, Senn S. 2021. Some plant growth promoting traits of *Streptomyces* species isolated from various crop rhizospheres with high root colonization ability of spinach (*Spinacia oleracea* L.). Appl Ecol Environ Res 19: 3069-3081. DOI: 10.15666/aeer/1904_30693081.

Ser HL, Tan LT, Palanisamy UD, Malek SNA, Yin WF, Chan KG, Goh BH, Lee LH. 2016. *Streptomyces antioxidans* sp. nov., a novel mangrove soil actinobacterium with antioxidative and neuroprotective potentials. Front Microbiol 16: 899. DOI: 10.3389/fmicb.2016.00899.

Setiawati S, Nuryastuti T, Sholikhak EN, Lisdiyanti P, Pratiwi SUT, Sulistiyanti TR, Ratnakomala S, Jumina, Mustofa. 2021. The potency of Actinomycetes extracts isolated from Pramuka Island, Jakarta, Indonesia as antimicrobial agents. Biodiversitas 22: 1104-1111. DOI: 10.13057/biodiv/d220304.

Shi S, Cui L, Zhang K, Zeng Q, Li Q, Ma L, Long L, Tian X. 2022. *Streptomyces marincola* sp. nov., a novel marine Actinomycete, and its biosynthetic potential of bioactive natural products. Front Microbiol 13: 860308. DOI: 10.3389/fmicb.2022.860308.

Siddharth S, Vittal RR. 2018. Evaluation of antimicrobial, enzyme inhibitory, antioxidant and cytotoxic activities of partially purified volatile metabolites of marine *Streptomyces* sp.S2A. Microorganisms 6 (3): 72. DOI: 10.3390/microorganisms6030072.

Sivalingam P, Hong K, Pote J, Prabakar K. 2019. Extreme environment *Streptomyces*: Potential sources for new antibacterial and anticancer drug leads. Intl J Microbiol 2019: 5283948. DOI: 10.1155/2019/5283948.

SuPei, WangDe-Xiang, DingShao-Xiong, ZhaoJing. 2014. Isolation and diversity of natural product biosynthetic genes of cultivable bacteria associated with marine sponge *Mycale* sp. from the coast of Fujian, China. Canadian J Microbiol 60 (4): 217-225. DOI: 10.1139/cjm-2013-0785.

Syed CS, Mounika PPN, Mounika Y, Kumar SS, Bai VT, Audipudi AV. 2017. Evaluation of antimicrobial and antibiotic sensitivity of Chilli root endophytic bacteria for ecofriendly Biofertilizer. Intl J Curr Microbiol App Sci 5: 45-53.

Tenebro CP, Trono DJVL, Vicera CVB, Sabido EM, Ysulat JA, Macaspac AJM, Tampus KA, Fabrigar TAP, Saludes JP, Dalisay DS. 2021. Multiple strain analysis of *Streptomyces* species from Philippine

marine sediments reveals intraspecies heterogeneity in antibiotic activities. Sci Rep 11: 17544. DOI: 10.1038/s41598-021-96886-4.

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG. 1997. The CLUSTAL_X windows interface: Flex- ible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res 25: 4876-4882. DOI: 10.1093/nar/25.24.4876.

Vishwanatha BT, Babu KG, Padmashri P, Malagi SB, Dandin CJ, Nayaka S. 2017. Isolation, identification and characterization of *Streptomyces* sp. SN-2. Biosci Biotechnol Res Asia 14 (4): 1401-1407. DOI: 10.13005/bbra/2585.

Wahyudi AT, Priyanto JA, Fijrina HN, Mariastuti HD, Nawangsih AA. 2019. *Streptomyces* spp. from rhizosphere soil of maize with potential as plant growth promoter. Biodiversitas 20 (9): 2547-2553. DOI: 10.13057/biodiv/d200916.

Wulandari W, Rahayu T. 2015. Aktivitas antibakteri isolat actinomycetes dari sampel pasir Gunung Merapi dengan lama fermentasi yang berbeda terhadap bakteri *Escherichia coli* multiresisten antibiotik. Bioeksperimen 1 (2): 53-59. DOI: 10.23917/bioeksperimen.v1i2.878.

Yuwantiningsih S, Margino S, Wahyuono S. 2015. Identification of antibiotic producing endophytic microbe isolates from a national park in Java island. Intl J Biotechnol 20 (2): 169-176. DOI: 10.22146/ijbiotech.24345.