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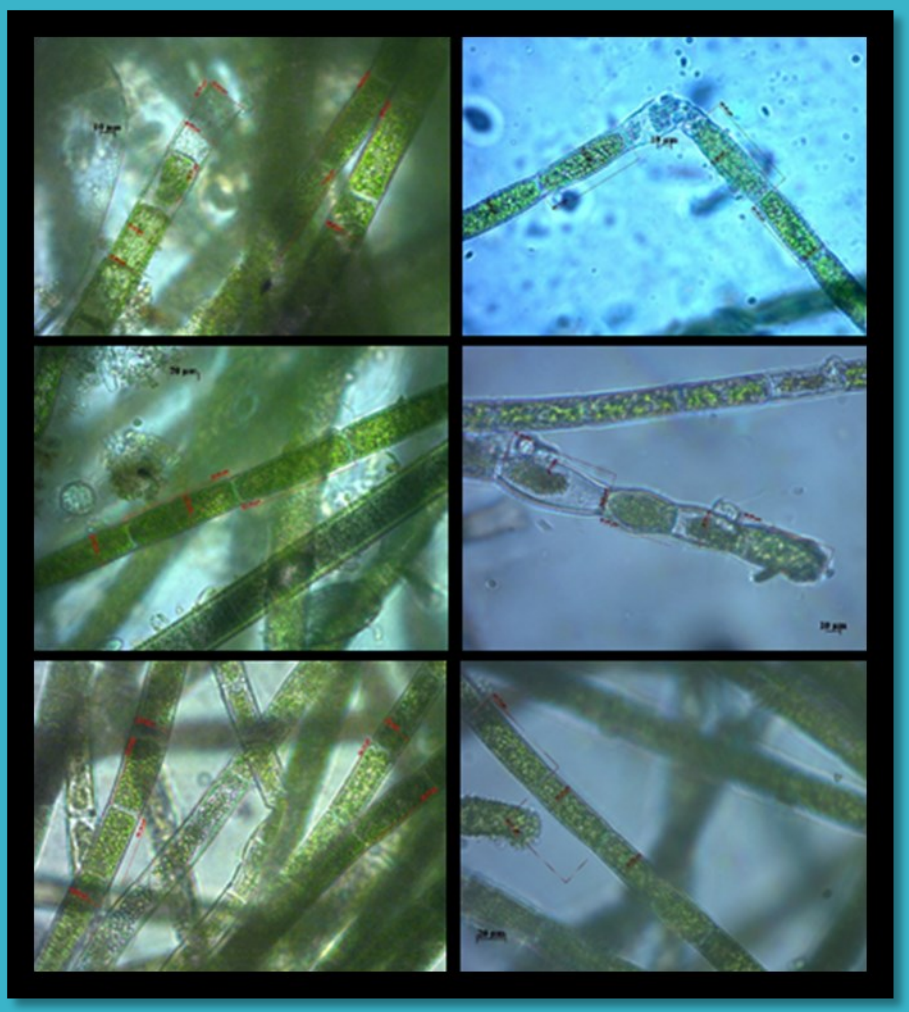

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Alamat (*Address*)
Pusat Penelitian Biologi-LIPI
Kompleks Cibinong Science Center (CSC-LIPI)
Jalan Raya Jakarta-Bogor KM 46,
Cibinong 16911, Bogor-Indonesia
Telepon (021) 8765066 - 8765067
Faksimili (021) 8765059
Email: berita.biologi@mail.lipi.go.id
jurnalberitabiologi@yahoo.co.id
jurnalberitabiologi@gmail.com

Keterangan foto cover depan (*Notes of cover picture*): Pertumbuhan *Oedogonium* sp. pada perlakuan cahaya yang berbeda. *Oedogonium* sp. Pada kultur Outdoor tampak lebih padat daripada kultur indoor, sesuai dengan halaman 309 (*Growth of Oedogonium sp. at different light treatments. Oedogonium* sp *in outdoor culture appeared denser than in indoor culture, as in page 309)*

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SELECTIVE ISOLATION OF *Dactylosporangium* AND *Micromonospora* FROM THE SOIL OF KARST CAVE OF SIMEULUE ISLAND AND THEIR ANTIBACTERIAL POTENCY

[Isolasi Selektif *Dactylosporangium* dan *Micromonospora* dari Tanah Gua Karst Pulau Simeulue dan Potensinya Sebagai Antibakteri]

Ade Lia Putri* ✉ **and I Nyoman Sumerta**

Mikrobiologi Division, Research Center for Biology-LIPI
Jl. Raya Jakarta-Bogor Km. 46, Cibinong, Bogor 16911
email: adelia.rikardi@gmail.com

ABSTRACT

Karst is a unique ecosystem that consists of a thin soil layer on the carbonate rocks. It has a diverse microorganism, especially actinomycetes group, which might potentially produce beneficial secondary metabolites that remain unknown. In this study, we were interested in isolating *Dactylosporangium* and *Micromonospora* that have been potentially reported as antibiotic sources. We used two methods using the chemical germicide (pretreatment 1.5% phenol) compared with the SDS-YE (0.05% SDS pretreatment). Thirty-nine isolates actinomycetes were successfully isolated from two samples of the karst cave soils. Thirteen isolates were obtained by using the germicide chemical method and closely related to the genera of *Dactylosporangium* and *Micromonospora*. On the other hand, the SDS-YE grew twenty-six isolates which closely related to 11 genera of actinomycetes (*Catenulispora, Nocardia, Rhodococcus, Ornithinimicrobium, Catellatospora, Dactylosporangium, Micromonospora, Streptacidiphilus, Streptomyces, Nonomuraea,* and *Streptosporangium*). These results suggest that 1.5% of phenol pretreatment could preserve the genera of *Dactylosporangium* and *Micromonospora,* while the *Streptomyces* and other rare actinomycetes were killed. Among all isolates, only seven showed the antibacterial activity on tested bacteria. Even though the antibacterial activity of those isolates was not high, the isolation of actinomycetes from a specific substrate is necessary to be conducted on exploring the richness of our natural resources.

Keywords: Actinomycetes, *Dactylosporangium, Micromponaspora,* phenol, karst

ABSTRAK

Karst adalah sebuah ekosistem yang terbentuk dari batuan karbonat yang dilapisi lapisan tanah yang tipis. Ekosistem ini memiliki microorganisme yang beragam, khusunya aktinomisetes yang mungkin berpotensi dalam menghasilkan metabolit sekunder bermanfaat yang belum diketahui khasiatnya. Pada penelitian ini, kami tertarik untuk mengisolasi genus *Dactylosporangium* dan *Micromonosporas* yang pernah dilaporkan berpotensi sebagai sumber antibiotik. Kami menggunakan dua metode yaitu metode kimia germisida (pra-perlakuan 1,5% fenol) dan dibandingkan dengan metode SDS-YE (pra-perlakuan 0,05% SDS). Tiga puluh sembilan isolat aktinomisetes berhasil diisolasi dari dua sampel tanah gua karst. Tiga belas isolat diperoleh dengan metode kimia germisida yang berhubungan dekat dengan dua marga yaitu *Dactylosporangium* dan *Micromonospora.* Disisi lain, SDS-YE menumbuhkan sebanyak 26 isolat yang termasuk kedalam 11 marga aktinomisetes (*Catenulispora, Nocardia, Rhodococcus, Ornithinimicrobium, Catellatospora, Dactylosporangium, Micromonospora, Streptacidiphilus, Streptomyces, Nonomuraea,* dan *Streptosporangium*). Hasil ini menggambarkan bahwa pra-perlakuan fenol 1,5% dapat mempertahankan marga *Dactylosporangium* dan *Micromonospora* sedangkan *Streptomyces* dan rare aktinomistes lainnya tidak dapat tumbuh. Dari keseluruhan isolat, hanya tujuh yang memiliki aktivitas antibakteri. Walaupun kemampuan isolat tersebut dalam menghambat pertumbuhan bakteri uji tidak tinggi, isolasi aktnomisetes dari substrat spesifik sangat dibutuhkan untuk mengungkap kekayaan sumber daya alam.

Kata kunci: aktinomisetes, *Dactylosporangium, Micromponaspora,* fenol, karst

INTRODUCTION

Karst is a unique ecosystem that consists of thin soil layering the carbonate rocks, such as dolomite and limestone. It has vulnerable resources providing many species habitats and also generating ecosystem services in carbon cycle (Goldscheider, 2019). The key role of ecosystem services in karst is driven by microorganisms that improve the soil properties, drive vegetation succession (Zhu *et al.,* 2012), and then become a restoration indicator (Hu *et al*., 2016). The microbial activity in karst is closely related to the karstification process altering the karst dynamic and stability (Lian, Yuan, and Liu, 2011).

Many researches have been done on the microbial community, which has a role in karst ecosystem services. Soil microbes in karst are restricted by carbon and phosphorus (Chen *et al.,* 2019) to control the pH by the acidification process, so that implies to their diversity (Yun *et al*., 2016). As a diverse organism, the status of microbial diversity of karst could become a biomonitoring process of groundwater (Pronk *et al.,* 2009), and microbes constructing biofilm in the cave play role as a biogenic process of the karst ecosystem (Borsodi

*Kontributor Utama
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et al., 2012). Meanwhile, for commercial use, Moreno *et al.* (2013) report that microbes in the cave of karst might potentially produce beneficial secondary metabolites for a human that remain unknown (Moreno *et al*., 2013). Therefore, we were interested in isolating these microbes, especially in the actinomycetes group, and screening for their antimicrobial activity.

The *Streptomyces* is the predominant member of actinomycetes used as a source antibiotic. The search for novel antibiotics and other bioactive compounds is important against pathogens. By isolation and characterization of novel actinomycetes from unique natural habitat, a novel metabolite and antibacterial ability could be obtained. In this study, we would like to explore the specific genera of *Dactylosporangium* and *Micromonospora* from the soils cave of karst. These genera are rare Actinobacteria that have a very robust function in medicine (Hirsch and Valdés, 2010; Tiwari and Gupta, 2012). They are usually used for antibiotic production and become a potential source for new drug discovery (Hirsch and Valdés, 2010; Tiwari and Gupta, 2012).

In the conventional isolation techniques, most of the isolates recovered on agar plates have been identified as genus *Streptomyces.* By simulating a new performed process, the pretreatment process in soil samples with chemicals such as phenol, the rare actinomycetes which are *Dactylosporangium* and *Micromonospora* could be obtained. This method has been used to selected genus *Dactylosporangium* from soil (Chiaraphongphon *et al.,* 2010; Thawa and Suriyachadkun, 2013; Thawai *et al.,* 2018) and genus *Micromonaspora* (Hayakawa *et al.,* 1991). Using appropriate isolation methods, we expect that the *Dactylosporangium* and *Micromonospora* from the soil of karst in Simeulue Island, Province of Aceh are isolated and screened of actinomycetes antibacterial activity.

MATERIALS AND METHODS

Sampling and pretreatment of soil

Two soil samples were collected from the Karst area, located Air Pinang village, Simeulue Island, Province of Aceh, Indonesia (Latitude: N: 02° 34'40.2", Longitude: E: 96°15'32.1"). These samples were stored in sterile plastic bags and kept at 4°C before transferring into the laboratory for isolation. The soil samples were air-dried for seven days at room temperature. Then, the soils were grounded with mortar and pestle. To exclude large mineral and organic particles, the soil samples were sieved.

Selective isolation of actinomycetes

1. SDS-YE method

One gram of each sample was treated with 6% yeast extract and 0.05% sodium dodecyl sulfate (SDS) (Hayakawa and Nonomura, 1989). Serial dilution was aseptically carried out up to 10^{-6}. One hundred µl of each diluted suspension was spread onto the plates of Humic Acid Vitamin (HV) agar medium supplemented with cycloheximide (50 µg/L) and nalidixic acid (20 µg/L) (Hayakawa and Nonomura, 1987). The plates were incubated at 30°C for 14-21 days.

2. Chemical germicide method (pretreatment with 1.5% phenol)

Each soil sample was prepared by mixing 1 g of soil into 9 ml of distilled water. The suspension was vigorously stirred for 10 minutes at room temperature. About 1 mL of supernatant was transferred into 9 mL of 1.5% phenol solution. Then, the mixture was maintained at 30°C for serial dilution up to 10^{-6} for the next step. After incubation for 30 minutes, 100 µl of the mixture was spread onto the surface of the HV agar supplemented with cycloheximide (50 µg/L) and nalidixic acid (20 µg/L) plates (Hayakawa *et al*., 1991). When the single colony grows, it was regrown at 30°C on the YSA medium for purification. Subsequently, the purified isolates were preserved and kept in 10% glycerol at -80°C as a stock culture.

Molecular identification of 16S rRNA gene sequence and phylogenetic analysis

Actinomycetes isolates were cultured on the YSA medium for 7-14 days. The colony grown on the agar plate was utilized for the he DNA extraction. Firstly, the biomass of actinomycetes was harvested by scraping off the mycelia and the spores of the colony from the YSA medium and put into a microtube for overnight at -20°C incubation. After

incubation, the mycelia and spores of actinomycetes were cleaned three times with distilled water (500 µl). The genomic DNA was then extracted following Franco-Correa *et al.* (2010) method. PCR was performed to amplify the 16S rRNA gene with a set of universal bacterial primers 27F (5' AGAGTTTGA TCMTGGCTCAG 3') and 1492R (5' TAC-GGYTACCTTGTTACGACTT 3') for full length. The PCR condition performed by an initial denaturation at 94°C for 1 minute followed by 30 cycles of amplification (denaturation at 95°C for 30 seconds, annealing at 50°C for 30 seconds, and elongation at 72°C for 1 minute 30 seconds) (Putri *et al.,* 2018). Then, the amplicons were sequenced at Macrogen, Republic of Korea. The sequencing results, AB1 files, were analyzed using the CromasPro program version 1.6 until the full fragments of 16S rRNA gene were obtained. Homology of the obtained sequences was compared in the EzTaxon-e database server (http://eztaxon-e.ezbiocloud.net) (Kim *et al.*, 2012) For phylogenetic tree, we used Molecular Evolution Genetics Analysis (MEGA) software version 6.0 with neighbor-joining method. The sequences were aligned with the reference sequences using Clustal W and the constructed phylogenetic tree was evaluated by bootstrap of neighbor-joining data based on 1000 resampling.

Screening of the actinomycetes isolates for antibacterial activity

The primary screening of the actinomycetes isolates for antibacterial activity against *Bacillus subtilis* (InaCC B1), *Escherichia coli* (InaCC B5), and *Staphylococcus aureus* (InaCC B4) performed by agar overlaid method (Gebreyohannes *et al.,* 2013) with modifications. Firstly, each tested bacteria isolates were prepared by inoculating into Muller-Hinton broth and shaken for 24 hours at 30°C in 120 rpm. Meanwhile, actinomycetes isolates were inoculated into ISP2 agar medium and incubated at 30°C for 7 days. This preculture actinomycetes was used for spot inoculation (6 mm diameter) on Muller -Hinton Agar (MHA) and incubated for 4 days at 30°C. Next, a semisolid MHA medium containing each tested bacteria was overlaid on the top of spot inoculation MHA and incubated for 24 hours at 37°C. The positive results were measured by the clear zone formation around the spot. The ratio of antibacterial activity was calculated by dividing the diameters of the clear zone and spot.

RESULTS

Selective isolation was carried out from two soil samples of the karst cave using the HVA medium. Actinomycetes were preliminarily selected from the HVA medium by morphological examination. Based on the morphological characteristics, they were categorized into the *Streptomyces* group or rare actinomycetes. From this selection, a total of 39 actinomycetes isolates were successfully isolated from 2 soil samples of the karst cave. Twenty-six isolates were isolated using the SDS-YE method, and 13 isolates were isolated using the chemical germicide method. Phenol treatment of soil suspension lowers the number of obtained actinomycetes than SDS-YE treatment. Among 39 isolates, only four isolates produced aerial spore masses on the YSA medium, which were isolated using the SDS-YE method.

16S rRNA gene sequencing analysis

All obtained isolates were identified based on 16S rRNA gene sequencing analysis. The partial 16S rRNA gene sequence analysis revealed diversity among these isolates, which were affiliated with 11 genera in six families. The isolates were belonged to Catenulisporaceae, Nocardiaceae, Intraspo-rangiaceae, Micromonosporaceae, Strepto-mycetaceae, Streptosporangiaceae (Tabel 1). Members of the Streptomyces group were confirmed to belong to the genus *Streptomyces,* while the rare actinomycetes group members were closely related to 10 genera, namely *Catenulispora, Nocardia, Rhodococcus, Ornithinimicrobium, Catellatospora, Dactylosporangium, Micromonospora, Strepta-cidiphilus, Nonomuraea,* and *Streptosporangium.*

Analysis of the gene of 16S rRNA sequences showed that a total of 11 genus actinomycetes were successfully isolated from soil samples using the SDS-YE method. These isolates were *Catellatospora, Catenulispora, Dactylosporangium, Micromono-spora, Nocardia, Nonomuraea, Ornithinimicrobium, Rhodococcus, Streptacidiphilus, Streptomyces,* and

Table 1. The actinomycetes isolates based on 16S rRNA gene sequence analysis (*Kelompok aktinomisetes berdasarkan analisis pemetaan gen 16S rRNA*)

Group	Family	Genus	Total
Rare actinomycetes	Catenulisporaceae	*Catenulispora*	1
	Nocardiaceae	*Nocardia*	2
		Rhodococcus	3
	Intrasporangiaceae	*Ornithinimicrobium*	2
	Micromonosporaceae	*Catellatospora*	2
		Dactylosporangium	7
		Micromonospora	16
	Streptosporangiaceae	*Nonomuraea*	1
		Streptosporangium	1
		Streptacidiphilus	1
Streptomyces	Streptomycetaceae	*Streptomyces*	3
	Total		**39**

Streptosporangium (Tabel 2). The most frequent genus that was found using the SDS-YE method was *Micromonaspora* (seven isolates), followed by *Rhodococcus, Dactylosporangium*, and *Streptomyces*. Only two isolates showed low similarity (< 98%) with their close relatives (Table 3) which were SASS 5(1) and SASS 5(8). Both of those isolates belong to the genus *Ornithinimicrobium* (Table 3). These isolates may represent a putative novel species, which shared only 97.33% and 97.41% similarity with *Ornithinimicrobium flavum* CPCC 203535(T). Phylogenetic analysis based on 16S rRNA gene sequences showed that some isolates clustered in a different branch of the closest genus. The isolates were SASS 3(10), SASS 3(14), SASS 5 (7), SASS 3(6), SASS 3(7), SASS 3(16), SASS 5(4), and SASS 3(3).

Meanwhile, the analysis of the sequences of 16S rRNA from 13 isolates was isolated using 1.5% phenol treatment and affiliated only within two genera. These isolates belong to the genus *Dactylosporangium* and *Micromonospora*. The genus of *Micromonospora* (nine isolates) was predominant, followed by *Dactylosporangium* (four isolates) (Table 3). The similarity values of these isolates were between 98.54% and 99.70%. The molecular identification was supported by the phylogenetic tree analysis based on a neighbor-joining tree. The 13 isolates were affiliation to two genera (*Dactylosporangium* and *Micromonospora*) (Figure 2). Four isolates showed distantly related to other known species of the nearest genus, which are SASG 3(5), SASG 3(3), SASG 5(4), and SASG 5(3). Further, a taxonomical study of these isolates compared with known species is needed.

Antibacterial activity

All of the actinomycetes isolates were preliminary screened their antibacterial ability against three bacteria (presence/absence of inhibition zone). Out of 39 actinomycetes isolates, only 7 isolates (0.18%) showed antibacterial activities against at least one of the tested bacteria (Table 4). Among seven isolates, six isolates inhibited gram-positive bacteria (*S. aureus* or *B. subtilis*), and only one isolate inhibited gram-negative bacteria (*E. coli*). The ratio of inhibition activity was very low from 1.17 to 1.33. Only one isolate could inhibit gram-negative bacteria identified as *Micromonospora rhizosphaerae* with inhibition zone 1.17. Six isolates inhibited at least one tested gram-positive bacteria which were identified as *Streptomyces cinereoruber* subsp. *fructofermentans*, *Catellatospora citrae, Dactylosporangium solaniradicis, Dactylosporangium tropicum, Rhodococcus antrifimi, Micromonaspora rhizosphaerae,* and *Dactylosporangium sucinum.*

Table 2. Actinomycetes isolated using SDS-YE method (*Aktinomisetes yang diisolasi dari metode SDS-YE*)

No	Isolate Code	Genus	Species	Strain No	Sequence Similarity (%)
1	SASS 3(14)	*Catellatospora*	*Catellatospora chokoriensis*	2-25/1(T)	99.70
2	SASS 3(10)		*Catellatospora citrea*	DSM 44097	99.12
3	SASS 5(7)	*Catenulispora*	*Catenulispora rubra*	Aac-30(T)	98.72
4	SASS 5(4)	*Dactylosporangium*	*Dactylosporangium solaniradicis*	NEAU-FJL2(T)	98.96
5	SASS 3(3)		*Dactylosporangium sucinum*	RY35-23	99.05
6	SASS 5(2)		*Dactylosporangium tropicum*	KB2-4(T)	99.26
7	SASS 3(1)	*Micromonospora*	*Micromonospora eburnea*	DSM 44814(T)	99.48
8	SASS 3(13)		*Micromonospora eburnea*	DSM 44814(T)	99.55
9	SASS 3(8)		*Micromonospora rhizosphaerae*	DSM 45431(T)	99.55
10	SASS 3(12)		*Micromonospora rhizosphaerae*	DSM 45431(T)	99.47
11	SASS 3(2)		*Micromonospora schwarzwaldensis*	HKI0641(T)	99.92
12	SASS 3(7)		*Micromonospora yasonensis*	DS3186(T)	99.04
13	SASS 3(16)		*Micromonospora yasonensis*	DS3186(T)	99.04
14	SASS 5(12)	*Nocardia*	*Nocardia anaemiae*	NBRC 100462(T)	99.56
15	SASS 3(11)		*Nocardia harenae*	WS-26(T)	98.79
16	SASS 5(13)	*Nonomuraea*	*Nonomuraea spiralis*	IFO 14097	99.27
17	SASS 5(1)	*Ornithinimicrobium*	*Ornithinimicrobium flavum*	CPCC 203535(T)	97.33
18	SASS 5(8)		*Ornithinimicrobium flavum*	CPCC 203535(T)	97.41
19	SASS 5(5)	*Rhodococcus*	*Rhodococcus antrifimi*	D7-21(T)	99.25
20	SASS 5(9)		*Rhodococcus antrifimi*	D7-21(T)	99.25
21	SASS 5(10)		*Rhodococcus gannanensis*	M1(T)	99.47
22	SASS 5(14)	*Streptacidiphilus*	*Streptacidiphilus rugosus*	AM-16(T)	99.71
23	SASS 3(4)	*Streptomyces*	*Streptomyces cinereoruber subsp. fructofermentans*	NBRC 15396(T)	99.11
24	SASS 3(5)		*Streptomyces cinereoruber subsp. fructofermentans*	NBRC 15396(T)	99.40
25	SASS 3(6)		*Streptomyces seranimatus*	YIM 45720(T)	98.79
26	SASS 5(11)	*Streptosporangium*	*Streptosporangium algeriense*	169(T)	99.05

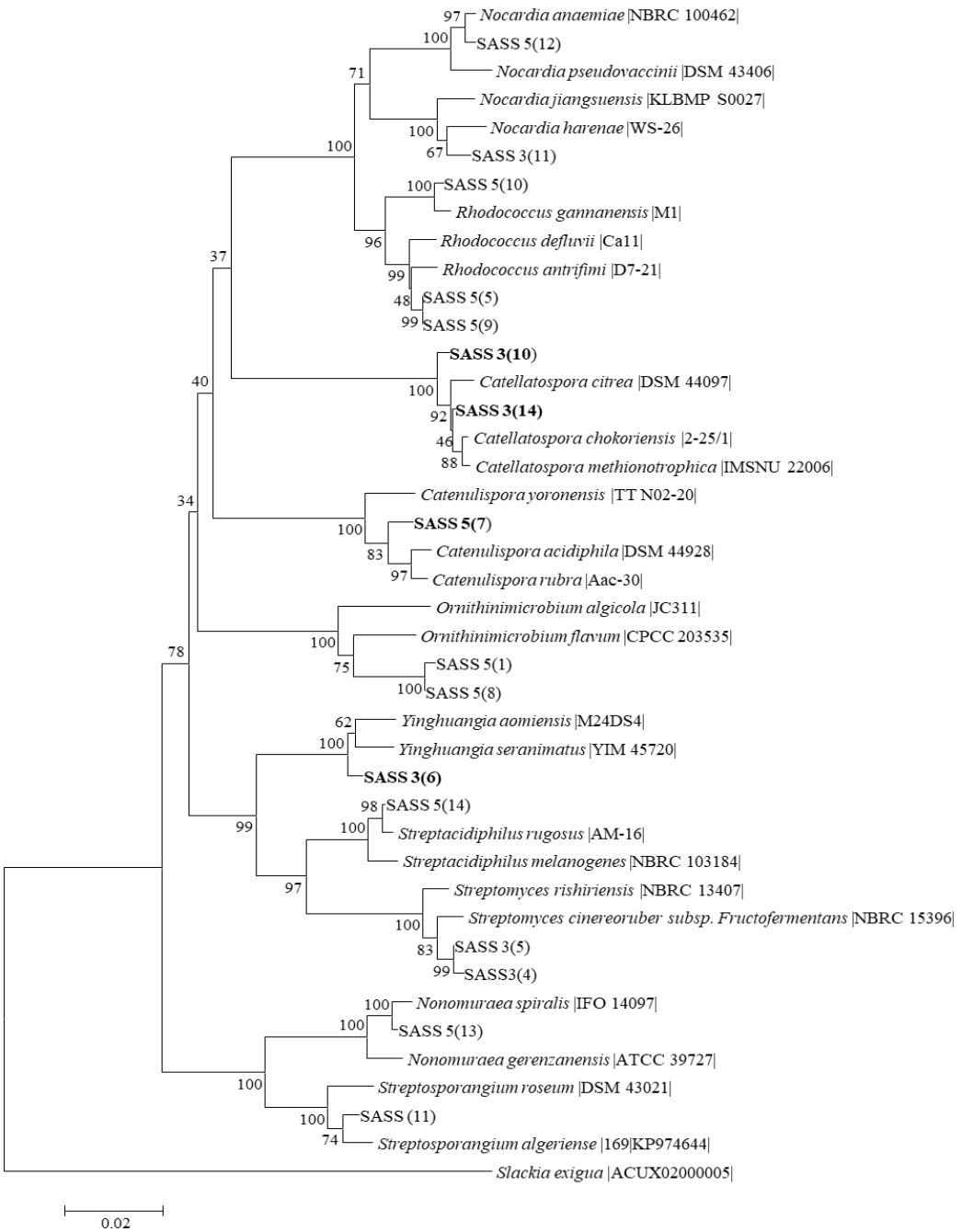

Figure 1. Phylogenetic tree of actinomycetes isolated from soil karst cave using the SDS-YE method and their closely related type strain within the family Catenulisporaceae, Nocardiaceae, Intrasporangiaceae, and Streptosporangiaceae. The bootstrap values are expressed as percentages 1000 replications and the bar represents 0.02 substitutions per nucleotide position. *Slackia exigua* ACUX02000005 was used as an outgroup sequence. (*Pohon filogenetik dari aktinomisetes yang diisolasi dari tanah pada gua karst menggunakan metode SDS-YE dan hubungan dekatnya dengan strain tipe pada famili Catenulisporaceae, Nocardiaceae, Intrasporangiaceae, dan Streptosporangiaceae. Nilai bootstrap dengan replikasi 1000 kali dan sebanyak 0.02 substitusi per posisi nukleotida yang ditunjukkan pada diagram. Slackia exigua ACUX02000005 digunakan sebagai outgroup*)

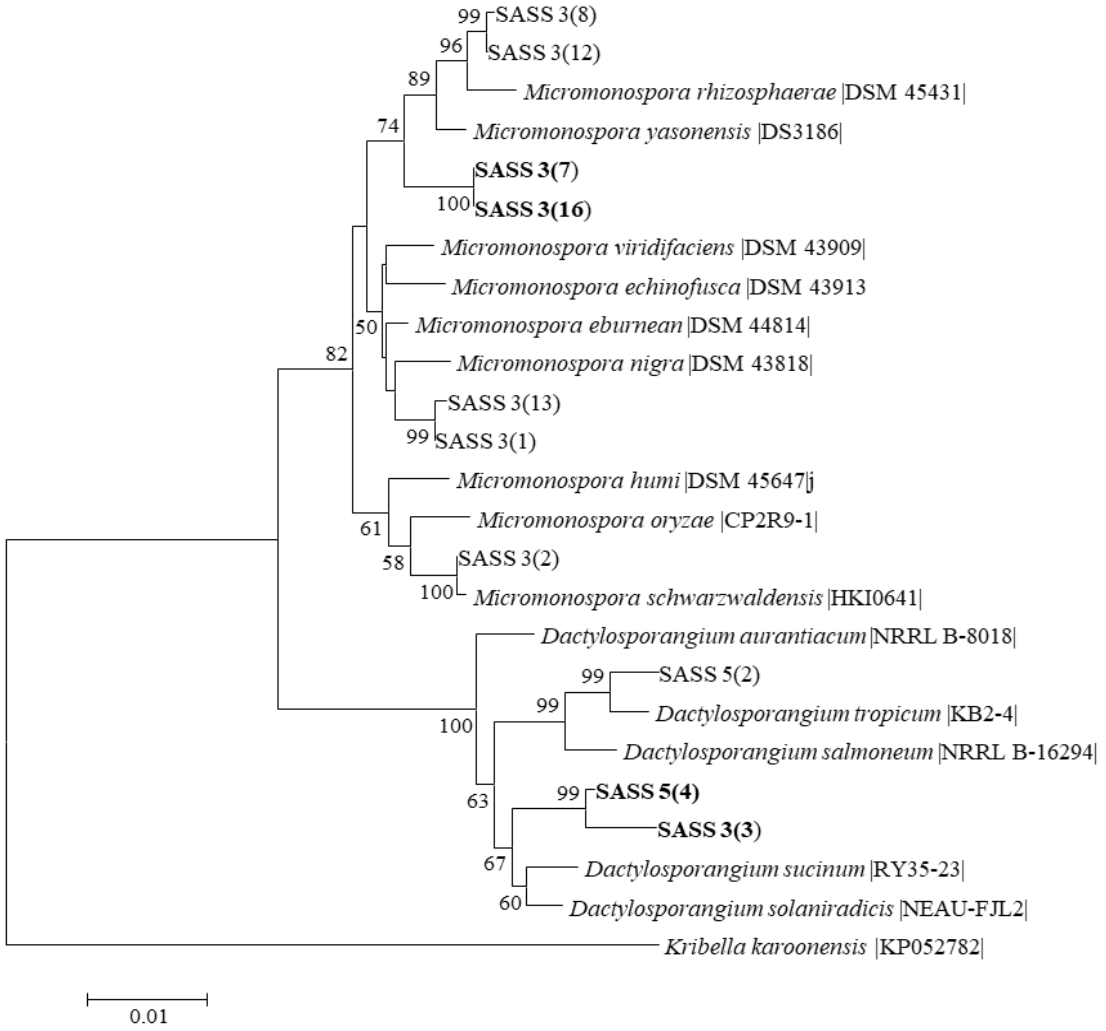

Figure 2. Phylogenetic tree of actinomycetes isolated from soil karst cave using the SDS-YE method and their closely related type strains within the family Micromonasporaceae. The bootstrap values are expressed as percentages 1000 replications. The bar represents 0.01 substitutions per nucleotide position. *Kribella karoonensis* KP052782 was used as an outgroup sequence. (*Pohon filogenetik dari aktinomisetes yang diisolasi dari tanah pada gua karst menggunakan metode SDS-YE dan hubungan dekatnya dengan strain tipe pada famili Micromona-sporaceae. Nilai bootstrap dengan replikasi 1000 kali dan sebanyak 0.01 substitusi per posisi nukleotida yang ditunjukkan pada diagram. Kribella karoonensis KP052782 digunakan sebagai outgroup*)

Table 3. Actinomycetes isolated using 1.5% phenol treatment. (*Aktinomisetes yang diisolasi menggunakan 1.5% phenol*)

No	Isolate Code	Genus	Species	Strain No	Sequence Similarity (%)
1	SASG 5(2)	*Dactylosporangium*	*Dactylosporangium darangshiense*	DLS-44	99.56
2	SASG 5(1)		*Dactylosporangium sucinum*	RY35-23	99.70
3	SASG 5(3)		*Dactylosporangium sucinum*	RY35-23	99.27
4	SASG 5(4)		*Dactylosporangium sucinum*	RY35-23	99.26
5	SASG 3(6)	*Micromonospora*	*Micromonospora eburnea*	DSM 44814	99.56
6	SASG 3(1)		*Micromonospora rhizosphaerae*	DSM 45431	99.33
7	SASG 3(7)		*Micromonospora rhizosphaerae*	DSM 45431	99.49
8	SASG 3(8)		*Micromonospora rhizosphaerae*	DSM 45431	99.49
9	SASG 3(9)		*Micromonospora rhizosphaerae*	DSM 45431	99.20
10	SASG 3(2)		*Micromonospora schwarzwaldensis*	HKI0641	99.63
11	SASG 3(4)		*Micromonospora soli*	SL3-70	99.20
12	SASG 3(3)		*Micromonospora yasonensis*	DS3186	99.05
13	SASG 3(5)		*Micromonospora yasonensis*	DS3186	98.54

DISCUSSION

The growth of actinomycetes is often disadvantaged by other fast-growing microorganisms as actinomycetes are slow-growing microorganism and easily to be contaminated. The suitable culture technique for specific isolation of actinomycetes to reduce the growth of other microbes or selecting particular group could be performed. The culture medium of actinomycetes is one of important part on isolation process. Humic acid vitamin (HV) agar is a well-known culture medium formulated for actinomycetes containing humic acid as the carbon and nitrogen sources (Hayakawa and Nonomura, 1987; Ruttanasutja and Pathom-Aree, 2015). This media supplemented with cycloheximide (50 µg/L) and nalidixic acid (20 µg/L) could inhibit the number of predominant bacterial and fungal contamination (Flora *et al.,* 2015; Hayakawa *et al*., 1991; Naikpatil and Rathod, 2011). On HV, the rare Actinomycetes group as well as *Streptomyces* grow well.

Performing pretreatment on the samples could select the certain species of microbes and minimize the contamination from other microbes (Jiang *et al.,* 2016; Kumar and Jadeja, 2016). Pretreatment using SDS-YE method is particularly for the isolation of general soil actinomycetes which easily grow the actinobacteria. In contrast, phenolic pretreatment could inhibit the growth of some genera actinomycetes and reduce number of non-actinomycetes (Kang *et al*., 2009). In comparison, the number of actinomycetes isolated pretreatment with 0.05% SDS (SDS-YE method) was higher than pretreatment with 1.5% phenol (germicide chemical method). This result is similar to the previous research by Khamna *et al.* (2009).

Pretreatment with 1.5% phenol give a selective result for the obtained actinomycetes group. It could preserve the growth of *Dactylosporangium* and *Micromonaspora,* while *Streptomyces* and other rare actinomycetes were eliminated. Phenol addition could reduce the growth of actinomycetes and bacteria as phenol is toxic to microbial cells and spores of microbes (Ruttanasutja and Pathom-Aree, 2015). This pretreatment has been previously used to select genus *Dactylosporangium* from soil (Chiaraphongphon *et al*., 2010; Thawai *et al*., 2011; Thawa and Suriyachadkun, 2013) and genus *Micromonaspora* (Hayakawa *et al*., 1991). It

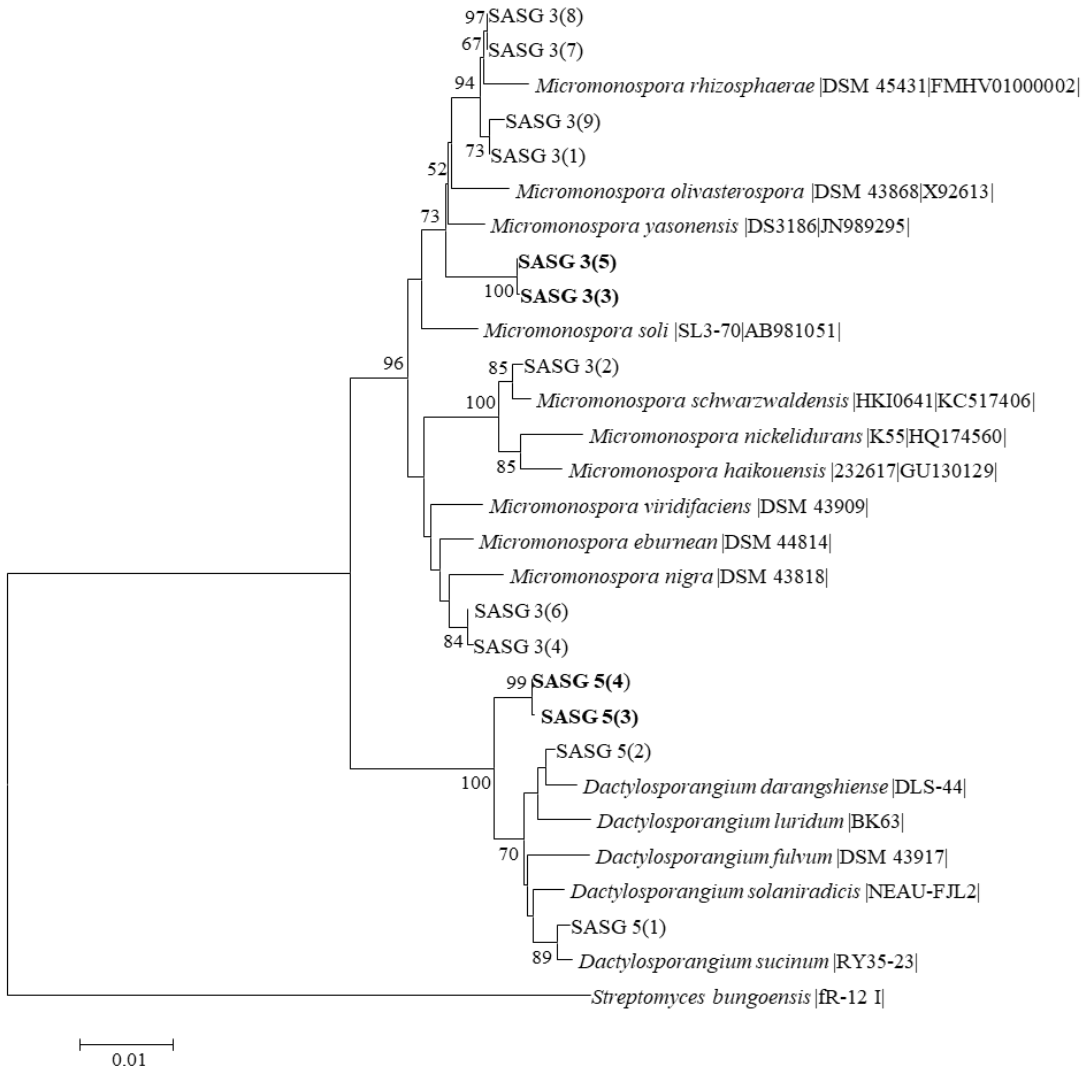

Figure 3. Phylogenetic tree of actinomycetes isolated from soil karst cave using 1.5% phenol pretreatment and their closely related type strains within the family Micromonasporaceae. The bootstrap values are expressed as percentages 1000 replications. The bar represents 0.01 substitutions per nucleotide position. *Streptomyces bungoensis* strain fR-12-I was used as an out-group sequence. (*Pohon filogenetik dari aktinomisetes yang diisolasi dari tanah pada gua karst menggunakan metode SDS-YE dan hubungan dekatnya dengan strain tipe pada famili Micromonasporaceae. Nilai bootstrap dengan replikasi 1000 kali dan sebanyak 0.01 substitusi per posisi nukleotida yang ditunjukkan pada diagram. Streptomyces bungoensis fR-12-I digunakan sebagai outgroup*)

Table 4. The screening results of the positive antibacterial activity from actinomycetes isolated from cave soil of karst. (*Hasil penapisan aktivitas antibakteri yang positif dari isolat aktinomisetes yang diisolasi dari tanah gua karst*)

Isolate code	Identification	Clear zone ratio		
		E. coli	*B. subtilis*	*S. aureus*
SASS 3(5)	*Streptomyces cinereoruber* subsp. *fructofermentans*	-	1.17	-
SASS 3(10)	*Catellatospora citrea*	-	-	1.17
SASS 5(4)	*Dactylosporangium solaniradicis*	-	1.33	1.17
SASS 5(5)	*Rhodococcus antrifimi*	-	1.17	-
SASS 5(2)	*Dactylosporangium tropicum*	-	1.33	-
SASG 3(9)	*Micromonospora rhizosphaerae*	1.17	-	-
SASG 5(4)	*Dactylosporangium sucinum*	-	-	1.17

also has been used to reduce the number of *Streptomyces* colonies on HV agar plates and could be beneficial to obtain other rare actinomycetes (Istianto *et al*., 2012). This method was also successfully used to explore the actinomycetes from tropical limestone caves and Thai coastal marine sediment (Niyomvong *et al*., 2012; Ruttana-sutja and Pathom-Aree, 2015). In this research, phenol pretreatment was successfully applied for isolating *Dactylosporangium* and *Micromonospora* in the soil of karst cave from Simeulue Island and was reported for the first time.

Meanwhile, on antibacterial ability, actinomycetes are known as the potential producer of many diverse metabolites. A combination of selective isolation and the screening process of acti-nomycetes from unexplored habitat is the way for discovering new natural products (Goodfellow and Fiedler, 2010). We selected genera of *Dactylosporangium* and *Micromonaspora* as they have reportedly produced antibiotic (Hirsch and Valdés, 2010; Naikpatil and Rathod, 2011; Tiwari and Gupta, 2012; Kumar *et al*., 2014; Kawuri and Darmayasa, 2019). In this study, we obtained only seven genera of actinomycetes against tested bacteria and particularly against gram -positive bacteria. Our results support previous studies which showed that the most of isolates of actinomycetes had activity against gram-positive bacteria (Charousova *et al*. 2019; Baskaran *et al.,* 2011).

Even though the ability of those isolates against bacteria was limited, isolation of actinomycetes from a specific substrate is necessary to be conducted on exploring the richness of our natural resources. That might be different environment types could produce a different type of secondary metabolites. In addition, actinomycetes diversity and their antibacterial activity may be influenced by the composition of the soil cave of karst since karst consists of thin soil layering the carbonate rocks as most of the microbial activity in karst was closely related to the karstification process altering the karst dynamic and stability (Lian *et al*., 2011).

CONCLUSION

Giving the phenol pretreatment on the isolation process could inhibit the growth of other actinomycetes except for *Dactylosporangium* and *Micromonospora.* Therefore, *a* 0.15% phenol pretreatment is appropriate for the selective isolation method for the genera of *Dactylosporangium* and *Micromonospora* from the cave soil of karst as SDS-YE method obtained more diverse actinomycetes genera. On the other hand, the antibacterial activity of all obtained actinomycetes isolates were low and represented only in seven isolates. Furthermore, the taxonomical study of the isolates and the other potential ability of these isolates were interested in studying in the forthcoming.

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REFERENCES

Baskaran, R., Vijayakumar, R. and Mohan, P.M., 2011. Enrichment method for the isolation of bioactive actinomycetes from mangrove sediments of Andaman Islands, *Indian Journal of Microbiology, 7* (1), pp. 26–32.

Borsodi, A. K., Knáb, M., Krett, G., Makk, J., Márialigeti, K., Eross, A. and Mádl-Szonyi, J., 2012. Biofilm bacterial communities inhabiting the cave walls of the buda thermal karst system, hungary. *Geomicrobiology Journal*, *29*(7), pp. 611–627.

Chen, H., Li, D., Mao, Q., Xiao, K. and Wang, K., 2019. Resource limitation of soil microbes in karst ecosystems. *Science of the Total Environment*, *650*, pp. 241–248.

Charousova, I., Medo, J., Hleba, L., Cisarova, M. and Sona, M., 2019. Antimicrobial activity of actinomycetes and characterization of actinomycin-producing strain KRG-1 isolated from Karoo, South Africa. *Brazilian Journal of Pharmaceutical Sciences,* 55;el 17249, pp. 1–11.

Chiaraphongphon, S., Suriyachadkun, C., Tamura, T. and Thawai, C., 2010. *Dactylosporangium maewongense sp. nov*., isolated from soil. *International Journal of Systematic and Evolutionary Microbiology*, *60*(5), pp. 1200–1205.

Flora, D.O., Adeyemi, A.I. and George, W.P., 2015. Hyoscyamine-producing marine Actinomycetes from Lagos Lagoon sediment. *Asian Pacific Journal of Tropical Biomedicine*, *5*(3), pp. 196–201.

Franco-Correa, M., Quintana, A., Duque, C., Suarez, C., Rodríguez, M. X. and Barea, J.-M., 2010. Evaluation of actinomycete strains for key traits related with plant growth promotion and mycorrhiza helping activities. *Applied Soil Ecology*, *45*(3), pp. 209–217.

Gebreyohannes, G., Moges, F., Sahile, S. and Raja, N., 2013. Isolation and characterization of potential antibiotic producing actinomycetes from water and sediments of Lake Tana, Ethiopia. *Asian Pacific Journal of Tropical Biomedicine*, *3*(6), pp. 426–435.

Goldscheider, N., 2019. A holistic approach to groundwater protection and ecosystem services in karst terrains. *Carbonates and Evaporites*, *34*(4), pp. 1241–1249.

Goodfellow, M. and Fiedler, H.P., 2010. A guide to successful bioprospecting: Informed by actinobacterial systematics. *Antonie van Leeuwenhoek, International Journal of General and Molecular Microbiology*, *98* (2), pp. 119–142.

Hayakawa, M. and Nonomura, H., 1987. Humic acid-vitamin agar, a new medium for the selective isolation of soil actinomycetes. *Journal of Fermentation Technology*, *65*(5), pp. 501–509.

Hayakawa, M. and Nonomura, H., 1989. A New method for the intensive isolation of actinomycetes from soil. *Actinomycetologica*, *3*(2), pp. 95–104.

Hayakawa, M., Sadakata, T., Kajiura, T. and Nonomura, H., 1991. New methods for the highly selective isolation of *Micromonospora* and *Microbispora* from soil. *Journal of Fermentation and Bioengineering*, *72*(5), pp. 320–326.

Hirsch, A. M. and Valdés, M., 2010. Micromonospora: An important microbe for biomedicine and potentially for biocontrol and biofuels. *Soil Biology and Biochemistry*, *42*(4), pp. 536–542.

Hu, N., Li, H., Tang, Z., Li, Z., Li, G., Jiang, Y. and Lou, Y., 2016. Community size, activity and C: N stoichiometry of soil microorganisms following reforestation in a Karst region. *European Journal of Soil Biology*, *73*, pp.77–83.

Istianto, Y., Setyo, R., Koesoemowidodo, A., Saputra, H., Watanabe, Y., Pranamuda, H. and Marwoto, B., 2012. Short Communication Application of phenol pretreatment for the isolation of rare actinomycetes from Indonesian soil. *Microbiology Indonesia, 6*(1), pp. 42–47.

Jiang, Y., Li, Q., Chen, X. and Jiang, C., 2016. Isolation and Cultivation Methods of Actinobacteria. *Actinobacteria - Basics and Biotechnological Applications*, pp. Chapter 2. pp 39–57. Intech Open Access Book. http://dx.doi.org/105772/61457

Kawuri, R. and Darmayasa, I.B.G., 2019. Bioactive compound from extract filtrat streptomyces sp.Sp1. as biocontrol of vibriosis on larvae of Macrobrachium rosenbergii shrimps. *Hayati Journal of Biosciences*, *26*(1), pp. 15 –25.

Khamna, S., Yokota, A. and Lumyong, S., 2009. Actinomycetes isolated from medicinal plant rhizosphere soils: Diversity and screening of antifungal compounds, indole-3-acetic acid and siderophore production Actinomycetes isolated from medicinal plant rhizosphere soils : diversity and screening of an. *World Journal of Microbiology and Biotechnology, 25*, pp. 649–655.

Kim, O., Cho, Y., Lee, K., Yoon, S., Kim, M., Na, H. and Chun, J., 2012. Introducing EzTaxon-e : a prokaryotic 16S rRNA Gene sequence database with phylotypes that represent uncultured species. *International Journal of Systematic and Evolutionary Microbiology*, *62*, pp. 716–721.

Kumar, P. S., Duraipandiyan, V. and Ignacimuthu, S., 2014. Isolation, screening and partial purification of antimicrobial antibiotics from soil *Streptomyces*. *Kaohsiung Journal of Medical Sciences*, *30*(9), pp. 435–446.

Kumar, R.R., and Jadeja, V.J., 2016. Isolation of Actinomycetes: A Complete Approach. *International Journal of Current Microbiology and Applied Sciences*, *5*(5), pp. 606–618.

Lian, B., Yuan, D.X. and Liu, Z.H., 2011. Effect of microbes on karstification in karst ecosystems. *Chinese Science Bulletin*, *56*(35), pp. 3743–3747.

Moreno, D.A., García, A.M., Naffakh, M., Fernández, M.A., Paz, M. I., Montero, F. and Moreno, A., 2013. *Cave Microbiomes: A Novel Resource for Drug Discovery*, *I* (2001), pp. 2–5.

Naikpatil, S.V. and Rathod, J.L., 2011. Selective isolation and antimicrobial activity of rare actinomycetes from mangrove sediment of Karwar. *Journal of Ecobiotechnology*, *3*(10), pp. 48–53.

Niyomvong, N., Pathom-Aree, W., Thamchaipenet, A. and Duangmal, K., 2012. Actinomycetes from tropical limestone caves. *Chiang Mai Journal of Science*, *39* (3), pp. 373–388.

Pronk, M., Goldscheider, N. and Zopfi, J., 2009. Microbial communities in karst groundwater and their potential use for biomonitoring. *Hydrogeology Journal*, *17*(1), pp. 37–48.

Putri, A. L., Lisdiyanti, P. and Kusmiati, M., 2018. Identifikasi Aktinomisetes Sedimen Air Tawar Mamasa, Sulawesi Barat Dan Aktivitasnya Sebagai Antibakteri Dan Pelarut Fosfat. *Jurnal Bioteknologi & Biosains Indonesia (JBBI)*, *5*(2), pp. 139–148.

Ruttanasutja, P. and Pathom-Aree, W., 2015. Selective isolation of cultivable actinomycetes from thai coastal marine sediment. *Chiang Mai Journal of Science*, *42*(1), pp. 89–104.

Thawa, C. and Suriyachadkun, C., 2013. *Dactylosporangium siamense* sp. nov., isolated from soil. *International Journal of Systematic and Evolutionary Microbiology*, *63*(11), pp. 4033–4038.

Thawai, C., Tanasupawat, S. and Kudo, T., 2011. *Dactylosporangium tropicum* sp. nov., isolated from soil. *61*(10), pp. 2358–2362.

Tiwari, K. and Gupta, R.K., 2012. Rare actinomycetes: a potential storehouse for novel antibiotics. *Critical Reviews in Biotechnology*, *32*(2), pp. 108–132.

Yun, Y., Wang, H., Man, B., Xiang, X., Zhou, J., Qiu, X. and Engel, A.S., 2016. The relationship between pH and bacterial communities in a single karst ecosystem and its implication for soil acidification. *Frontiers in Microbiology*, *7*, pp. 23–32.

Zhu, H., He, X., Wang, K., Su, Y. and Wu, J., 2012. Interactions of vegetation succession, soil biochemical properties and microbial communities in a Karst ecosystem. *European Journal of Soil Biology*, *51*, pp. 1–7.

Pedoman Penulisan Naskah Berita Biologi

Berita Biologi adalah jurnal yang menerbitkan artikel kemajuan penelitian di bidang biologi dan ilmu-ilmu terkait di Indonesia. Berita Biologi memuat karya tulis ilmiah asli berupa makalah hasil penelitian, komunikasi pendek dan tinjauan kembali yang belum pernah diterbitkan atau tidak sedang dikirim ke media lain. Masalah yang diliput harus menampilkan aspek atau informasi baru.

Tipe naskah

1. **Makalah lengkap hasil penelitian (*original paper*)**
   Naskah merupakan hasil penelitian sendiri yang mengangkat topik yang *up to date*. Tidak lebih dari 15 halaman termasuk tabel dan gambar. Pencantuman lampiran seperlunya, namun redaksi berhak mengurangi atau meniadakan lampiran.
2. **Komunikasi pendek (*short communication*)**
   Komuniasi pendek merupakan makalah hasil penelitian yang ingin dipublikasikan secara cepat karena hasil termuan yang menarik, spesifik dan atau baru, agar dapat segera diketahui oleh umum. Hasil dan pembahasan dapat digabung.
3. **Tinjauan kembali (*review*)**
   Tinjauan kembali merupakan rangkuman tinjauan ilmiah yang sistematis-kritis secara ringkas namun mendalam terhadap topik penelitian tertentu. Hal yang ditinjau meliputi segala sesuatu yang relevan terhadap topik tinjauan yang memberikan gambaran '*state of the art*', meliputi temuan awal, kemajuan hingga issue terkini, termasuk perdebatan dan kesenjangan yang ada dalam topik yang dibahas. Tinjauan ulang ini harus merangkum minimal 30 artikel.

Struktur naskah

1. **Bahasa**
   Bahasa yang digunakan adalah Bahasa Indonesia atau Inggris yang baik dan benar.
2. **Judul**
   Judul diberikan dalam bahasa Indonesia dan inggris. Judul ditulis dalam huruf tegak kecuali untuk nama ilmiah yang menggunakan bahasa latin, Judul harus singkat, jelas dan mencerminkan isi naskah dengan diikuti oleh nama serta alamat surat menyurat penulis dan alamat email. Nama penulis untuk korespondensi diberi tanda amplop cetak atas (*superscript*). Jika penulis lebih dari satu orang bagi pejabat fungsional penelitian, pengembangan agar menentukan status sebagai kontributor utama melalui penandaan simbol dan keterangan sebagai kontributor utama dicatatan kaki di halaman pertama artikel.
3. **Abstrak**
   Abstrak dibuat dalam dua bahasa, bahasa Indonesia dan Inggris. Abstrak memuat secara singkat tentang latar belakang, tujuan, metode, hasil yang signifikan, kesimpulan dan implikasi hasil penelitian. Abstrak berisi maksimum 200 kata, spasi tunggal. Di bawah abstrak dicantumkan kata kunci yang terdiri atas maksimum enam kata, dimana kata pertama adalah yang terpenting. Abstrak dalam Bahasa Inggris merupakan terjemahan dari Bahasa Indonesia. Editor berhak untuk mengedit abstrak demi alasan kejelasan isi abstrak.
4. **Pendahuluan**
   Pendahuluan berisi latar belakang, permasalahan dan tujuan penelitian. Perlu disebutkan juga studi terdahulu yang pernah dilakukan terkait dengan penelitian yang dilakukan.
5. **Bahan dan cara kerja**
   Bahan dan cara kerja berisi informasi mengenai metode yang digunakan dalam penelitian. Pada bagian ini boleh dibuat sub-judul yang sesuai dengan tahapan penelitian. Metoda harus dipaparkan dengan jelas sesuai dengan standar topik penelitian dan dapat diulang oleh peneliti lain. Apabila metoda yang digunakan adalah metoda yang sudah baku cukup ditulis sitasinya dan apabila ada modifikasi maka harus dituliskan dengan jelas bagian mana dan hal apa yang dimodifikasi.
6. **Hasil**
   Hasil memuat data ataupun informasi utama yang diperoleh berdasarkan metoda yang digunakan. Apabila ingin mengacu pada suatu tabel/ grafik/diagram atau gambar, maka hasil yang terdapat pada bagian tersebut dapat diuraikan dengan jelas dengan tidak menggunakan kalimat 'Lihat Tabel 1'. Apabila menggunakan nilai rata- rata maka harus menyertakan pula standar deviasinya.
7. **Pembahasan**
   Pembahasan bukan merupakan pengulangan dari hasil. Pembahasan mengungkap alasan didapatkannya hasil dan arti atau makna dari hasil yang didapat tersebut. Bila memungkinkan, hasil penelitian ini dapat dibandingkan dengan studi terdahulu.
8. **Kesimpulan**
   Kesimpulan berisi infomasi yang menyimpulkan hasil penelitian, sesuai dengan tujuan penelitian, implikasi dari hasil penelitian dan penelitian berikutnya yang bisa dilakukan.
9. **Ucapan terima kasih**
   Bagian ini berisi ucapan terima kasih kepada suatu instansi jika penelitian ini didanai atau didukungan oleh instansi tersebut, ataupun kepada pihak yang membantu langsung penelitian atau penulisan artikel ini.
10. **Daftar pustaka**
    Tidak diperkenankan untuk mensitasi artikel yang tidak melalui proses *peer review*. Apabila harus menyitir dari "laporan" atau "komunikasi personal" dituliskan '*unpublished*' dan tidak perlu ditampilkan di daftar pustaka. Daftar pustaka harus berisi informasi yang *up to date* yang sebagian besar berasal dari *original papers* dan penulisan terbitan berkala ilmiah (nama jurnal) tidak disingkat.

Format naskah

1. Naskah diketik dengan menggunakan program Microsoft Word, huruf New Times Roman ukuran 12, spasi ganda kecuali Abstrak spasi tunggal. Batas kiri-kanan atas-bawah masing-masing 2,5 cm. Maksimum isi naskah 15 halaman termasuk ilustrasi dan tabel.
2. Penulisan bilangan pecahan dengan koma mengikuti bahasa yang ditulis menggunakan dua angka desimal di belakang koma. Apabila menggunakan Bahasa Indonesia, angka desimal ditulis dengan menggunakan koma (,) dan ditulis dengan menggunakan titik (.) bila menggunakan bahasa Inggris. Contoh: Panjang buku adalah 2,5 cm. Lenght of the book is 2.5 cm. Penulisan angka 1-9 ditulis dalam kata kecuali bila bilangan satuan ukur, sedangkan angka 10 dan seterusnya ditulis dengan angka. Contoh lima orang siswa, panjang buku 5 cm.
3. Penulisan satuan mengikuti aturan *international system of units*.
4. Nama takson dan kategori taksonomi ditulis dengan merujuk kepada aturan standar yang diakui. Untuk tumbuhan menggunakan *International Code of Botanical Nomenclature* (ICBN), untuk hewan menggunakan International *Code of Zoological Nomenclature* (ICZN), untuk jamur *International Code of Nomenclature for Algae*, *Fungi and Plant* (ICFAFP), *International Code of Nomenclature of Bacteria* (ICNB), dan untuk organisme yang lain merujuk pada kesepakatan Internasional. Penulisan nama takson lengkap dengan nama author hanya dilakukan pada bagian deskripsi takson, misalnya pada naskah taksonomi. Penulisan nama takson untuk bidang lainnya tidak perlu menggunakan nama author.
5. Tata nama di bidang genetika dan kimia merujuk kepada aturan baku terbaru yang berlaku.
6. Untuk range angka menggunakan en dash (–), contohnya pp.1565–1569, jumlah anakan berkisar 7–8 ekor. Untuk penggabungan kata menggunakan hyphen (-), contohnya: masing-masing.
7. Ilustrasi dapat berupa foto (hitam putih atau berwarna) atau gambar tangan (*line drawing*).
8. Tabel
   Tabel diberi judul yang singkat dan jelas, spasi tunggal dalam bahasa Indonesia dan Inggris, sehingga Tabel dapat berdiri sendiri. Tabel diberi nomor urut sesuai dengan keterangan dalam teks. Keterangan Tabel diletakkan di bawah Tabel. Tabel tidak dibuat tertutup dengan garis vertikal, hanya menggunakan garis horisontal yang memisahkan judul dan batas bawah.

8. Gambar
   Gambar bisa berupa foto, grafik, diagram dan peta. Judul gambar ditulis secara singkat dan jelas, spasi tunggal. Keterangan yang menyertai gambar harus dapat berdiri sendiri, ditulis dalam bahasa Indonesia dan Inggris. Gambar dikirim dalam bentuk .jpeg dengan resolusi minimal 300 dpi, untuk *line drawing* minimal 600dpi.
9. Daftar Pustaka
   Sitasi dalam naskah adalah nama penulis dan tahun. Bila penulis lebih dari satu menggunakan kata 'dan' atau *et al*. Contoh: (Kramer, 1983), (Hamzah dan Yusuf, 1995), (Premachandra *et al*., 1992). Bila naskah ditulis dalam bahasa Inggris yang menggunakan sitasi 2 orang penulis maka digunakan kata 'and'. Contoh: (Hamzah and Yusuf, 1995). Jika sitasi beruntun maka dimulai dari tahun yang paling tua, jika tahun sama maka dari nama penulis sesuai urutan abjad. Contoh: (Anderson, 2000; Agusta *et al*., 2005; Danar, 2005). Penulisan daftar pustaka, sebagai berikut:

   a. **Jurnal**
      Nama jurnal ditulis lengkap.
      Agusta, A., Maehara, S., Ohashi, K., Simanjuntak, P. and Shibuya, H., 2005. Stereoselective oxidation at C-4 of flavans by the endophytic fungus *Diaporthe* sp. isolated from a tea plant. *Chemical and Pharmaceutical Bulletin*, *53*(12), pp.1565–1569.
   b. **Buku**
      Anderson, R.C. 2000. *Nematode Parasites of Vertebrates, Their Development and Tramsmission*. 2nd ed. CABI Publishing. New York. pp. 650.
   c. **Prosiding atau hasil Simposium/Seminar/Lokakarya.**
      Kurata, H., El-Samad, H., Yi, T.M., Khammash, M. and Doyle, J., 2001. Feedback Regulation of the Heat Shock Response in *Eschericia coli*. *Proceedings of the 40th IEEE Conference on Decision and Control*. Orlando, USA. pp. 837–842.
   d. **Makalah sebagai bagian dari buku**
      Sausan, D., 2014. Keanekaragaman Jamur di Hutan Kabungolor, Tau Lumbis Kabupaten Nunukan, Kalimanan Utara. Dalam: Irham, M. & Dewi, K. eds. *Keanekaraman Hayati di Beranda Negeri*. pp. 47–58. PT. Eaststar Adhi Citra. Jakarta.
   e. **Thesis, skripsi dan disertasi**
      Sundari, S., 2012. Soil Respiration and Dissolved Organic Carbon Efflux in Tropical Peatlands. *Dissertation*. Graduate School of Agriculture. Hokkaido University. Sapporo. Japan.
   f. **Artikel online.**
      Artikel yang diunduh secara online ditulis dengan mengikuti format yang berlaku untuk jurnal, buku ataupun thesis dengan dilengkapi alamat situs dan waktu mengunduh. Tidak diperkenankan untuk mensitasi artikel yang tidak melalui proses peer review misalnya laporan perjalanan maupun artikel dari laman web yang tidak bisa dipertangung jawabkan kebenarannya seperti wikipedia.
      Himman, L.M., 2002. A Moral Change: Business Ethics After Enron. San Diego University Publication. *http:ethics.sandiego.edu/LMH/oped/Enron/index.asp*. (accessed 27 Januari 2008) bila naskah ditulis dalam bahasa inggris atau (diakses 27 Januari 2008) bila naskah ditulis dalam bahasa indonesia

Formulir persetujuan hak alih terbit dan keaslian naskah
Setiap penulis yang mengajukan naskahnya ke redaksi Berita Biologi akan diminta untuk menandatangani lembar persetujuan yang berisi hak alih terbit naskah termasuk hak untuk memperbanyak artikel dalam berbagai bentuk kepada penerbit Berita Biologi. Sedangkan penulis tetap berhak untuk menyebarkan edisi cetak dan elektronik untuk kepentingan penelitian dan pendidikan. Formulir itu juga berisi pernyataan keaslian naskah yang menyebutkan bahwa naskah adalah hasil penelitian asli, belum pernah dan tidak sedang diterbitkan di tempat lain serta bebas dari konflik kepentingan.

Penelitian yang melibatkan hewan dan manusia
Setiap naskah yang penelitiannya melibatkan hewan (terutama mamalia) dan manusia sebagai obyek percobaan/penelitian, wajib menyertakan *'ethical clearance approval*' yang dikeluarkan oleh badan atau pihak berwenang.

Lembar ilustrasi sampul
Gambar ilustrasi yang terdapat di sampul jurnal Berita Biologi berasal dari salah satu naskah yang dipublikasi pada edisi tersebut. Oleh karena itu, setiap naskah yang ada ilustrasinya diharapkan dapat mengirimkan ilustrasi atau foto dengan kualitas gambar yang baik dengan disertai keterangan singkat ilustrasi atau foto dan nama pembuat ilustrasi atau pembuat foto.

Proofs
Naskah *proofs* akan dikirim ke penulis dan penulis diwajibkan untuk membaca dan memeriksa kembali isi naskah dengan teliti. Naskah proofs harus dikirim kembali ke redaksi dalam waktu tiga hari kerja.

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Naskah dikirim secara online ke website berita biologi: *http://e-journal.biologi.lipi.go.id/index.php/berita_biologi*

Alamat kontak
Redaksi Jurnal Berita Biologi, Pusat Penelitian Biologi-LIPI
Cibinong Science Centre, Jl. Raya Bogor Km. 46 Cibinong 16911
Telp: +61-21-8765067, Fax: +62-21-87907612, 8765063, 8765066,
Email: berita.biologi@mail.lipi.go.id
jurnalberitabiologi@yahoo.co.id atau
jurnalberitabiologi@gmail.com

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