Isolation and Antibacterial Activities of Actinomycetes from Rhizosphere Plant Cane *(Saccharum officinarum)* on *Escherichia coli* and *Staphylococcus aureus*

Wahyu Nur Sulistyanto [1)], Guntur Trimulyono [2)]
Program Studi Biologi, Fakultas Matematika dan Ilmu Pengetahuan Alam
Universitas Negeri Surabaya, Indonesia.
email: wahyunut@gmail.com

Abstract

The phenomenon of increasing human bacterial infections and increased resistance to antibiotics encourages the exploration of more antibiotic producers. One source of antibiotics comes from the Actinomycetes bacteria, the source of isolation comes from the rhizosphere of sugarcane plants because it is known in the rhizosphere to produce exudates that are beneficial to bacteria. The purpose of this study was to obtain Actinomycetes isolates and determine the antibacterial abilities of selected isolates. Initial isolation was carried out using Starch Casein Agar (ScA) media, then color grouping, Gram test and cell morphology observation. The next stage is testing the antibacterial activity carried out using the well method. Data obtained in the form of basic characters Actinomycetes, inhibition zone diameter and antibacterial activity count. The results showed that 4 isolates had antibacterial ability against test bacteria, among others, ACB34a isolates, ACB44c isolates, ACB44c isolates and ACB55c isolates. Based on the results of measurement and calculation of antibacterial activity against E. coli, it can be known in succession, among others, ACB44c isolates (9.62 mm2 / 10µl) and ACB54c (0.38 mm2 / 10µl), then antibacterial activity against S. aureus. among others, ACB44c (27.33 mm2 / 10µl), ACB54c (26.41 mm2 / 10µl), ACB55c (8.04 mm2 / 10µl), and ACB34a (8.04 mm2 / 10µl).

Keywords: *Actinomycetes; Antibacterial; Characterization; Rizosphere of sugar cane*

1. INTRODUCTION

The increased of disease infections and antibiotics resistance have encouraged the exploration of more antibiotic-producing sources. Actinomycetes are known to have antibacterial abilities and contribute a lot to produce antibiotics as well as bioactive molecules that influence the industrial world (Sateesh et al., 2011).

In the study of Krismawati et al. (2015) Streptomycetes members of Actinomycetes can be found in the rhizosphere and non-rhizosphere of magrove vegetation. Several isolates were found to be able to produce antibiotics against several test bacteria. The types of bacterial compounds found were thought to be Erytromycin, Tetracyclin, Rimfampicyn, Polymyxin and Chloramphenicol. According to Raningsih (2015), antimicrobial effects on plasma membranes affect their integrity. Increased permeability and leakage of cells due to damaged cell membranes causes the release of intracellular material.

According to Khanna et al. (2011), microbial diversity in the rhizosphere or around cultivated plants is a direct result of interactions and adaptations between plants and microbes. The results of interactions and adaptations are possible adaptations or resistance to plant toxins, have specific secondary metabolites producing certain antibiotics. Soils around the roots or rhizosphere have organic material that encourage the development of active microbes rather than ordinary soils or bulk soils (Geetanjali and Jain, 2016).

Rhizosphere of sugarcane (*Saccharum officinarum*) has loose soil structure with smooth air aeration, tolerant of pH 6-7.5 even up to pH 8 and perfect root development (Indrawanto et al., 2010). This soil condition affect the presence of Actinomycetes. Niswati et al. (2008) explained that exudates found in the rhizosphere affect the bacterial population. Sugarcane exudates are related to the presence of nitrogen, phosphate and amino acid composition. Exudates released depend

Received 14 February 2019 | Received in revised form 15 March 2019 | Accepted 29 March 2019 | Published online 1 April 2019

on the type of plant, monocot plants can release more exudate than dicotyledonous plants (Kato et al., 1997). The presence of Actinomycetes in plant rhizosphere has been explored on rice plants (Ambarwati, 2012), mangrove (Krismawati et al., 2015), banana (Sudarma, 2010); eggplant, potatoes, corn, cabbage, coffee, teak (Susilowati et al., 2007). The purpose of this study was to isolate Actinomycetes from sugarcane rhizosphere and determine their antibacterial ability against *Escherichia coli* and *Staphylococcus aureus*.

2. RESEARCH METHOD

This was an observational study conducted from January to March 2019. Soil samples were obtained from the rhizosphere of the sugar cane plant (S. officinarum) in the Bangkal Kamal area. The implementation of isolation, initial screening, continued color grouping screening, antibacterial ability test and basic characterization were carried out in the Microbiology Laboratory of the Department of Biology.

The tools used were soil tester, incubator, test tube, haemocytometer, centrifuge, vortex, oven, petri dish, ose, cotton, microscope, glass cover, object glass, measuring cup, beaker glass, spirtus lamp, aluminum foil, water bath, woll yarn, filter paper and tissue paper, pestle mortar, analytical scales. The materials used in this study include, ScA media, Oatmeal, Escherichia coli FNCC 0091 and Staphylococcus aureus FNCC 0047, NaCl, crystal violet, methylene blue, iodint, safranin, sterile distilled water, 75% alcohol and 96% alcohol, Nyastin (Fungicide)), Nutrient Agar, Nutrient Broth, starch, K2HPO4, KNO3, Casein, MgSO4.7H2O, Agar, 3% H2O3, sucrose.

The procedure carried out in the study included making Oatmeal media with composition (10 g soluble starch, 2 g K2HPO4, 2 g KNO3, 0.3 g Casein, 0.05 g MgSO4.7H2O, 0.02 g CaCO3, 0.01 g FeSO4.7H2O, 15 g Agar, 1000 mL aquadest water and pH 7 (Mohseni et al., 2013), Sca media with composition (50 g oatmeal, 5 g sucrose, 20 g agar and 1000 mL aquades), NA, NB and test bacterial preparation for *E. coli* and *S. Aureus.*

Bacterial preparation was done by growing *E. coli* dan *S. aureus* on NB then the bacteria was counted its cell density using haemocytometer. The next stage was taking soil samples with five sampling points forming zigzag patterns in sugar cane gardens, measuring soil temperature, pre-treatment (heating 50 ° C soil samples) in the oven to kill gram negative bacteria and other non-target spores, isolating isolates which grows in ScA media and color grouping. Color grouping is a grouping of isolates grown on Oatmeal media so that it is based on the color of the mycelium substrate, the color of the aerial mycelium and mycelium pigmentation.

The stages were continued with purification of isolates which showed the key character of Actinomycetes. Key characteristics of members of the Actinomycetes include the character of the colonies in the ScA media, appearance of the mycelium in the agar media Oatmeal, cell morphology and gram staining. The suspected isolates then undergo antibacterial ability tests using difusion method. The isolates used in this test were suspected Actinomycetes on NB medium for 48 hours which were centrifuged. As much as 10 µL of supernatant applied in the diffusion method. The zone of inhibition formed around the well then observed and measured (Usmati and Marwati, 2007) .

3. RESULT AND DISCUSSION

The results of the initial isolation were successfully obtained 37 isolates and 23 of them were thought to be Actinomycetes. The results of measurements of soil temperature using soil tester showed a temperature of 30° C with a pH of 6.5.

Received 14 February 2019 | Received in revised form 15 March 2019 | Accepted 29 March 2019 | Published online 1 April 2019

Table 1. Results of Isolation and Initial Screening of Actinomycetes Bacteria (Starch Casein Agar) and Colony Morphology)

No	Isolates	Characteristics						
		Growth in ScA Medium	Koloni					
			Color	Shape	Edge	Elevation	Surface	Optic
1.	ACB14a	+	White	Circular	Entire	Convex	Rough	Opaque
2.	ACB14b	+	White	Circular	Entire	Convex	Rough	Opaque
3.	ACB15	+	White	Circular	Entire	Convex	Rough wrinkled	Opaque
4.	ACB16	+	White	Circular	Filament	Ranset	Rough	Opaque
5.	ACB24	+	White	Circular	Entire	Convex	Rough	Opaque
6.	ACB25a	+	White	Circular	Entire	Convex	Rough	Opaque
7.	ACB25b	+	Kream	Circular	Entire	Convex	Rough	Opaque
8.	ACB25c	+	Kream	Circular	Entire	Convex	velvety	Opaque
9.	ACB26a	+	Kream	Circular	Entire	Convex	Rough	Opaque
10.	ACB26b	+	White	Circular	Entire	Convex	Rough	Opaque
11.	ACB26c	+	Non bacterial Actinomycetes (slimy)					
12.	ACB27	+	White	Circular	Entire	Convex	Rough	Opaque
13.	ACB34a	+	White	Circular	Entire	Convex	Rough	Opaque
14.	ACB34b	+	White	Circular	Entire	Convex	Rough	Opaque
15.	ACB35a	+	Non bacterial Actinomycetes (slimy)					
16.	ACB35b	+	Non bacterial Actinomycetes (slimy)					
17	ACB35c	+	White	Circular	Entire	Pulvinate	Rough	Opaque
18.	ACB35d	+	No growth during re-cultur					
19.	ACB36	+	White	Circular	Entire	Convex	Rough	Opaque
20.	ACB44a	+	No growth during re-cultur					
21.	ACB44b	+	No growth during re-cultur					
22.	ACB44c	+	White	Circular	Entire	Convex	Rough	Opaque
23.	ACB45a	+	Kream	Circular	Entire	Convex	Rough	Opaque
24.	ACB45b	+	Non bacterial Actinomycetes (slimy)					
25.	ACB46a	+	Kream	Circular	Entire	Convex	Rough	Opaque
26.	ACB46b	+	No growth during re-cultur					
27.	ACB54a	+	White	Circular	Entire	Convex	Rough	Opaque
28.	ACB54b	+	White	Circular	Entire	Convex	Rough	Opaque
29.	ACB54c	+	Krim	Circular	Entire	Convex	Rough	Opaque
30.	ACB55a	+	No growth during re-cultur					
31.	ACB55b	+	Non bacterial Actinomycetes (slimy)					
32.	ACB55c	+	White	Circular	Entire	Convex	Rough	Opaque
33.	ACB55d	+	No growth during re-cultur					
34.	ACB56a	+	No growth during re-cultur					
35.	ACB56b	+	No growth during re-cultur					
36.	ACB57a	+	White	Circular	Entire	Convex	Rough	Opaque
37.	ACB57b	+	Non bacterial Actinomycetes (slimy)					

At the stage of color grouping, the data showed the appearance or color of mycelium substrate, aeral and pigmentation. As much as 13 isolates showed a color appearance, those were ACB14a, ACB14b, ACB25b, ACB25c, ACB26a, ACB34a, ACB36, ACB44c, ACB45a, ACB46a, ACB54c, ACB55c dan ACB57a.

Received 14 February 2019 | Received in revised form 15 March 2019 | Accepted 29 March 2019 | Published online 1 April 2019

Table 2. Advanced Screening Color Grouping Actinomycetes (Oatmeal Agar), Gram Tests, and Cell Morphology)

No	Isolates	Characteristics					
		Oatmeal Agar					
		Growth	Substrat Mycellium	Aeral Mycellium	Pigmentation of mycelium	Cell Morpho	Gram
1.	ACB14a	+	Pink	Pink	-	Filamen	+
2.	ACB14b	+	White	Green	purple	Filamen	+
3.	ACB15	+	White	Grey	-	Filamen	-
4.	ACB16	+	Slimy	Green	-	Filamen	-
5.	ACB24	+	White	White	-	Filamen	-
6.	ACB25a	+	White	White	-	Filamen	-
7.	ACB25b	+	Greyish pink	Greyish pink	-	Filamen	+
8.	ACB25c	+	White	Grey	Golden yellow	Filamen	+
9.	ACB26a	+	White	-	-	Filamen	+
10.	ACB26b	+	White	Grey	-	Filamen	-
11.	ACB27	+	Pink	Pink	-	Filamen	-
12.	ACB34a	+	Pink	Pink	-	Filamen	+
13.	ACB34b	+	White	White	-	Filamen	-
14.	ACB35c	+	White	Pink	-	Filamen	-
15.	ACB36	+	Pink	Pink	-	Filamen	+
16.	ACB44c	+	Pink	White	-	Filamen	+
17	ACB45a	+	White	White	Pink	Filamen	+
18.	ACB46a	+	White	Grey	-	Filamen	+
19.	ACB54a	+	White	Grey	-	Filamen	-
20.	ACB54b	+	White	Grey	-	Filamen	-
21.	ACB54c	+	Grey	Grey	Golden yellow	Filamen	+
22.	ACB55c	+	White	Greyish pink	-	Filamen	+
23.	Acb57a	+	Greyish green	White	-	Filamen	+

The suspected Actinomycetes were then tested for antibacterial abilities. The test was carried out by calculating the inhibition zone (Usmati and Marwati, 2007). Four isolates that showed positive result were ACB34a, ACB44c, ACB54c and ACB55c. Table 3 and 4 showing antibacterial ability and activity of the selective isolates.

Table 3. Antibacterial ability

No	Antibacterial ability						
	Isolates	*E. coli*	Diameter *Clear zone*	Category*	*S. aureus*	Diameter *Clear zone*	Category*
1.	ACB14a	-	-	-	-	-	-
2.	ACB14b	-	-	-	-	-	-
3.	ACB25b	-	-	-	-	-	-
4.	ACB25c	-	-	-	-	-	-
5.	ACB26a	-	-	-	-	-	-
6	ACB34a	-	-	-	+	3,2 mm	weak
7.	ACB36	-	-	-	-	-	-
8.	ACB44c	+	3,5 mm	weak	+	5,9 mm	mediocre
9.	ACB45a	-	-	-	-	-	-
10.	ACB46a	-	-	-	-	-	-
11.	ACB54c	+	0,7 mm	weak	+	5,8 mm	mediocre
12.	ACB55c	-	-	-	+	3,2 mm	weak
13.	ACB57a	-	-	-	-	-	-

*Categorization according David and Stout (1971)

Received 14 February 2019 | Received in revised form 15 March 2019 | Accepted 29 March 2019 | Published online 1 April 2019

Table 4. Antibacterial activity

Antibacterial activity				
Isolate	*E. coli*	Inhibition Activity (mm²/10µl)	*S. aureus*	Inhibition Activity (mm²/10µl)
ACB34a	-	-	+	8,04 mm²/10µl
ACB44c	+	9,62 mm²/10µl	+	27,33 mm²/10µl
ACB54c	+	0,38 mm²/10µl	+	26,41 mm²/10µl
ACB55c	-	-	+	8,04 mm²/10µl

Isolation of Actinomycetes from the rhizosphere of sugarcane (*Saccharum officinarum*) was carried out with five sampling points (zig-substance pattern). Based on the data, 13 out of 37 isolated bacteria were suspected Actinomycetes. These 13 isolates were ACB14a, ACB14b, ACB25a, ACB26a, ACB34a, ACB36, ACB44c, ACB45a, ACB46a, ACB54c, ACB55c and ACB57a.

Several screening stages were carried out to obtain all of 13 isolates. Those stages consist of 50° C pre-treatment to kill non-target bacteria or non Actinomycetes (Sembiring, 2000), screening on ScA media and mycelium screening on the Oatmeal medium (Krismawati et al., 20015); (Retnowati et al., 2017). The appearance of colonies, mycelium, tests on initial screening has a distinctive appearance that can differentiate from other bacteria. These characteristics are related to colonies that appear rough, dry, gram-positive, color mycelium, distinctive pigmentation and cell morphology in the form of filaments.

Environmental factors showed soil temperature of 30°C, pH 6.5, and clay texture. Environmental factors such as soil conditions support the presence of Actinomycetes to live (Hasyim et al., 2013; Goodfellow et al., 1988). In addition, according to Niswati et al. (2008); Lagos et al. (2015); Sinma et al. (2015) Actinomycetes and plants have reciprocity or mutual benefit.

Sugar cane produce exudates containing amino acids, sugar, vitamins, tannins and others substances that affect the Actinomycetes or rhizobacteria. On the other hand, Actinomycetes provide benefits in the form of active degradation of organic matter, suppress plant pathogen infections and provide phosphate nutrition.

Adaptations and interactions to certain toxic substances lead to secondary metabolites production to specific antibiotics (Khanna et al., 2011). This open up the opportunity to utilize a metabolite product in other fields, in this study against *E. coli* and *Staphylococcus aureus*.

Four isolates of Actinomycetes were able to inhibit the growth of test bacteria. Actinomycetes are a group of bacteria that produce extracellular secondary metabolites so that they are utilized in the source of antibacterial compounds without going through cell breaks (Sharma et al., 2014). Actinomycetes bacteria inhibit cell wall synthesis from test bacteria which causes a decrease in cell osmotic pressure so that test bacteria become inhibited (Jawetz et al., 2007).

A centrifugation at 3000x rpm for 15 minutes was carried out before application.
The supernatant is used as a stock of antibacterial sources that are implanted in wells. Isolates that show antibacterial ability by showing clear zones around the well.

Isolates AcB34a showed a weak inhibition toward S. *aureus* with 3.2 mm clear zone, while isolate ACB44c showed weak inhibition toward E. coli with 3.5 mm clear zone. However ACB44c showed moderate ability in inhibit S. aureus with 5.9 mm inhibition zone. Similar result showed in ACB54c isolate that showed poor activity towards E.coli (0,7 mm) but moderate to S. aureus (5,8 mm). The last isolate, ACB55c, considered as weak againts S. aureus with a 3.2 mm inhibition zone.

It can be seen from the result that isolates which have the highest values to the lowest in sequence include ACB44c isolates (9.62 mm2 / 10µl) and ACB 54c (0.38 mm2 / 10µl), against E. coli, then ACB44c isolates (27.33 mm2 / 10µl), ACB54c (26.41 mm2 / 10µl), ACB55c (8.04 mm2 / 10µl), and ACB34a (8.04 mm2 / 10µl) against S. aureus. The value of the calculation showed the compounds had

Received 14 February 2019 | Received in revised form 15 March 2019 | Accepted 29 March 2019 | Published online 1 April 2019

capabilities that were classified as moderate to low.

According to Kumala et al. (2015), clear zones which appear to be bactericidal regions, namely areas where extracts or supernatants containing a compound that can kill test bacteria (E. coli and S. aureus). Whereas zones formed outside the clear zone or bactericidal area are regions of the clear zone which only inhibit the test bacteria (Kumala et al., 2015). In this study the only visible zone is the clear zone which is the extract area of the supernatant which kills the test bacteria.

This finding is supported by previous research conducted by Ambarwati (2012) showing the antibacterial ability of Actinomycetes from rhizosphere isolation of rice plants to E. coli. There were 3 isolates from 11 isolates who had the ability to inhibit *E. coli* with the details of one isolate in the strong category, one isolate in the moderate category and one isolate in the weak category.

According to Meklat et al. (2011), Actinomycetes antibiotics were coded by the polyketide synthetase (PKS) gene precisely PKS-I, PKS-II and enedyne-PKS. The gene code that produces Actinomycetes antibiotics can be assumed to produce various antibiotic compounds. Goodfellow et al. (1988), explained that each type of strain of Actinomycetes can produce a typical antibiotic compound. Examples are actinohordin, streptomycin, erithromycin, bialaphos, tetracenomycin, rifampicin. Pure actinomycetes can show various chemical structures, among others, anthraeyclines, aminoglyuises, glycopeptides, βlactams, polynes, macrolides, tetracyclins and polyether. These compounds have important function the health field.

The mechanism of inhibition for bacteria depends on the type of compound. It is known that Actinomycetes produce a lot of metabolites harmful to bacteria. There are many compounds that have role to inhibit E. coli and S. aureus. The compounds produced by plant rhizosphere Actinomycetes erytromycin, tetracycline, rifampicin with peptide compounds, flavonoids and alkaloids. The mechanism involving the attacks on RNA (protein synthesis) and cell wall structure (cell lysis). This is based on research conducted by Krismawati et al. (2015) and Masda (2018) with similarity of isolation sources, namely plant rhizosphere and the same test bacteria (E. coli and S. aureus).

In the study of Krismawati et al., (2015) the isolation of Actinomycetes in the mangrove rhizosphere tested on E. coli and S. aureus showed that several isolates produced metabolites such as erytromycin, tetracycline, rifampicin through Thin Layer Chromatography (TLC) methods. Then in the Masda study (2018) the isolation of Actinomycetes in the rhizosphere of bitter plants produced isolates of several isolates which had the ability to inhibit the bacteria E. coli and S. aureus. At the stage of the research, TLC was carried out and the groups of peptides, flavonoids and alkaloids were found. In general, these compounds interfere with the instability of hydrogen bonds with the complexes formed so that permeability fusion of bacterial cells is disrupted and lysis occurs.

The diameter of the inhibitory zone in each selected isolate on test bacteria were different. Several factors affect the diameter of the clear zone are the media composition, temperature, agar thickness (Brady and Katz, 1990); Suriani et al, 2013); (Mujahid et al., 2016). The incubation temperature also affects the enzyme activity related to denaturation and optimal bacterial growth temperature. Bacteria do not grow when they are above the maximum or below the minimum (Suriani et al., 2013). Then the composition of growth media such as carbon sources, nitrogen and special additives to the media influence bacterial or zone of growth (Mujahid et al., 2016). The medium thickness affect the visibility of the clear zone (Brady and Katz, 1990).

4. CONCLUSION

As much as 13 Actinomycetes isolates were obtained from, 4 among them showed the inhibition activity againts E.coli and S.aereus which range in low to moderate category.

5. REFERENCES

Al-Alsaadi A., Noora M.H. and Eman M.J. 2013. Isolation and Identification of Streptomyces From Different Sample of Soils. *Journal of Biology and Medical Science-JBMS*. 1: 31-36.

Ambarwati. 2007. Studi Actinomycetes Yang Berpotensi Menghasilkan Antibiotik Dari Rhizosfer Tumbuhan Putri Malu (Mimosa

Received 14 February 2019 | Received in revised form 15 March 2019 | Accepted 29 March 2019 | Published online 1 April 2019

Pudica L.) Dan Kucing-Kucingan (*Acalypha indica* L). *Jurnal Penelitian Sains & Teknologi*. 8 (1): 1-14.

Ambarwati T. dan Azizah G. 2009. Isolasi Actinomycetes Dari Tanah Sawah Sebagai Penghasil Antibiotik. *Jurnal Penelitian Sains dan Teknologi*. 10 (2): 101 – 111.

Ambarwati, Tanti A., Langkah S. dan Subagus W. 2012. Uji Aktivitas Antifungi Isolat Actinomycetes Yang Berasosiasi Dengan Rizosfer Padi (*Oriza sativa*). *Jurnal Kesehatan*. 5 (2): 139-148.

Armaida E. dan Siti K. 2016. Karakterisasi Actinomycetes yang Berasosiasi dengan Porifera (*Axinella* spp.) dari Perairan Pulau Lemukutan Kalimantan Barat. *J. Protobiont*. 5(1): 68-73.

Athalye M., M Goodfellow., Lacey J. and White R.P. 1985. Numerical Classification of Actinomadura and Nocardiopsis. *Journal of Systematic Bacteriology*. 35 (1): 86-98.

Brady M.S. and Katz S.E. 1990. Factors Influencing Optimalization of Diffusion Assays for Antibiotics. *J Assoc Off Anal Chem*. 73(2): 202-205.

David W.W. and Stout T.R. 1971. Disc Plate Method of Microbiological Antibiotic Assay. Applied Microbiology. 22 (4): 666-670.

Geetanjali and Pranay J. 2016. Antibiotic Production by Rhizospheric Soil Microflora - A Review. *IJPSR*. 7 (11): 4304-4314.

Goodfellow M., Williams S.T. and Mordarski M. 1988. *Actinomycetes In Biotechnology*. San Diego: Academic Press Inc.

Holt J.G., Sneath PH., Krieg and Noel R. 1994. *Bergey's Manual of Determinative Bacteriology: Ninth Ed*. USA: William and Walkins Baltimore.

Indrawanto C., Purwono, Siswanto, Syakir M. S., Joni M., Joko P. dan Widi R. 2010. *Budidaya dan Pasca Panen Tebu*. Jakarta: ESKA Media.

Jawetz M. dan Adelberg. 2007. *Mikrobiologi Kedokteran*. EGC: Jakarta. 165-174.

Kato K., Yasuhiro A. dan Hiroshi H. 1997. Effect of exudates released from seed and seedling root of common bean (Phaseolus vulgaris L.) on proliferation of Rhizobium sp. (Phaseolus). *Soil Sci. Plant Nutr*. 43: 275-283.

Khanna M., Renu S. and Rup L. 2011. Selective Isolation of Rare Actinomycetes Producing Novel Antimicrobial Compounds. *International Journal of Advanced Biotechnology and Research*. 2 (2): 357-375.

Krismawati H., Langkah Sembiring. dan Subagus W. 2015. Streptomycetes Penghasil Antibiotik yang Berasosiasi dengan rhizosfer beberapa Spesies Mangrove. *Plasma*. 1 (2): 59-70.

Kumala T., Afghani J. dan Puji A. 2015. Uji Aktivitas Antibakteri Isolat Actinomycetes 9ISP1 Dari Spons Asal Perairan Pulau Randayan. *JKK*. 4 (2): 30-36.

Lagos M.L., Maruyama F., Nanniperi P., Mora M.L., Ogram A. and Jorquera M.A. 2015. Current Overview on The Study of Bacteria in The Rhizosphere by Modern Molecular Techniques: A Mini–Review. *Journal of Soil Science and Plant Nutrition*. 15 (2): 504-523.

Masda N.R. 2018. *Potensi Metabolit Sekunder Isolat Actinomycetes SM-2 Dari Rizosfer Andrographis paniculata Sebagai Penghasil Senyawa Antibakteri*. Skripsi (tidak di terbitkan). Marough: Universitas Hasanuddin Makassar.

Meklat A.N., Sabaou A., Zitouni F., Mathiey and A. Lebrihi. 2011. Isolation, Taxonomy, and Antagonistic Properties of Halophilic Actinomycetes in Saharan Soils of Algeria. *Applied and Environmental Microbiology*. 77 (18): 6710–6714.

Received 14 February 2019 | Received in revised form 15 March 2019 | Accepted 29 March 2019 | Published online 1 April 2019

Mohseni M., Norouzi H., Hamedi J. and Roohi A. 2013. Screening of Antibacterial Producing Actinomycetes from Sediment of The Caspian Sea. *Int J Mol Cell Med Spring*. 2 (2): 64-71

Mujahid T.Y., Syed A.S., Abdul W., Javeria M., Nuzhat A. and Tanveer A. 2015. Effects of Different Physical and Chemical Parameters on Phosphate Solubilization Activity of Plant Growth Promoting Bacteria Isolated from Inidgenous Soil. *Journal of Pharmacy and Nutrition Sciences*. 5: 64-70.

Niswati A., Sri, dan Mas ASA. 2008. Populasi Mikroba Pelarut Fosfat dan P-tersedia pada Rizosfir beberapa Umur dan Jarak dari Pusat Perakaran Jagung (Zea mays L.). *Jurnal Tanah Trop*. 13 (2): 123-130.

Nurkanto A. dan Andria A. 2015. Identifikasi molekular dan karakterisasi morfo-fisiologi actinomycetes penghasil senyawa antimikroba. *Jurnal Biologi Indonesia*. 11 (2):195-203.

Pujiati. 2014. Isolasi Actinomyctes Dari Tanah Kebun Sebagai Bahan Petunjuk Praktikum Mikrobiologi. *Jurnal Flore*. 1(2):42-46.

Raningsih N.M. 2015. *Aktivitas Antibakteri Actinomycetes terhadap Methicillin Resistant Staphylococcus aureus (MRSA)*. Thesis (Tidak diterbitkan). Denpasar: Universitas Udayana.

Retnowati Y., Langkah Sembiring, Sukarti M., Tjut S. dan Endang S.S. 2017. Isolasi dan Uji Aktivitas Antibakteri Actinomycetes dari Rhizosfer Bakau di Hutan Bakau Torosiaje Gorontalo. Seminar Nasional Pendidikan Biologi dan Saintek. Surakarta: Universitas Muhammadiyah Surakarta.

Sateesh V., Naikpatil J.L. and Rathod. 2011. Selective Isolation and Antimicrobial Activity of Rare Actinomycetes from Mangrove Sediment of Karwar. *Journal of Ecobiotechnology*. 2 (10): 48-53.

Sembiring L., Ward A.C. dan Goodfellow M. 2000. Selective Isolation and Characterisation of Members of the Streptomyces violaceusniger Clade Associated with the Roots of Paraserianthes falcataria. Antonie Van Leeuwenhoek. 78: 353-366.

Sinma K., Nurak T. and Khucharoemphaisan K. 2015. Potentiality of Endophytic Actinomycetes. *KMITL Sci. Tech*. J. 15 (2): 88-97.

Sudarma I.M. 2010. Seleksi Dan Pemanfaatan Actinomycetes Sebagai Mikroba An Tagonis Yang Ramah Lingkungan Terhadap Fusarium oxysporum Sp. Cubense Secara In Vitro. *ECOTROPHIC*. 5 (2): 104 – 107.

Suriani S., Soemarno dan Suharjono. 2013. Pengaruh Suhu dan pH Laju Pertumbuhan Lima Isolat Bakteri Anggota Genus Pseudomonas yang diisolasi dari Ekosistem Sungai Tercemar Deterjen di sekitar Kampus Universitas Brawijaya. *J-PAL*. 3(2): 58-62.

Susilowati D.N., Ratih D.H. dan Erny Y. 2007. Isolasi dan karakterisasi aktinomycetes penghasil antibakteri enteropatogen escherechia coli K1.1, Pseudomonas pseudomallei 02 05, dan Listeria monocytogenes 5407. *Jurnal AgroBiogen*. 3(1): 15-23.

Usmati S. dan Tri M. 2007. Seleksi dan Optimasi Proses Produksi Bakteriosin Dari Lactobacillus sp. *J. Pascapanen*. 4 (1): 27-37.

Received 14 February 2019 | Received in revised form 15 March 2019 | Accepted 29 March 2019 | Published online 1 April 2019